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Scyllo-inositol in normal aging human brain: ^1H magnetic resonance spectroscopy study at 4 Tesla

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Abstract

The *scyllo*-inositol and *myo*-inositol concentrations of 24 normal human subjects were measured *in vivo* using ^1H magnetic resonance spectroscopy at 4 T. Single-voxel short-echo ($TE = 15$ ms) metabolite spectra were collected from the white matter region of the corona radiata. Test–retest studies performed on 10 normal subjects demonstrated coefficient of variation for *scyllo*-inositol measurement of 37%, compared with 6% for *N*-acetyl aspartate. Comparisons between old and young subjects showed higher concentration of *scyllo*-inositol and *myo*-inositol in older subjects and a trend for a correlation between *scyllo*-inositol and *myo*-inositol levels across subjects.

Keywords

scyllo-inositol; *myo*-inositol; taurine; 4 T; spectroscopy; human brain; white matter; aging

INTRODUCTION

Previous *in vivo* proton magnetic resonance spectroscopy (^1H MRS) studies have reported findings of abnormal *scyllo*-inositol (*s*-Ins) level in normal brains¹ and in patients with different neuropathologies.^{2–5} These studies were performed at various magnetic fields, ranging from 1.5 to 4 T. One study performed at 7 T demonstrated improved visibility of *s*-Ins in the ^1H MR spectra in the human brain at higher fields.⁶ In general, reliable detection of *s*-Ins is limited because of its low concentration in human brain (<1 mM). In addition, a complete spectral overlap with taurine (Tau) at lower magnetic fields (i.e. 1.5–2 T) imposes an additional limitation for *s*-Ins quantification. At higher magnetic field strength, the overlap of the singlet resonance line of *s*-Ins (from six equivalent protons) with the multiplet resonance pattern of Tau is substantially reduced due to a greater spectral dispersion of the Tau multiplets (compare numerical simulations of the spectral patterns at 4 and 1.5 T in Fig. 1). Furthermore, signal-to-noise ratios of singlet resonances, such as the singlet of *s*-Ins, generally increase with higher magnetic fields,⁷ while the same is not generally true for more complex spectral patterns of multiplets, such as Tau and *myo*-inositol (*m*-Ins). Because the overlap from *s*-Ins and Tau resonances is reduced and intensity of the *s*-Ins singlet increases with increasing magnetic field strength, measurements of *s*-Ins should become more accurate at higher magnetic fields.

s-Ins and its stereoisomer, *m*-Ins, are the two most ubiquitous forms of inositol, present in brain, liver and kidney. *s*-Ins is the second largest isomer in abundance after *m*-Ins, which contributes over 90% to the total inositol content of mammalian cells⁸ (therefore *myo*-inositol and inositol

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nomenclature is used interchangeably in the majority of the literature). In the brain, inositol is involved in osmoregulatory processes⁹ (in its free form) and in neural receptor signaling systems¹⁰ (via its phosphorylated derivatives). Compared with *m*-Ins, the information on *s*-Ins measurements in human brain is rather limited. Currently, findings from brain MRS studies suggest an increase of *s*-Ins in patients with mitochondrial enzyme deficiency⁴ and certain brain tumors.⁵ Abnormally high levels of *s*-Ins were also reported in a case study on one healthy subject¹ and two patients with human immunodeficiency virus (HIV).³ Lower levels of *s*-Ins and *m*-Ins were detected in hepatic encephalopathy using both MRS and high-performance liquid chromatography (HPLC).² In some cases the reports have suggested that there is a positive correlation between *s*-Ins and *m*-Ins concentration both in normal controls and pathology, implying a metabolic relationship between the two metabolites.

Recently, we reported ¹H MRS measurements of glutamate and glutamine levels in healthy human brain.¹¹ Subsequently, we observed changes in the spectral region associated with *s*-Ins, although this had not been postulated *a priori*, and therefore we re-analyzed the data with inclusion of *s*-Ins and Tau resonances in the fitting routine. In addition, we included prior knowledge of macromolecular content into the fitting routine to improve metabolic quantification.¹² Our first goal in this study was to explore whether *s*-Ins could be reliably identified in ¹H MR spectra of normal subjects using improved sensitivity and resolution at 4 T. Our second goal was to determine if *s*-Ins levels increase with age. In addition, we sought to confirm previous reports of a correlation between *s*-Ins and *m*-Ins.^{4,13}

MATERIALS AND METHODS

Human subjects

The study included 24 normal individuals (11 males, 13 females) aged 24–71 years. The population was divided into a younger group of 10 subjects (age range 24–29 years) and an older group of 14 subjects (age range 46–71 years). Ten subjects between 24 and 46 years old were scanned twice within a week to determine reliability and reproducibility of the single voxel ¹H MRS measurements. Written informed consents approved by the committees of human research at the University of California, San Francisco and University of California at Berkeley were obtained from each subject prior to the measurements.

MR methods

Experiments were performed on a 4 T Inova Infinity system (Varian) using a volume head coil provided by the manufacturer. A three-dimensional magnetization prepared FLASH (fast low angle shot) sequence (*TR/TE/TI* = 9/5/300 ms) was used to acquire anatomical *T*₁-weighted images for the purpose of placement of the MRS voxel in the corona radiata region and for tissue segmentation. ¹H MRS data were acquired using a stimulated echo acquisition mode (STEAM) sequence¹⁴ (*TE* = 15 ms, *TM* = 10 ms, voxel size = 2*2*2 cm³) with optimized volume selection Shinnar–Le Roux (SLR) pulses¹⁵ (4.5 kHz bandwidth, 2 ms duration), which were used for the excitation in combination with the magnetic field gradient (0.53 G/cm). Each spectrum consisted of 2048 points within 2000 Hz bandwidth and eight averages collected 32 times (total 256 scans) using water suppression with the VAPOR scheme¹⁶ and a *TR* = 2s. One reference spectrum without water suppression was collected at *TR* = 2s from each subject for computations of metabolite concentration,¹⁷ eddy current and phase corrections.¹⁸ The extent of motion contribution to the spectral quality was monitored via block acquisition, where individual spectra were collected prior to averaging, and variation of *N*-acetyl aspartate signal intensity in the phase sensitive mode was used as a subject motion reference. The variation between phase changes due to physiological/physical motion was similar in younger and older subjects. Spectroscopy data were zero-filled to 4096 points and a 2 Hz Gaussian line broadening filter was applied prior to Fourier transformation. Baseline correction and fitting was performed

with FITT software,¹⁹ which utilizes prior knowledge of spectral patterns. The following metabolites were included in the prior knowledge: NAA, *s*-Ins, *m*-Ins, Tau, choline, glutamate and glutamine. Glucose was not included in the fitting procedure, as its three main peaks occur at 3.44, 3.79 and 5.23 ppm²⁰—all sufficiently far from *s*-Ins (at 3.34 ppm). Numerical simulations of metabolic spectra (demonstrated in Fig. 1) and simulations for prior knowledge fitting routine were performed using GAMMA software.²¹ Prior knowledge of chemical shifts for the fitting was derived from the literature.^{12,20} Similar to Christiansen *et al.*,¹⁷ resonance intensities of the metabolites were expressed relative to the concentration of water, accounting for the compartmental distribution of water in gray matter, white matter and cerebrospinal fluid (CSF). Tissue segmentation was performed using Matlab^{TM} software SPM2 (Wellcome Department of Cognitive Neurology, London, UK). Computations of metabolic concentrations did not account for longitudinal relaxation, since relaxation measurements would have been prohibitively long and no values for T_1 of *s*-Ins, Tau or *m*-Ins at 4 T are available in the literature. Wilcoxon tests were used to determine the significance of metabolite differences between the young and old subjects. The level of significance was $p < 0.05$.

RESULTS

Figure 2(A) displays a T_1 -weighted coronal image used for selection of the 8 cm³ volume of interest (VOI) in the predominantly white matter of corona radiata. Figure 2(B) shows simulations of spectral patterns based on prior knowledge, which was used for metabolic fitting. A representative experimental ¹H MR spectrum from an elderly subject (age =68 years) and the corresponding fitted spectrum are shown in Fig. 2(C) and (D), respectively. For comparison, representative experimental and fitted spectra from a younger subject (age =26 years) are depicted in Fig. 2(E) and (F), respectively. The raw spectrum (Fig. 2C) from the older subject exhibits a distinct resonance peak at 3.35 ppm, which is consistent with the reported *s*-Ins chemical shift *in vivo*.⁴ Table 1 lists average concentrations of *s*-Ins *m*-Ins and Tau referenced to cerebral water and ratios to creatine for both the young and old groups. The NAA results are also listed for comparison. Furthermore, average coverage of white matter, gray matter and cerebrospinal fluid (CSF) in the VOI is also given for each group. On average, *s*-Ins concentration was 0.30 ± 0.10 mM in the younger subjects and 0.43 ± 0.15 mM in the older subjects. For comparison, *m*-Ins was 3.93 ± 1.13 mM in the younger subjects and 4.69 ± 0.69 mM in the older subjects. Group differences of both metabolites were significant ($p < 0.05$). The group effects remained significant for both metabolites when expressed as ratios to creatine. Also listed in Table 1 are the variabilities of the metabolite measurements expressed as coefficients of variation. The coefficients of variation for *s*-Ins and *m*-Ins were markedly higher than that of NAA. Figure 3 depicts a positive correlation between *s*-Ins and *m*-Ins concentrations, which showed a trend towards significance ($R = 0.40$; $p = 0.06$).

DISCUSSION

The main findings of this study were higher levels of *s*-Ins and *m*-Ins and concomitantly reduced NAA of predominantly white matter regions of corona radiata in normal older subjects compared with younger subjects. The increase of *s*-Ins in the older population is difficult to interpret because little is currently known about the pathways or functionality of this metabolite in the human brain. It is not known whether brain *s*-Ins is synthesized *in situ* or how much of it is transported into the brain via the blood stream. The majority of literature supports the role of *m*-Ins as a precursor of *s*-Ins,²² together with the results of this study that also suggest a positive correlation between *s*-Ins and *m*-Ins. Therefore it is reasonable to interpret *s*-Ins level variations in the context of the overall inositol metabolism in the brain. Since ¹H MRS can only detect metabolic concentrations in the millimolar range, extracellular inositol that is present in micromolar concentration²³ is virtually undetectable. The intracellular inositol concentration in the human brain is maintained via three distinct mechanisms: (1) transport of

inositol across the plasma membrane; (2) *de novo* synthesis from D-glucose 6-phosphate; and (3) inositol efflux mediated via a volume-sensitive organic osmolyte channel.²³ Any three of those processes maybe compromised in the course of normal aging process. Our finding of increased *m*-Ins in normally aged brain agrees with results by Chang *et al.*,²⁴ albeit their findings were primarily in gray matter. Nevertheless, changes of *s*-Ins levels, either absolute or relative to *m*-Ins, may be important in studies of neurodegenerative diseases such as Alzheimer's dementia that exhibit elevated concentrations of *myo*-inositol.²⁵

The coefficient of variation for *s*-Ins was markedly higher than for NAA (37 vs 6%). This was expected, because the cerebral concentration of *s*-Ins is significantly lower than that of NAA, resulting in a lower signal-to-noise ratio and thus reduced reliability. Furthermore, large biological variability of *s*-Ins may also contribute to poor reproducibility of *s*-Ins measurements.²² On the other hand, similar reproducibility for *m*-Ins and *s*-Ins (34 and 37%, respectively) is unexpected because *m*-Ins has a much higher concentration in the brain than *s*-Ins. Another MRS study at 4 T by Bartha *et al.* reported a 7% NAA coefficient of variation and reproducibility for *m*-Ins that was about 16%. This study displayed very similar results for NAA; however, the *m*-Ins reproducibility was markedly better in their study. These differences may be due to the different region of the brain and the larger voxel size used in this study, causing non-negligible partial volume effects.

A major limitation of this study is the possibility for error in fitting small signal intensities around zero is not random, but skewed towards positive values because negative signal amplitudes (emission) are excluded per definition. This may cause age-effects to be underestimated, especially in subjects with low concentrations of *s*-Ins. Another limitation of this study is that the test–retest study for metabolite measurements involved only younger subjects and did not cover the entire age range of the population in this study. Therefore, it cannot be ruled out that increased *s*-Ins variability in older individuals may mimic in part an age-related increase.

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Abbreviations used

HIV

human immunodeficiency virus

HPLC

high-performance liquid chromatography

m-Ins

myo-inositol

NAA	<i>N</i> -acetylaspartate
<i>s</i>-Ins	<i>scyllo</i> -inositol
STEAM	stimulated echo acquisition mode
Tau	taurine
VOI	volume of interest

Figure 1.

Spectral simulation of *s*-Ins, *m*-Ins and Tau resonances (STEAM with $TE = 15\text{ ms}$, and $TM = 10\text{ ms}$): (A) at 1.5 T with $T_2^* = 120\text{ ms}$ and (B) at 4 T with $T_2^* = 60\text{ ms}$. The concentration ratio of simulated metabolites *m*-Ins:Tau: *s*-Ins = 6:6:1 for both magnetic fields (*s*-Ins concentration was scaled down six times with respect to *m*-Ins and Tau to fit vertically into the figure. Shorter time dampening constant, T_2^* , for a higher field, reflects increased effects of susceptibility at higher field and thus broader linewidth.)

Figure 2.

(A) Representative T_1 -weighted coronal image used for selection of VOI (corona radiata). (B) Complete prior knowledge spectrum with metabolic, macromolecular and lipid contribution. (C) Representative experimental ^1H MR spectrum (thin line) from a normal healthy control (age =68 years) and (D) fitted spectrum (thick line). (E) Representative experimental ^1H MR spectrum (thin line) from a normal healthy control (age =26 years) and (F) computational fit (thick line)

Figure 3.

Correlation between *s*-Ins and *m*-Ins concentrations. A least squares fit depicts the trend in positive correlation between *m*-Ins and *s*-Ins ($R=0.40$, $p=0.06$). Solid circles represent data from older subjects; open circles show data from the younger subjects

Metabolite concentrations and ratios to creatine (mean $\pm$ SD), relative voxel composition in young (mean age 26 years) and older (mean age 56 years) normal subjects. Also listed are coefficients of variation (CV) as index of measurement reproducibility

Table 1

	WM/GM/CSF	[s-Ins], mM	[m-Ins], mM	[Tau], mM	[NAA], mM	s-Ins/Cr	m-Ins/Cr	Tau/Cr	NAA/Cr
Young (<i>n</i> =10)	0.79/0.20/0.01	0.30 $\pm$ 0.10	3.93 $\pm$ 1.13	1.40 $\pm$ 0.58	8.99 $\pm$ 0.48	0.05 $\pm$ 0.02	0.69 $\pm$ 0.22	0.25 $\pm$ 0.11	1.58 $\pm$ 0.14
Older (<i>n</i> =14)	0.80/0.18/0.02	0.43 $\pm$ 0.15*	4.69 $\pm$ 0.69*	1.48 $\pm$ 0.59	8.39 $\pm$ 0.60*	0.07 $\pm$ 0.02*	0.80 $\pm$ 0.16*	0.26 $\pm$ 0.12	1.44 $\pm$ 0.10*
CV, % (<i>n</i> =10)	28.0/7.5/60.2	37.0	34.5	55.4	5.6	36.3	43.1	61.1	8.0

* Significant difference between groups, *p* < 0.05.
WM, white matter; GM, gray matter.