COMMUNICATIONS

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^{1}H NMR Detection of Vitamin C in Human Brain In Vivo

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Vitamin C (ascorbate) is well established as an essential nutrient that functions as an antioxidant. Since it is present in the human brain at detectable concentrations, this study was designed to detect and quantify ascorbate in the human brain in vivo using ^{1}H NMR spectroscopy (MRS). Ascorbate was consistently detected in all five study subjects, and was measured using MEGA-PRESS difference editing. The in vivo resonance pattern was consistent with that of ascorbate based on position, line width, peak pattern, and relative intensity. Metabolites with a potential for coediting were assessed using phantom solutions. The putative resonances of myo-inositol, lactate, glycerophosphocholine, phosphocholine, and phosphoethanolamine were detected at positions distinct from those of ascorbate. This study represents the first in vivo detection of vitamin C in the human brain using ^{1}H MRS. A concentration of 1.3 ± 0.3 μmol/g (mean ± SD, *N* = 4) was estimated. Magn Reson Med 51:225–229, 2004. © 2004 Wiley-Liss, Inc.

Key words: vitamin C; ascorbic acid; human; brain; magnetic resonance spectroscopy

The importance of vitamin C (ascorbic acid) in protecting against various diseases that have their origins in oxidative stress has been established over the past 25 years. While vitamin C is known primarily for its role in preventing scurvy, it has also been credited with lowering risks of chronic degenerative disease and cancer (1). Vitamin C occurs physiologically as the ascorbate anion (Asc). The brain, spinal cord, and adrenal glands have the highest Asc concentrations of all tissues in the body, which suggests that Asc plays a key role in brain function. Brain Asc has been linked to neurotransmitter (glutamate) release and uptake (2). Since vitamin C levels in the blood may be suboptimal in large sections of the population, the recommended daily allowances are being revisited (1).

^{1}H NMR spectroscopy (MRS) is uniquely qualified for measuring metabolite concentrations in live, intact tissue (i.e., in vivo). Since the concentration of Asc in the human brain (~1 μmol/g (2)) is high enough to be detectable, the purpose of this study was to evaluate the feasibility of detecting and quantifying Asc in the human brain using ^{1}H MRS. Edited spectroscopy is routinely utilized to uncover coupled resonances, such as Asc from underneath stronger resonances. Figure 1 and Table 1 illustrate the structural and chemical shift information for Asc that is pertinent to edited ^{1}H MRS. The close proximity (in terms of hertz) of the coupled resonances make edited detection of Asc particularly challenging. Increased magnetic fields (B_0) are helpful in overcoming this challenge because they result in increased separation of coupled resonances. ^{1}H MRS MEGA-PRESS (sequence element for editing placed within point resolved spectroscopy) editing was recently applied in vivo at high fields to quantify the anti-oxidant glutathione (3) and the neurotransmitter GABA (4), which have in vivo concentrations and spectroscopic properties similar to those of vitamin C. Therefore, this technique was optimized for detection of vitamin C at 4T.

MATERIALS AND METHODS

Five normal volunteers (two males and three females, average age = 21 years) gave informed consent for this study, which was conducted according to procedures approved by the Institutional Review Board. All experiments were performed with a 4T, 90-cm-bore magnet (Oxford Magnet Technology, Oxford, UK) interfaced to a Varian INOVA spectrometer (Varian, Palo Alto, CA) equipped with gradients capable of switching to 40 mT/m in 400 μs (Sonata, Siemens, Erlangen, Germany) and a surface ^{1}H quadrature transceiver (5). The subjects were positioned supine inside the magnet with the RF transceiver subjacent to their occipital lobe. First, localizer multislice rapid acquisition with relaxation enhancement (RARE) images (TR = 4.0 s, TE = 60 ms, echo train length = 8, matrix = 256 × 128, two averages, slice thickness = 2 mm, and five slices) were obtained to select a cubic volume of interest (VOI) (3 × 3 × 3 cm^3) centered on the midsagittal plane in the occipital lobe. All first- and second-order coils were shimmed using FAST(EST)MAP (6), which consistently resulted in water line widths of 8 Hz. After the spectrometer frequency was adjusted, difference editing using MEGA-PRESS was performed as described below. Each acquisition (NEX = 1) was stored separately in memory, and was then frequency- and phase-corrected based on the individual NAA signal prior to summation of all 512 acquisitions (TR = 4.5 s). Frequency correction was never more than 6 Hz. Processing was performed using spectrometer built-in software.

MEGA-PRESS difference editing (3) was optimized for edited detection of the C6H$_2$ resonance of Asc (3.73 ppm), via J-coupling to the C5H resonance (4.01 ppm). Spectra acquired from a phantom containing Asc using stimulated echo acquisition mode (STEAM) (7) revealed that the C4 (4.50 ppm) and C6 protons gave rise to more intense resonances than the C5 proton at 4.01 ppm (Fig. 1). Therefore, the C5 proton at 4.01 ppm was chosen as the target for the editing inversion pulse in MEGA-PRESS (8). An optimal TE of 112 ms was determined using the Asc phantom. This

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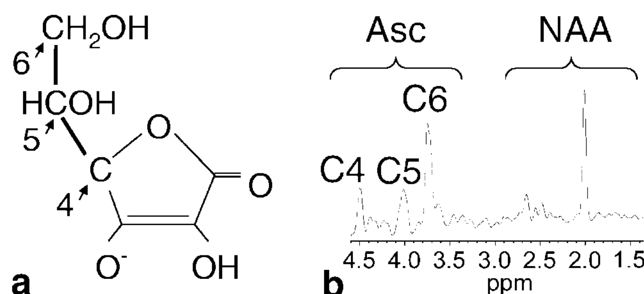

FIG. 1. Structure and ^{1}H MRS spectrum of ascorbate: (**a**) chemical structure and (**b**) ^{1}H MRS spectrum acquired from a phantom solution containing 10 mM ascorbate and 5 mM NAA (STEAM (16) with VAPOR water suppression, 27 ml volume, TE = 5 ms, TR = 4.5 s, NEX = 4).

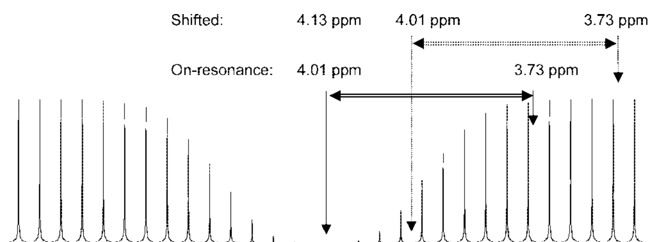

FIG. 2. Illustration of the editing pulse placement required in order to avoid detection of noncoupled spins at 3.73 ppm in difference-edited spectra at 4T. The inversion pulse profile was measured via the water suppression profile. ^{1}H spectra from an aqueous phantom were acquired while the offset of the inversion pulse was incremented within ±75 Hz of the water frequency in 5-Hz steps. The horizontal double arrows correspond to the frequency separation between the Asc C6H$_2$ at 3.73 ppm and the C5H at 4.01 ppm (47 Hz). When the editing pulse is placed at 4.01 ppm (labeled “on-resonance” (solid arrows)), a small amount of excitation can occur at 3.73 ppm, especially if small frequency drifts are incurred. The frequency of the editing pulses was therefore set to 4.13 ppm (labeled “shifted” (dotted arrows)), which moved the resonance positions relative to the editing profile, as illustrated by the dashed arrows and the top row labels. In the “shifted” case, complete inversion was not accomplished at 4.01 ppm, which led to increased reliability at the cost of reduced editing efficiency.

long TE accommodated a longer editing pulse (a 40-ms Gaussian inversion) with a more selective frequency bandwidth (40 Hz). Unfortunately, this 40-ms Gaussian centered on C5H at 4.01 ppm can result in partial excitation of resonances at 3.73 ppm, which can lead to signal contributions from the more intense glutamate and glutamine H2 resonances. Figure 2 illustrates the inversion profile of the 40-ms Gaussian pulse via the water suppression profile of MEGA-PRESS. Note that if complete inversion at 4.01 ppm is accomplished in the middle of the profile, a small amount of excitation may occur at 3.73 ppm, as indicated by the solid arrows. Shifting the offset of the inversion pulse by approximately 20 Hz, or to 4.13 ppm, minimized such partial excitation, although it also reduced editing efficiency (dashed arrows in Fig. 2). The offset of the editing pulse was therefore set to 4.13 ppm in order to minimize potential subtraction artifacts at 3.73 ppm, and to accommodate a safety margin of 10 Hz for B_0 shift during in vivo studies. RF power (B_1) for the inversion (editing) pulses was determined in each case by placing the offset of the editing pulse on the water resonance, and incrementing B_1 until optimal water suppression was observed. Water suppression using variable power radiofrequency pulses with optimized relaxation delays (VAPOR) (7) and outer volume saturation were incorporated before the MEGA-PRESS technique was applied, as described previously (3).

Asc concentration was estimated by measuring the heights of the maxima of edited Asc and edited aspartyl NAA resonances both in vivo and in a phantom spectrum, which was line-broadened to match the in vivo line widths (i.e., accordingly line broadened), and contained known concentrations of Asc and NAA. The intensities measured as such in the phantom spectrum were used to calibrate the ratio for calculating in vivo concentrations, assuming an in vivo NAA concentration of 10 μmol/g.

Potential contamination of the edited Asc signal at 3.73 ppm was evaluated by consulting metabolite assignment tables (9,10). Based on known resonance positions and J-coupling, metabolite spin systems capable of coediting with Asc, and producing resonances in the spectral region between 1 and 4 ppm, were identified. Table 2 summarizes the metabolites that may conceivably contribute resonances to the edited spectrum. Glucose (Glc) and glycogen contribute resonances that may be coedited immediately adjacent to 3.73 ppm. Glutamate (Glu) and glutamine (Gln) were identified as most likely to contribute resonances near 3.73 ppm as a result of partial excitation by the editing pulse on every other scan. Although certain metabolites (N-acetylaspartate (NAA), myo-inositol (Ins), lactate (Lac), glycero-phosphocholine (GPC), phosphoryl choline (PC), phosphoryl ethanolamine (PE), and glycerol-phosphoethanolamine (GPE)) should be resolved from Asc, they are capable of coediting with Asc because they all have coupling partners within the bandwidth of the editing pulse. In addition, the strong creatine (Cr) resonance is likely to be affected by the editing pulse and contribute a resonance near 3.9 ppm. Therefore, spectra acquired by MEGA-PRESS difference editing were acquired from phantoms (37°C, pH = 7.1) containing the aforementioned metabolites. NAA was included in most phantoms to serve as an intensity reference and to verify the correct editing technique. Since GPC and PC could not be buffered (11), they were not pH-balanced or warmed. However, it is likely that pH in these phantoms was neutral, because GPC and PC are not expected to influence pH substantially, and NAA was not included in these phan-

Table 1
^{1}H Chemical Shifts of Ascorbate*

Group	Multiplicity	Chemical shift
C6H$_2$	M	3.73
C5H	M	4.01
C4H	D	4.50
C3		
C2		
C=O		

*From (17), Table 5, page 172.
D, doublet; M, multiplet.

Table 2
Metabolites That Potentially Co-edit in MEGA-PRESS Editing Experiments Designed to Detect Asc and Their Concentrations in the Phantom Solutions*

Metabolite	Group	Multiplicity	Chemical shift	Phantom composition
Asc[a]				1.5 mM Asc
				10 mM NAA
Ins	^{1}CH	DD	3.52	7 mM Ins
	^{2}CH	T	4.05	10 mM NAA
	^{3}CH	DD	3.52	
Lac	^{2}CH	Q	4.10	1 mM Lac
	$^3CH_3$	D	1.31	10 mM NAA
GPC[b]	$^7CH_2$	M	4.31	~1 mM GPC
	$^8CH_2$	M	3.66	
PC	$^1CH_2$	M	4.28	10 mM PC
	$^2CH_2$	M	3.64	
GPE	N		3.29	Not available
	O		4.11	
PE	$^1CH_2$	M	3.98	2 mM PE
	$^2CH_2$	M	3.22	2 mM NAA
Cr/PCr	$N(CH_3)$	S	3.03	10 mM Cr
	$^2CH_2$	S	3.91	
Glc	α ^{6}CH	DD	3.83	1 mM Glc
	α $^{6'}$CH	DD	3.75	10 mM NAA
	β ^{6}CH	DD	3.88	
	β $^{6'}$CH	DD	3.71	
Glu	^{2}CH	DD	3.74	9 mM Glu
				10 mM NAA
Gln	^{2}CH	T	3.75	3 mM Gln
				10 mM NAA
NAA[c]	^{2}CH	DD	4.38	5 mM NAA
	$^3CH_2$	DD	2.67	
		DD	2.49	

*From 9, 10.
[a]See Table 1 for groups, multiplicities, and chemical shifts.
[b]Choline moiety; the Glycerol moiety was expected to be less likely to contribute.
[c]Aspartate moiety.
D, doublet; DD, double-doublet; M, Multiplet; Q, quartet; S, singlet; T, triplet.

toms. Table 2 details the composition of the phantoms. Typically, physiologic concentrations of the metabolites were used.

Finally, although the macromolecule (MM) spin system is unlikely to contribute to the edited signal near 3.73 ppm (11,12), one experiment was dedicated to acquiring a "metabolite-nulled" spectrum (4,8). An inversion pulse was applied before the MEGA-PRESS editing scheme, and the time between the inversion pulse and MEGA-PRESS (TIR = 0.66 s) was set experimentally to the value where the strongest metabolite signals (NAA, Cr, and Cho) were nulled in subspectra. Since macromolecules recover faster than metabolites, they appear in the metabolite-nulled (i.e., MM) spectrum. To minimize the effect of slightly different metabolite T_1's leading to residual metabolite signal in the inversion-recovery (IR) measurement, a shorter repetition time (TR = 2 s) was used, which further saturated the metabolite signals while allowing substantial recovery of macromolecule signals. Frequency and phase corrections were based on interleaved, inversion-naïve scans.

RESULTS

The $C6H_2$ resonance of Asc was consistently detected at 3.73 ppm in all in vivo studies, as judged from the consistent spectral pattern at 3.73 ppm, which mimicked that measured in an accordingly line-broadened phantom spectrum (Fig. 3). In all four subjects measured at TR = 4.5 s, the editing resulted in resonance intensity at 3.73 ppm. This was consistent with an Asc concentration of 1.3 ± 0.3 μmol/g (mean ± SD, $N = 4$), as judged from the relative intensities of Asc and NAA (aspartyl moiety) in vivo and in vitro. Thus, the in vivo resonance pattern was consistent with that of Asc based on position, line width, peak pattern, and relative concentration. The striking repeatability of the edited in vivo spectra was noteworthy (Fig. 3).

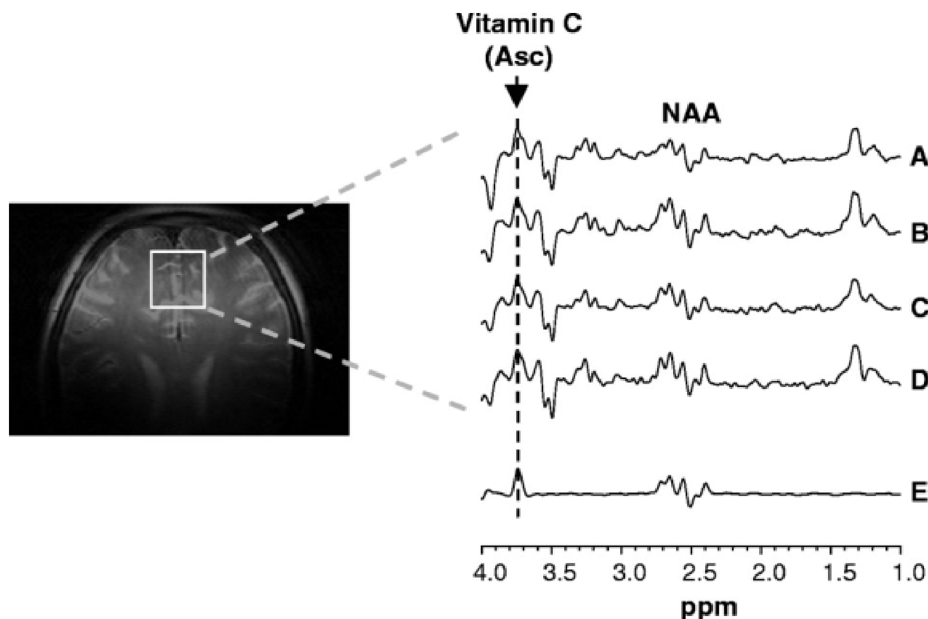

FIG. 3. RARE image (TR = 4.0 s, TE = 60 ms, echo train length = 8, matrix = 256 × 128, two averages, slice thickness = 2 mm, five slices) illustrating the VOI (3 × 3 × 3 cm^3). Also shown are edited in vivo ^{1}H MRS spectra obtained using MEGA-PRESS (TE = 112 ms, TR = 4.5 s, NEX = 512) in four volunteers (**a–d**), and the accordingly line-broadened spectrum from a phantom containing 1.5 mM Asc and 10 mM NAA (**e**).

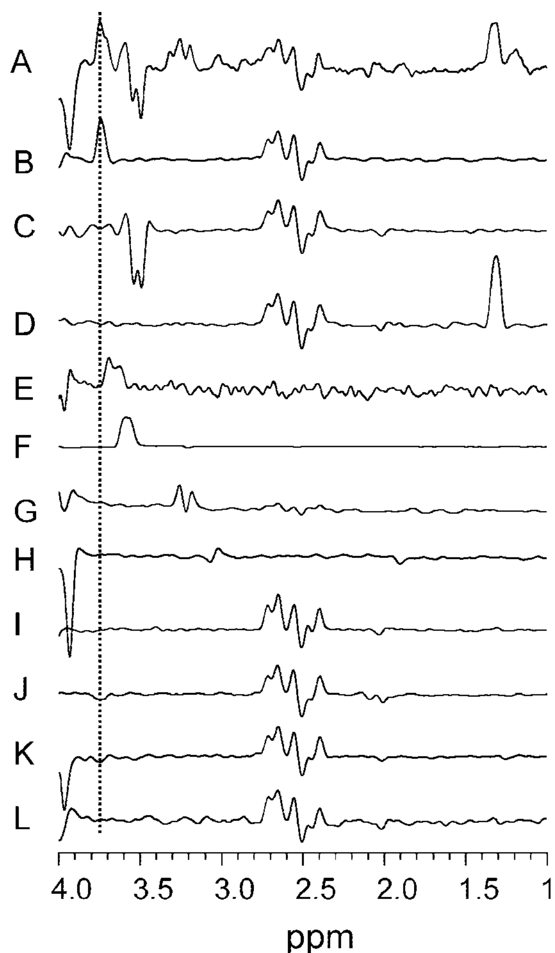

FIG. 4. Representative in vivo spectrum (A) and accordingly line-broadened spectra acquired from phantoms containing the following metabolites (which are capable of coediting or contributing subtraction artifacts using MEGA-PRESS as described): Asc + NAA (B), mIns + NAA (C), Lac + NAA (D), GPC (E), PC (F), PE (G), Cr (H), Gluc + NAA (I), Glu + NAA (J), Gln + NAA (K), and NAA (L). The concentrations of the metabolites are listed in Table 2.

Several coedited resonances that were resolved from those of Asc were consistently detected in vivo, while contamination from subtraction artifacts was not observed near 3.73 ppm (Figs. 3 and 4). When compared with the in vivo spectrum, spectra acquired from the Ins, Lac, GPC, PC, and PE phantoms indicated that these coedited metabolites accounted for the corresponding coedited resonances (Fig. 4). Furthermore, a resonance near 3.3 ppm that was consistently detected but was resolved from PE, may have been contributed by GPE. Cr contributed the expected subtraction signal near 3.9 ppm. Using the present methodology, coediting of resonances from Glc, Glu, and Gln was negligible (Fig. 4). A 10-Hz B_0 drift in the direction most likely to increase signal contribution was simulated experimentally with the Glu phantom, and resulted in a small resonance of opposite sign relative to the Asc resonance. While the expected (13) MM resonance near 1.2 ppm was detected in the nulled (MM) spectrum, the absence of a resonance in the vicinity of 3.7 ppm suggested a minimal contribution of macromolecules to the Asc resonance (Fig. 5).

DISCUSSION

In this study, vitamin C was detected by ^{1}H MRS in the human brain in vivo for the first time. The ^{1}H MRS editing technique described herein facilitated the detection of a strikingly reproducible spectral pattern (Fig. 3). The edited resonance detected in vivo at 3.73 ppm clearly resembled that detected in solutions containing Asc. Since J-modulation at the long TE used in this application of MEGA-PRESS inherently yields a specific spectral pattern for Asc, this resemblance is strong evidence that the resonance detected at 3.73 ppm is indeed Asc. Metabolites that could be mistaken for vitamin C were identified and rejected as significant contributors to this resonance. Great care was taken to desensitize the editing technique to contamination, even in the presence of B_0 drift (4,14). In fact, editing efficiency was compromised in order to ensure that glutamate and glutamine would not contribute signal near 3.73 ppm, with a safety margin allowing for a ±10 Hz drift in B_0. Experimental in vivo B_0 drifts (<6 Hz) were well within this safety margin. In addition to facilitating B_0 and phase correction, storing data on a scan-by-scan basis enabled continuous surveillance of the B_0 drift.

Several coedited resonances were putatively identified (Fig. 4) in the reproducible spectral pattern observed in vivo. These resonances may extend the usefulness of this technique to additional metabolites, such as GPC and GPE. The aspartyl moiety of NAA detected here may increase our understanding of the relative contributions from the acetyl vs. aspartyl moiety, in addition to serving as an internal reference. With the application of LCModel to these edited spectra (3), GPC and GPE may be resolved from PC and PE, respectively.

The relative intensities of the Asc and NAA aspartyl resonances in vivo and in vitro indicated an in vivo Asc concentration of 1 mM, in excellent agreement with that reported in the literature (2). The application of LCModel to the fitting of edited spectra (3) should yield a more precise value for the concentration of vitamin C, due to the fine structure of the resonances in these spectra. Although some uncertainty may result from T_2 relaxation, small changes in vitamin C concentration may be measurable, especially given the high repeatability and quality demonstrated in the in vivo spectra.

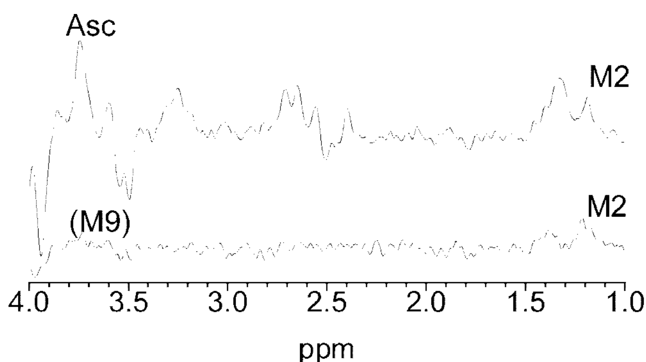

FIG. 5. Metabolite-nulled experiment. The edited (metabolite) spectrum (top trace) was acquired as in Fig. 3 (TE = 112 ms, NEX = 512), except for a 2-s TR, and interleaved with a metabolite-nulled (MM) spectrum (TIR = 0.66 s, bottom trace).

In the present study, a high magnetic field (4T) was advantageous for the detection of the coupled Asc resonance. Further improvements are likely to be achieved at 7T, provided the loss in sensitivity due to the shortening in T_2 does not outweigh the advantages.

Although a coupled MM resonance in the vicinity of 3.73 ppm is not suspected (12), coupling to a more recently identified macromolecule resonance (M9) at 3.77 ppm (15) has not been studied. Therefore, we included an IR experiment in this study. The observation of the edited resonance near 1.2 ppm (M2) in vivo (Fig. 5), which was expected as a result of connectivity to 4.23 (12), demonstrated sufficient MM detection to overrule substantial contamination from M9, which was not detected in vivo. The appearance of a strong Asc resonance in the interleaved metabolite spectrum, together with the negligible signal near 3.7 ppm in the MM spectrum, supports our conclusion that MM contamination of the Asc signal is negligible with the present methods.

In vivo quantification of Asc concentration, as described herein, could aid researchers in revisiting the recommended daily allowances of vitamin C. Since Asc is an antioxidant, in vivo quantification of this metabolite may also increase our understanding of certain diseases, such as neurodegenerative diseases and cancers, in which oxidative stress has been implicated. In addition, nine compounds of biological interest appear to be simultaneously measurable using the proposed editing scheme, which increases the usefulness of such editing methods.

CONCLUSIONS

This study establishes that vitamin C can be detected reproducibly in the human brain in vivo using ^{1}H MRS. Concentration can by quantified by appropriate fitting procedures (3), which renders the detection of changes on the order of 5–10% conceivable. With this new measurement technique, one can investigate the influence of dietary vitamin C manipulations on tissue Asc concentrations in humans, as well as the role of Asc in the pathogenesis of several neurodegenerative disorders and cancer.

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