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ORIGINAL RESEARCH

N-acetylaspartate-to-creatine ratio in twelve brain locations among healthy men and women with different levels of education

Jelena Ostojic 1, Dusko Kozic 2, Sergej M. Ostojic 3 4*

1 Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
2 Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
3 Faculty of Sport and Physical Education, University of Novi Sad, Novi Sad, Serbia
4 University of Belgrade School of Medicine, Belgrade, Serbia

* Correspondence:
Professor Sergej M. Ostojic, MD, PhD
ORCID ID: http://orcid.org/0000-0002-7270-2541
FSPE Applied Bioenergetics Lab, University of Novi Sad
Lovcenska 16, Novi Sad 21000, Serbia.
Phone: (++381)-21-450-188 | Fax: (++381)-21-450-199
E-mail address: sergej.ostojic@chess.edu.rs

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Highlights

• The link between education and N-acetylaspartate-to-creatine ratio (NAA/Cr) in specific brain locations remains currently unknown
• We found that having more years of formal education was related to higher NAA/Cr levels in specific brain regions of healthy men and women
• Education has been recognized as a strong predictor of NAA/Cr levels in both white and brain matter of specific brain regions
Education contributed up to 72% to the variance in the NAA/Cr levels when controlled for age and gender

Abstract
Brain bioenergetics could be affected by many intrinsic and extrinsic factors, yet no link has been established between markers of bioenergetics in specific brain regions and educational level. In this cross-sectional study, we evaluated N-acetylaspartate-to-creatine ratio (NAA/Cr), a biomarker of brain functionality, using multivoxel proton magnetic resonance spectroscopy in twelve brain locations among healthy volunteers (n = 51; 30 females; age 47.8 ± 16.3 years) who earned either a high school diploma (36 out of 51) or a college degree (15 out of 51). Hierarchical multiple regression analysis revealed that our model as a whole (including level of education as a predictor variable, and age and gender as control variables) explained significant percentage of the variance in the NAA/Cr levels at 8 out of 12 specific brain locations (P < 0.05). This was highlighted in left anterior mesial cortex where the model explained 63.1% of the variance in brain NAA/Cr, with education and age make significant contributions (72.6% and 48.5%, respectively) to our model (P < 0.05). Having superior brain N-acetylaspartate-to-creatine ratio appears to be related with higher education in healthy men and women.

Keywords: N-acetylaspartate; creatine; bioenergetics; brain; biomarker; education

Introduction
The human brain is a high energy-consuming organ, and delicate homeostasis of brain bioenergetics appears to be affected by many intrinsic and extrinsic factors. Impaired brain bioenergetics is often associated with aging, neurodegenerative diseases, neoplasms or inherited disorders [1], with concentrations of different energy-related brain metabolites routinely used as indicators of brain functionality. N-acetylaspartate-to-creatine ratio (NAA/Cr) has proved to be a relevant marker to evaluate brain-specific bioenergetics in the setting of impaired energy metabolism and neuronal injury of various genesis [2]. A link between NAA and brain functionality is well described in healthy adults [for detailed review see Ref. 3], with NAA/Cr positively correlated with various measures of cognitive abilities, including processing speed, memory and verbal intelligence. NAA/Cr appears to be associated with aging, with lower brain NAA/Cr values found in healthy adults of advanced age [4]. The whole-brain N-acetylaspartate also correlates with education [5], implying innate tissue characteristics that predispose an individual to higher intellectual achievement. Glodzik and co-workers [5] have shown that education was associated with larger intracranial volume and higher whole brain concentration of N-acetylaspartate, possibly reflecting higher neuronal integrity in individuals with higher education. However, the link between education and NAA/Cr in specific brain locations remains currently unknown. In this cross-sectional study, we evaluated NAA/Cr in twelve brain locations among healthy adults with different educational levels, while controlling for age and gender as effect modifiers.

Methods
Fifty-one healthy adult volunteers (30 females) were recruited to take part in this cross-sectional study. The minimal sample size \((n = 42)\) was calculated according to power analysis for correlation point biserial model, with the effects size set at 0.50, a two-tail alpha level of 0.05, and a study power of 0.95 (G-Power 3, Heinrich Heine University Düsseldorf, Düsseldorf, Germany). Participants were recruited through printed and electronic advertisements on notice boards at various sites at the University of Novi Sad. After contacting the investigators, participants received the participant information sheet explaining the procedure and the goal of the study as well as the exclusion criteria. All participants completed at least a high school education, and were free from acute or chronic diseases, alcohol abuse, neurologic or psychiatric disorders, and any therapeutic treatment, as evaluated by the pre-participation medical survey and Mini-Mental State Examination test. The participants signed a fully-informed written consent, with all study protocols were approved by the local Institutional Review Board in accordance with the Declaration of Helsinki. Each individual underwent a standard magnetic resonance (MR) imaging of the brain to rule out eventual structural alterations, and proton MR spectroscopic examination. MR imaging was performed on 1.5 T scanner (Siemens Avanto Tim, Erlangen, Germany) using matrix head coil (receiver coil) in circularly polarized mode. Sagittal T1 weighted spin-echo sequences with TR/TE of 511/8.7 milliseconds, axial T2 weighted turbo spin-echo (TSE) sequences with TR/TE of 8590/98 ms, coronal T2 TSE TR/TE 5170/105 3 mm slice thickness, were obtained in orthogonal orientation for image guided localization of the spectroscopic imaging slab. Axial FLAIR with TR/TE 8840/109, 5.0 mm slice thickness was obtained to exclude any pathological process. Proton 2D MR spectroscopic imaging data sets were acquired with point-resolved spectroscopy TR/TE 1500/135 ms. The CSI slab size (field of view 160 × 160 × 160 mm; VOI 80 × 80 × 80 mm, thickness 10 mm) was positioned parallel to the axial images, immediately above the corpus callosum along the anterior-posterior commissure to encompass the semioval white matter and the cortical gray matter (Figure 1). The locations that have been chosen represent typical locations for monitoring brain metabolism changes with aging [6], with region-of-interest spectra extracted for assessment of the regional and inter-subject variation in the metabolite peak areas and ratios. This large volume is generally fitted tightly between the ventricle and the skull, with little room for variations in inter- and intrasubject positioning [6]. Number of phase encoding steps (scan resolution) was 16 in all directions (R-L, A-P and F-H). Interpolation resolution was 16 in all directions resulting in VOI of 10 × 10 × 10 mm. Number of acquisitions were four, scan time was 7 min and 12 s. The Weighted phase-encoding scheme was applied. Interfering signal contributions from areas outside the VOI were suppressed by 6 saturation regions, manually positioned along the margin of the VOI. The homogeneity of the magnetic field is optimized over the VOI using an automatic, volume selective shimming method. We took care to position the region of interest in the same way in every subject in order to achieve the highest possible level of reproducibility, taking into account anatomical variations. A two-tailed \(t\) test for independent samples was employed to compare NAA/Cr levels between groups with different education level. Hierarchical multiple regression model with a stepwise method was additionally employed to predict a quantitative outcome at each of 12 brain locations (NAA/Cr) from a predictor variable (level of education), while controlling for potential confounding variables (age, gender). The data were analyzed using the statistical software SPSS for Mac, version 24.0 (SPSS Inc., Chicago, IL). A statistical significance was established at \(P < 0.05\).
- *Figure 1 about here* -

**Results**

The mean age of participants was 47.8 ± 16.3 years (95% confidence interval [CI] 43.6–52.0 years). The highest grade or level of school the participants have completed was a high school diploma in 70.6% participants (36 out of 51) or a college degree (or above) in 29.4% participants (15 out of 51). Participants who completed a college education had significantly higher tissue NAA/Cr as compared to high-school counterparts at L2 (1.90 ± 0.18 vs. 1.70 ± 0.21; \( P = 0.002 \)), L3 (1.76 ± 0.18 vs. 1.44 ± 0.18; \( P < 0.001 \)) and L8 (1.82 ± 0.19 vs. 1.64 ± 0.31; \( P = 0.02 \)), respectively. Hierarchical multiple regression analysis revealed that our model as a whole (including level of education as a predictor variable, and age and gender as control variables) explained significant percentage of the variance in tissue NAA/Cr at the majority of specific brain locations evaluated (8 out of 12) \( (P < 0.05) \) (Table 1). This was highlighted for L3 location (left mesial cortex with mostly gray matter of the anterior region), where our model explained 63.1% of the variance in brain NAA/Cr, with the evaluation of the contribution of each independent variable revealed that education and age make significant contributions (72.6% and 48.5%, respectively) to our model \( (P < 0.05) \), while gender made no significant contribution \( (12.4\%; P > 0.05) \). Other regions with relevant link of education to brain NAA/Cr were L1, L2, L4, L7-L9 and L12, with contribution of education to our model varies from 27.1% to 50.3% \( (P < 0.05) \).

- *Table 1 about here* -

**Discussion**

We found that having more formal education was related to higher NAA/Cr levels in specific brain regions of healthy men and women, with the level of education recognized as a strong predictor of brain NAA/Cr levels. Specifically, NAA/Cr in mesial cortex gray matter at the level of the centrum semiovale appeared to be notably different among participants with different educational levels, and education contributed up to 72% to the variance in the NAA/Cr levels when controlled for age and gender. This might indicate a possible consequence of education on brain functionality in specific regions. Alternatively, elevated NAA/Cr levels in individuals with higher education accomplished could reflect inherent characteristics of the brain tissue that make a person prone to higher academic achievement.

Several recent studies evaluated an association between different markers of brain bioenergetics and education. Glodzik and co-workers [5] reported a strong relationship between education and whole-brain N-acetylaspartate (WBNAA) levels, with education was associated with higher WBNAA in a cohort of 51-70 years old cognitively healthy adults. Education, a commonly used proxy for cognitive reserve, appears to mitigate age-related decline in frontal cortex N-acetylaspartate levels in neurologically healthy older adults \( (n = 135, \text{ mean age } 66 \text{ years}) \) [7], while NAA/Cr specifically decreases in the cortical, semiolal, and temporal regions with normal aging [4]. In addition, NAA/Cr values correlates well with cognitive ability in 40 healthy university students \( (\text{mean age } 21.1 \text{ years}) \) [8], yet several studies suggest possible gender differences in brain bioenergetics correlates of brain function [9,10]. Here, we corroborated previous research suggesting superior neuronal fitness in individuals with higher education, yet our
results expand previous findings and suggest that education made a unique significant contribution while controlling for age and gender. While the previous study [5] reported a strong effect of education on WBNAA levels ($\beta = 1.90$) here we demonstrated a medium-to-strong association between education and location-specific NAA/Cr levels (beta coefficients up to 0.73). This might be due to rather advanced age of subjects recruited for the previous study (71.7 ± 8.2 years) [5], with advanced age perhaps potentiate NAA-education interconnection. We also demonstrated a location specificity for NAA/Cr-education link, with education singled out as a most important predictive variable for three specific locations in the centrum semiovale (L2, L3 and L8) that correspond to the left anterior white matter, and the right anterior and left middle mesial cortex grey matter. This perhaps indicate that cognitive stimulation (attained by higher education) helps to maintain higher levels of NAA/Cr in cortical and subcortical brain regions involved in learning and processing activity [11]. As a brain-specific marker of mitochondrial bioenergetics [2], NAA/Cr seems to positively respond to training-driven stimulation of mitochondrial biogenesis in the human brain [12], making it a possible proxy of education-related brain biodynamics. We could speculate that education stimulates NAA synthesis within the body of neurons through modulating experience-dependence brain plasticity [13]. Preliminary study confirmed that adult men performing a short-term educational task display increases in hippocampal NAA [14] yet more permanent changes may be necessary to maintain high NAA levels [5]. Does elevated NAA/Cr accompanies other education-related attributes of brain complexity (e.g. neural efficiency, high degree of cortical convolution, dense inter-neuronal connections, more complex dendritic system) remain currently unknown. Recent studies demonstrated that higher left frontal cortex-connectivity contributes to cognitive reserve in both healthy and pathological aging [15,16], suggesting neuroprotective effects of experience and stimulating lifestyles on cognitive function [17,18] with enhanced mitochondrial bioenergetics might play a role. While the focus was on educational level for the present study, we demonstrated that age also made a significant contribution (48.5%) to our hierarchical multiple regression model. It appears that NAA/Cr is negatively correlated with age for all 12 brain locations evaluated. This is in accordance with previous studies reporting decreased NAA/Cr levels with aging in the cortical, semiiovale, and temporal regions [2-4], therefore confirming NAA/Cr as a viable biomarker of age-related metabolic changes in the brain.

There are several limitations to this study. First, we used a dichotomic categorization of education, and the quality of education or other cognitive correlates of learning (e.g. intelligence, memory) were not evaluated for this study, also factors other than education that can affect NAA/Cr levels (e.g. socioeconomic background, lifestyle behaviors including diet and physical activity). Second, the study sample was relatively small and recruited participants who completed high school or higher levels of formal education while not being able to examine a population with a more varied educational background (e.g. non-formal education, elementary school graduation). Third, we used NAA/Cr as a single biomarker of brain metabolism and other more sophisticated measurements of tissue bioenergetics (e.g. stronger MR scanner > 1.5 T, comprehensive profile of brain metabolites, functional MRI) are missing. Fourth, we were unable to compare groups for absolute creatine levels, or acquire unsupressed water spectra, with higher levels of creatine could lead to lower NAA/Cr ratio. Reyngoudt and co-workers reported an increase in creatine (referenced to water) with age [19], implying rather complex metabolic changes with aging and
education which requires further investigation. Finally, the present study was cross-sectional in nature and therefore it was not possible to address cause-effect relationship between education and NAA/Cr levels.

In conclusion, higher brain N-acetylaspartate-to-creatine ratio appears to be associated with higher education in healthy men and women aged 20 to 78 years, with education recognized as a strong predictor of NAA/Cr levels in both white and brain matter of specific brain regions. Longitudinal studies addressing short- and long-term effects of education on brain metabolism are highly warranted in both normal population and clinical environment.

**Conflict of Interest** The authors declare that they have no conflict of interest

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References


FIGURE CAPTION

Figure 1. Axial magnetic resonance image showing the outlines of the spectroscopic VOI and typical position of 12 voxel locations in the centrum semiovale. Six locations were in bilateral anterior, middle and posterior regions containing predominantly white matter (voxel numbers 1, 2, 5, 6, 9, 10) and six locations were in bilateral mesial cortex with mostly gray matter of the anterior, middle and posterior regions (voxel numbers 3, 4, 7, 8, 11, 12).
Table 1. The average brain NAA/Cr levels and coefficients of determination ($R^2$) for predicting $N$-acetylaspartate-to-creatine ratio at 12 different brain locations (L1 to L12) from a predictor variable (level of education), while controlling for potential confounding variables (age and gender). Beta coefficients describe the individual contribution of each independent variable to the model. Asterisk (*) indicates statistical significance at $P < 0.05$. CI – confidence interval.

<table>
<thead>
<tr>
<th>Location</th>
<th>NAA/Cr Mean ± SD</th>
<th>95% CI</th>
<th>Adjusted $R^2$</th>
<th>Standardized beta coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Education</td>
<td>Age</td>
</tr>
<tr>
<td>L1</td>
<td>1.69 ± 0.24</td>
<td>1.63–1.75</td>
<td>0.44*</td>
<td>0.35*</td>
</tr>
<tr>
<td>L2</td>
<td>1.76 ± 0.22</td>
<td>1.70–1.82</td>
<td>0.37*</td>
<td>0.50*</td>
</tr>
<tr>
<td>L3</td>
<td>1.54 ± 0.23</td>
<td>1.48–1.60</td>
<td>0.63*</td>
<td>0.73*</td>
</tr>
<tr>
<td>L4</td>
<td>1.68 ± 0.25</td>
<td>1.61–1.74</td>
<td>0.32*</td>
<td>0.35*</td>
</tr>
<tr>
<td>L5</td>
<td>1.97 ± 0.33</td>
<td>1.88–2.06</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>L6</td>
<td>2.09 ± 0.31</td>
<td>2.01–2.18</td>
<td>0.09</td>
<td>0.18</td>
</tr>
<tr>
<td>L7</td>
<td>1.65 ± 0.27</td>
<td>1.58–1.73</td>
<td>0.30*</td>
<td>0.27*</td>
</tr>
<tr>
<td>L8</td>
<td>1.69 ± 0.29</td>
<td>1.62–1.78</td>
<td>0.13*</td>
<td>0.32*</td>
</tr>
<tr>
<td>L9</td>
<td>2.05 ± 0.36</td>
<td>1.96–2.15</td>
<td>0.40*</td>
<td>0.28*</td>
</tr>
<tr>
<td>L10</td>
<td>2.02 ± 0.29</td>
<td>1.94–2.10</td>
<td>0.31</td>
<td>0.16</td>
</tr>
<tr>
<td>L11</td>
<td>1.66 ± 0.25</td>
<td>1.59–1.72</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>L12</td>
<td>1.69 ± 0.23</td>
<td>1.62–1.76</td>
<td>0.23*</td>
<td>0.30*</td>
</tr>
</tbody>
</table>