



## Get Clarity On Generics

Cost-Effective CT & MRI Contrast Agents



FRESENIUS  
KABI

WATCH VIDEO

# AJNR

## Serial Whole-Brain N-Acetylaspartate Concentration in Healthy Young Adults

D.J. Rigotti, M. Inglese, J.S. Babb, M. Rovaris, B. Benedetti, M. Filippi, R.I. Grossman and O. Gonen

*AJNR Am J Neuroradiol* 2007, 28 (9) 1650-1651

doi: <https://doi.org/10.3174/ajnr.A0712>

<http://www.ajnr.org/content/28/9/1650>

This information is current as of August 19, 2025.

# Serial Whole-Brain *N*-Acetylaspartate Concentration in Healthy Young Adults

D.J. Rigotti  
M. Inglese  
J.S. Babb  
M. Rovaris  
B. Benedetti  
M. Filippi  
R.I. Grossman  
O. Gonen

**SUMMARY:** Although the concentration of *N*-acetylaspartate (NAA) is often used as a neuronal integrity marker, its normal temporal variations are not well documented. To assess them over the 1–2 year periods of typical clinical trials, the whole-brain NAA concentration was measured longitudinally, over 4 years, in a cohort of healthy young adults. No significant change (adjusted for both sex and age) was measured either interpersonally or intrapersonally over the entire duration of the study.

**P**roton MR spectroscopy ( $^1\text{H}$ -MR spectroscopy) is often used to increase the specificity of MR imaging with the levels of several detectable brain metabolites.<sup>1</sup> Key among them is *N*-acetylaspartate (NAA), which is second only to glutamate as the most abundant amino acid in the mammalian brain.<sup>2</sup> Because it is almost exclusive to neuronal cells,<sup>3,4</sup> it is regarded as a marker for their health and concentration,<sup>1,5</sup> and  $^1\text{H}$ -MR spectroscopy, therefore, is often part of clinical studies of neurologic disorders.<sup>6–8</sup> Cost and complexity, however, often restrict such studies to 1–2 years, with many months between samples.<sup>9</sup>

Because when attempting to quantify neurodegeneration the NAA level of controls is frequently the implicit reference, it is important to establish its normal temporal variations in healthy subjects, that is, its sensitivity. Unfortunately, most  $^1\text{H}$ -MR spectroscopy studies to date used either single-voxel or 2D multivoxel volumes of interest.<sup>10</sup> Covering only 10% or less of the brain, they required image-guidance to visible pathology and, therefore, are susceptible to misregistration errors in serial studies; implicitly assume changes to occur only at MR imaging-visible abnormalities; and preclude studies near the skull to prevent lipid contamination, excluding most of the cortex.<sup>11</sup> Consequently, despite many reports on NAA variations in different diseases and brain regions, to our knowledge, its normal global temporal variations are unknown.

To test the hypothesis that the NAA level of healthy adults is stable, the present study addressed all 3 of the above issues by following whole-brain (WBNA) concentration serially, at 2–3 time points, over 2–4 years. Such durations frequently coincide with patients' follow-up schedule in many chronic neurologic disorders and are common in their treatment clinical trials.

## Experimental Design

### Human Subjects

Fourteen healthy volunteers (9 women) 29.6  $\pm$  8.6 years old (range, 18–50 years), were recruited. "Healthy" was deemed based on negative

answers to a questionnaire listing 28 neurologic disorders and unremarkable T1- and T2-weighted MR imaging, as determined by a neuroradiologist (R.I.G.). MR imaging and WBNA were performed on each, at enrollment, 20  $\pm$  6 months later, and for 7 of the 14 an additional 21  $\pm$  3 months after the second time point. All of the subjects gave institutional review board-approved written informed consent.

### MR Imaging and Brain Volume

All of the experiments were done on a Vision 1.5T whole-body clinical MR imager (Siemens, Erlangen, Germany) using its standard, manufacturer provided, circularly polarized volume head coil. Each subject's brain volume ( $V_B$ ) was obtained from the sagittal T1-weighted magnetization-prepared rapid acquisition of gradient echo (TE/TR/TI: 7.0/14.7/300 ms; 128 sections; 1.5-mm thick each; 256  $\times$  256 matrix; 210  $\times$  210 mm<sup>2</sup> FOV) imaging using our MIDAS package, as described elsewhere.<sup>12</sup>

### MR Spectroscopy: WBNA Quantification

The total amount of brain NAA ( $Q_{\text{NAA}}$ ) was measured in a 1.5T Vision imager (Siemens). Shimming to a consistent 15  $\pm$  4 Hz whole-head water line was followed by nonlocalizing TE/TI/TR at 0/0.97/10 seconds for  $^1\text{H}$ -MR spectroscopy.<sup>13</sup> Absolute quantification was done by phantom replacement against a reference 3-L sphere of 1.5  $\times$  10<sup>-2</sup> moles of NAA in water. Subject and reference NAA peaks,  $S_S$  and  $S_R$ , were integrated and  $Q_{\text{NAA}}$  obtained as follows<sup>13</sup>:

$$1) \quad Q_{\text{NAA}} = 1.5 \times 10^{-2} \cdot \frac{S_S}{S_R} \cdot \frac{V_S^{180^\circ}}{V_R^{180^\circ}} \text{ moles,}$$

where  $V_R^{180^\circ}$  and  $V_S^{180^\circ}$  are the transmitter voltages into 50  $\Omega$  for non-selective 1-ms 180 $^\circ$  inversion pulses on the reference and subject, reflecting relative coil loading.

To account for natural brain size variations, the global NAA concentration, a specific volume-independent metric suitable for cross-sectional comparison, was used:

$$2) \quad \text{WBNA} = Q_{\text{NAA}}/V_B \text{ mM.}$$

Intrasubject and intersubject variability have previously been shown to be better than  $\pm 7\%$ .<sup>11,13</sup>

### Statistical Analyses

The intrasubject change in WBNA between each pair of time points was computed as the value at the second time point minus the first, expressed as a percentage of the value at the first. Two time points were compared with respect to the mean level and between-subject WBNA variance.

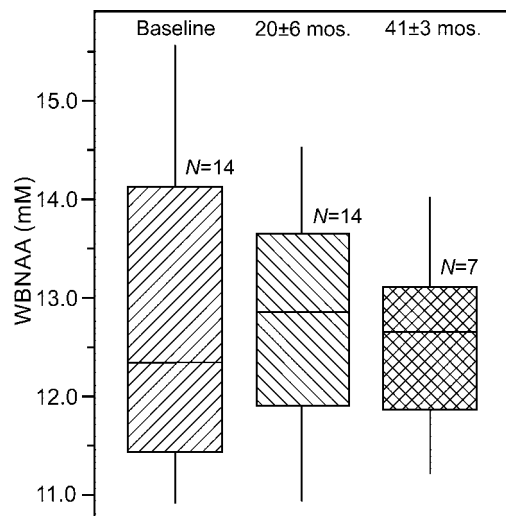
Received March 27, 2007; accepted after revision May 1.

From the Department of Radiology (D.J.R., M.I., J.S.B., R.I.G., O.G.), New York University School of Medicine, New York, NY; Neuroimaging Research Unit (M.R., B.B., M.F.), Department Neurology, Scientific Institute and University, San Raffaele Hospital, Milan, Italy.

This research was supported by National Institutes of Health grants EB01015, NS050520, CA92547, and NS29029.

Please address correspondence to Oded Gonen, Department of Radiology, New York University School of Medicine, 650 First Ave, 6th Floor, New York, NY 10016; e-mail: oded.gonen@med.nyu.edu

DOI 10.3174/ajnr.A0712



**Fig 1.** Box plots showing the first, second (median), and third quartiles (box),  $\pm 95\%$  (whiskers), of the WBNA distributions at baseline, first, and second follow-up time points (the 7 subjects at the third time point are a subset of the original 14).

Restricted maximum likelihood was used to estimate within- and between-subject variance components within a mixed model analysis of variance framework that modeled WBNA as a function of subject identification and time point, represented as random and fixed classification factors, respectively. A paired sample *t* test was used to compare time point pairs with respect to the mean WBNA level.

The estimated between- and within-subject variance components and the observed correlation between longitudinal measures on a subject were used to compute the precision that can be expected when the yearly rate of WBNA change is estimated using a linear mixed-model regression analysis with data from *K* equally spaced time points over 2 years for each of *N* subjects. This permitted us to determine the *N* needed to detect any specific annual rate of WBNA change with either 80% or 90% statistical power at the 2-sided 5% significance level.

## Results

Intrasubject  $V_B$  changed by an annual average of  $-0.25\%$ , consistent with previous reports.<sup>14</sup> Box plots of the WBNA concentrations, shown in Fig 1, exhibit very similar median NAA levels between the time points with average  $\pm$  SD of  $12.5 \pm 1.4$ ,  $12.8 \pm 1.4$ , and  $12.6 \pm 1.2$  mmol/L, at baseline, first, and second follow-ups, respectively.

The intersubject distribution of WBNA values at baseline was not different from the first or second follow-ups with respect to its mean ( $P > .5$ ) or variation ( $P > .3$ ). The mean intrasubject WBNA level changes,  $1.7\% \pm 9.0\%$  and  $0.5\% \pm 12\%$  from baseline to first and second follow-up ( $n = 14$  and 7, respectively) and  $-0.3\% \pm 9.0\%$  between second and third

time points ( $N = 7$ ) were similar to previous reports for back-to-back and day-to-day changes.<sup>13</sup> Based on this variability, estimates of the number of subjects needed to detect various annual percentages of WBNA changes sampling *K* equally spaced time points over 2 years are compiled in the Table.

## Discussion

This study quantifies, to the best of our knowledge, for the first time, the longitudinal course of the global brain NAA concentration in the healthy human brain over a 1- to 2-year period typical of most clinical trials of neurologic disorders. The results support the hypothesis that the concentration and health of neuronal cells are stable in this 20- to 50-year-old population to within the  $\pm 6\%$ – $8\%$  precision of the WBNA method,<sup>13</sup> regardless of exact age or sex. Because WBNA is a ratio (see Eq 2) and its denominator,  $V_B$ , is stable to within  $\pm 0.25\%$ ,<sup>14</sup> the intersubject and intrasubject variability can be assigned entirely to the  $Q_{NAA}$  quantification.

Knowledge of these variations can be used to aid the design of trials in which NAA is used as a surrogate marker. Specifically, how many measurements, on how many subjects, for how long, are needed to detect specific change? The Table shows, for example, that 3 measurements over 2 years on 11 patients suffices to establish 6% annual change with 90% power.

The WBNA approach also has 3 main unavoidable limitations. First, it is inherently nonlocation specific. Second, the age range, 18–50 years, studied here is most appropriate for neurologic disorders that afflict younger patients. Finally, because NAA decline has been associated with both neuronal loss and/or dysfunction, though its changes are specific to this cell type, they are not deterministic of the type of damage.

## References

- Ross BD, Bluml S. Magnetic resonance spectroscopy of the human brain. *Anat Rec* 2001;265:54–84
- Birken DL, Oldendorf WH. N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. *Neurosci Biobehav Rev* 1989;13:23–31
- Simmons ML, Frondoza CG, Coyle JT. Immunocytochemical localization of N-acetyl-aspartate with monoclonal antibodies. *Neuroscience* 1991;45:37–45
- Moffett JR, Namboodiri MA, Cangro CB, et al. Immunohistochemical localization of N-acetylaspargate in rat brain. *Neuroreport* 1991;2:131–34
- Arnold DL, de Stefano N, Matthews PM, et al. N-acetylaspargate: usefulness as an indicator of viable neuronal tissue. *Ann Neurol* 2001;50:823; discussion 824–25
- Miller DH, Grossman RI, Reingold SC, et al. The role of magnetic resonance techniques in understanding and managing multiple sclerosis. *Brain* 1998;121:3–24
- Sormani MP, Bruzzi P, Comi G, et al. MRI metrics as surrogate markers for clinical relapse rate in relapsing-remitting MS patients. *Neurology* 2002;58:417–21
- Filippi M, Dousset V, McFarland HF, et al. Role of magnetic resonance imaging in the diagnosis and monitoring of multiple sclerosis: consensus report of the White Matter Study Group. *J Magn Reson Imaging* 2002;15:499–504
- Filippi M, Grossman RI. MRI techniques to monitor MS evolution: the present and the future. *Neurology* 2002;58:1147–53
- Brooks WM, Friedman SD, Stidley CA. Reproducibility of 1H-MRS in vivo. *Magn Reson Med* 1999;41:193–97
- Inglese M, Ge Y, Filippi M, et al. Indirect evidence for early widespread gray matter involvement in relapsing-remitting multiple sclerosis. *Neuroimage* 2004;21:1825–29
- De Santi S, de Leon MJ, Rusinek H, et al. Hippocampal formation glucose metabolism and volume losses in MCI and AD. *Neurobiol Aging* 2001;22:529–39
- Gonen O, Viswanathan AK, Catalaa I, et al. Total brain N-acetylaspargate concentration in normal, age-grouped females: quantitation with non-echo proton NMR spectroscopy. *Magn Reson Med* 1998;40:684–89
- Scallin RI, Frost C, Jenkins R, et al. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Arch Neurol* 2003;60:989–94

**Estimated sample sizes for 80% or 90% power at 2-sided 5% significance level to detect specific yearly rates of WBNA change with *K* ( $\geq 3$ ) equally spaced scans per subject over 2 years**

Yearly Rate of Change, mM (%) <sup>*</sup>	Sample Size (80% Power)				Sample Size (90% Power)			
	<i>K</i> = 3	<i>K</i> = 4	<i>K</i> = 5	<i>K</i> = 6	<i>K</i> = 3	<i>K</i> = 4	<i>K</i> = 5	<i>K</i> = 6
0.25 (2%)*	57	52	46	42	76	68	61	55
0.51 (4%)*	16	15	13	12	21	19	17	16
0.77 (6%)*	9	8	8	7	11	10	9	8
1.03 (8%)*	6	6	5	5	7	7	6	6

\* Yearly rate of millimoles in percentage assumes a mean baseline WBNA concentration of 12.8 mmol/L.<sup>11</sup>