HHS Public Access

Author manuscript

Neuroimage. Author manuscript; available in PMC 2017 August 15.

Published in final edited form as:

Neuroimage. 2016 August 15; 137: 45–51. doi:10.1016/j.neuroimage.2016.05.014.

Physiological neuronal decline in healthy aging human brain – an in vivo study with MRI and short echo-time whole-brain ^{1}H MR spectroscopic imaging

Xiao-Qi Ding[a,*], **Andrew A. Maudsley**[b], **Mohammad Sabati**[b,c], **Sulaiman Sheriff**[b], **Birte Schmitz**[a], **Martin Schütze**[a], **Paul Bronzlik**[a], **Kai G. Kahl**[d], and **Heinrich Lanfermann**[a]

[a] Institute of Diagnostic and Interventional Neuroradiology, Hannover Medical School, Hannover, Germany

[b] Department of Radiology, University of Miami School of Medicine, Miami, FL, USA

[c] Department of Radiology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

[d] Department of Psychiatry, Social Psychiatry and Psychotherapy, Hannover Medical School, Hannover, Germany

Abstract

Knowledge of physiological aging in healthy human brain is increasingly important for neuroscientific research and clinical diagnosis. To investigate neuronal decline in normal aging brain eighty-one healthy subjects aged between 20 to 70 years were studied with MRI and whole-brain ^{1}H-MR spectroscopic imaging. Concentrations of brain metabolites N-acetyl-aspartate (NAA), choline (Cho), total creatine (tCr), myo-inositol (mI), and glutamine+glutamate (Glx) in ratios to internal water, and the fractional volumes of brain tissue were estimated simultaneously in eight cerebral lobes and in cerebellum. Results demonstrated that an age-related decrease in gray matter volume was the largest contribution to changes in brain volume. Both lobar NAA and the fractional volume of gray matter (FVGM) decreased with age in all cerebral lobes, indicating that the decreased NAA was predominantly associated with decreased gray matter volume and neuronal density or metabolic activity. In cerebral white matter Cho, tCr, and mI increased with age in association with increased fractional volume, showing altered cellular membrane turn-over, energy metabolism, and glial activity in human aging white matter. In cerebellum tCr increased while brain tissue volume decreased with age, showing difference to cerebral aging. The observed age-related metabolic and microstructural variations suggest that physiological neuronal decline in aging human brain is associated with a reduction of gray matter volume and neuronal density, in combination with cellular aging in white matter indicated by microstructural alterations and altered energy metabolism in the cerebellum.

Corresponding author: Xiao-Qi Ding, Ph.D., M.D., Institute of Diagnostic and Interventional Neuroradiology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany, Tel.: 0049 511 532 3401, Fax: 0049 511 532 5876, ding.xiaoqi@mh-hannover.de.

Keywords

whole-brain MR spectroscopic imaging; normal aging; neuronal metabolic activity; N-acetyl-aspartate (NAA); choline (Cho); total creatine (tCr)

Introduction

Knowledge of physiological aging in healthy human brain is increasingly important for neuroscientific research and clinical diagnosis. As a complex and heterogeneous process, cerebral aging in humans involves a large variety of molecular changes and multiple neuronal networks. Many structural and functional studies have been carried out to investigate how cognitive abilities result from dynamic interactions in large-scale cortical network under the influences of aging or diseases (Lustig et al., 2003; Romero-Garcia et al., 2014). It has been reported that normal aging has indirect effects on cognition that are associated with brain markers such as gray matter (GM) thickness and volume, white matter (WM) hyperintensities, fractional anisotropy, and resting-state functional connectivity, with markers varying across cognitive domains (Hedden et al., 2014). Neurodegenerative disorders are found to be associated with specific patterns of gray matter atrophy within distinct functional connectivity networks, which involve nearly all gray matter (Seeley et al., 2009). Age-related changes of metabolite concentrations could provide information about human brain aging at the molecular level, because the observed brain metabolites of N-acetyl-aspartate (NAA), choline (Cho), total creatine (tCr), myo-inositol (mI), glutamine (Gln), and glutamate (Glu), are related to neurometabolic activity as well as neuronal integrity (NAA), membrane turnover (Cho), energy metabolism (tCr), gliosis (mI), or neurotransmitter function (Glu) (Barker et al., 2009; Grachev and Apkarian, 2001). Numerous ^{1}H-MR spectroscopy studies on aging brains have been reported; however, due to limitations in the spatial coverage of the acquisition techniques used, most of these studies have been carried out on one or a few small brain regions with varying results (Haga et al., 2009). Only in a retrospective study Maudsley et al. used a whole brain ^{1}H-MR spectroscopic imaging (wbMRSI) acquisition with an intermediate echo time (TE) to study age-related metabolite changes within the whole brain, with the metabolite concentrations being reported in an institutional unit over bilaterally averaged lobar structures (Maudsley et al., 2012). Moreover, few reports have examined associations between age-related changes of metabolite concentrations and brain tissue volume. This report describes a prospective study on healthy subjects that used MRI and a recently established short-TE wbMRSI acquisition (Ding et al., 2015) to estimate age-related changes in metabolite concentrations and in the fractional volume of brain tissue , with the aim of investigating physiological neuronal decline and to obtain reference data for studies of brain disorders.

Material and methods

Subjects

Ninety-six healthy volunteers were recruited from the local population. All subjects had no neurological disorder or other systemic diseases according to a self-report. To exclude potential cognitive or psychiatric impairments each subject received two screening tests

prior to the MR examination: 1) The Beck Depression Inventory (BDI-II (Steer et al., 1999); and 2) The DemTect (Kalbe et al., 2004). Subjects with abnormal results of screening tests (n = 3), incomplete MR examinations (n = 3), excess body weight (body mass index ≥ 30, n = 8) or brain morphological alterations (n = 1) were excluded. Eighty-one subjects with age distributed between 20 to 70 years (46 females and 35 males, mean age 44 ± 14 years, n > 12 subjects with at least 6 males and 6 females per decade) were finally included. Local Institution Review Board approved the study and written consent was obtained from each participant before the examinations.

MR examination and data processing

MR examinations were carried out at 3T (Verio, Siemens, Erlangen, Germany) with a twelve-channel phased-array receive-only head coil. The MRI scan protocol included a T2-weighted turbo spin echo (TSE) sequence, a T1-weighted 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo) acquisition at 1-mm isotropic resolution, and a volumetric spin-echo planar spectroscopic imaging (EPSI) sequence (TR/TE = 1550/17.6 ms, 50 x 50 voxels in-plane and 18 slices, over a field-of-view of 280 × 280 × 180 mm^3) with parallel imaging acquisition and GRAPPA reconstruction. The acquisition included a second dataset obtained without water suppression that was used for several processing functions and normalization of the metabolite concentrations as described previously (Ding et al., 2015). The scans of TSE, MPRAGE, and EPSI were obtained with the same angulation so that the same anatomic structures could be identified. T2- and T1-weighted images were inspected by two experienced neuroradiologists to exclude subjects with morphological abnormalities. Metabolite image reconstruction was carried out from the EPSI data by using the MIDAS (Metabolic Imaging and Data Analysis System) software (Maudsley et al., 2006; Maudsley et al., 2009) to obtain volumetric metabolite maps for NAA (combined with N-acetylaspartylglutamate), tCr, Cho, mI, and Glx (= glutamate + glutamine), together with corresponding maps of spectral linewidth as previously described (Maudsley et al., 2012). The processing also included calculation of the fractional tissue volume contributed to each MRSI voxel, by applying a tissue segmentation procedure to the T1-weighted MPRAGE data to map gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF), followed by a resampling and convolution by the MRSI spatial response function to coincide with the MRSI voxel volume and location. All resultant individual images were spatially transformed and interpolated to a standard spatial reference (Collins et al., 1998) at 2-mm isotropic resolution, which was associated with a brain atlas that mapped individual brain lobes and the cerebellum (Ding et al., 2015; Maudsley et al., 2006).

Regional metabolite concentrations and fractional tissue volume

Relative concentrations of the individual metabolites were estimated as ratios of the metabolite signal to that of tissue water and presented as [NAA], [Cho], [tCr], [Glx] and [mI], with the number of protons per molecule accounted for in the spectral analysis. The concentrations, presented in institutional units (i.u.), together with the spectral linewidth (LW), the fractional volumes of the CSF (FVCSF) and brain tissue (FVBT), and the fractional volumes of the GM (FVGM) and WM (FVWM) were obtained by using multi-voxel analysis based on nine atlas-defined anatomical regions: the left and right frontal lobe (LFL and RFL), temporal lobe (LTL and RTL), parietal lobe (LPL and RPL), occipital lobe

(LOL and ROL), and the cerebellum (Cbl). In addition, lobar metabolite concentrations in pure GM and pure WM were generated by linear regression of the metabolite concentrations against the normalized GM fraction and extrapolating to 100% or 0% GM (i.e., pure WM). To distinguish mean values reported from regional integration and that from the regression analysis, the former is termed the "native" relative concentration. Subsequently, mean values for regional metabolite concentrations, the extrapolated concentrations in pure GM and WM, as well as all the fractional volumes were derived by averaging corresponding values of the subjects within each decade of age. Correction for CSF volume contribution was applied as $Met' = Met/(1-f_{csf})$ for $0 < f_{csf} < 0.3$, where Met is the uncorrected metabolite value and f_{csf} is the fractional volume of CSF in the MRSI voxel. Voxels with spectral line width (LW) greater than 12 Hz or a voxel with fcsf > 0.3, were excluded from the calculation.

Statistical analysis—One-way analysis of variance (ANOVA) was used to test the hypothesis that the mean metabolite concentrations for males and females in nine brain regions were equal. Multiple comparisons were performed using post hoc Bonferroni test. Linear regression analysis was used to estimate age dependencies of regional metabolite concentrations, the spectral linewidth, and the fractional volumes. Pearson's correlation test was used to test for possible correlations between age-related regional NAA concentration as well as extrapolated gray matter NAA (NAAgm) and the relative GM volume. For multiple correlation tests Bonferroni corrections were made by setting the significance level, alpha, to be 0.05/k, where k was the number of the correlation tests. Therefore, $k = (5+1)\times 9 = 54$ and alpha = 0.05/54 = 0.0009 for linear regression correlations of 5 metabolites and the spectral linewidth to age in 9 native brain regions; $k = 2\times 9+2\times 8 = 34$ and the alpha = 0.05/34 = 0.0015 for linear regression correlations of FVBT and FVCSF in 9 brain areas, FVGM and FVWM in 8 brain lobes to age; $k = 2\times 8 = 16$ and alpha = 0.05/16 = 0.003 for Pearson's correlations of regional NAA concentration as well as NAAgm to fractional GM volume in 8 brain lobes. For linear regression correlations of 5 metabolites to age in extrapolated 8 lobar pure GM or pure WM $k= 2\times 5\times 8 = 80$ and alpha = 0.05/80 = 0.0006. Results with $p <$ alpha were considered to be statistically significant, and not significant but showing a tendency with alpha $< p < 0.01$. Statistical analyses were performed with SPSS version 21 (SPSS IBM, New York, U.S.A).

Results

Example metabolite images of NAA, Cho, tCr, Glx and mI with corresponding T1-weighted images (T1w) at two axial sections around the level of centrum semiovale are shown in Fig. 1, which were obtained from female subjects of 25 (Fig.1A) and 70 years old (Fig.1B), note that the signal intensities of NAA maps of the older volunteer are lower and those in Cho, tCr, and mI are slightly higher in comparison to those of the younger volunteer, indicating qualitatively the age-dependencies of the metabolite concentrations. Also included in Fig. 1C are example MR spectra from a voxel selected in occipital cortex and in parietal white matter of the younger. The metabolite concentrations of NAA, Cho, tCr, Glx, and mI were found to be different across brain regions. Bonferroni-corrected multiple comparisons found that the metabolite concentrations of different brain regions were significantly different from each other, while no significant gender differences were found. Differences were also found

for white matter [NAA], [tCr] and [Glx] between left and right temporal lobes. Therefore, for linear regression analysis or Pearson's correlation tests the male and female metabolite concentrations were combined, while the values of each brain regions, as well as the corresponding right and left lobes were used separately.

Mean values by decade of regional [NAA], [Cho], [tCr], [mI], and [Glx] with corresponding spectral linewidths are given in supplemental Tables S1 and S2. These results demonstrate differences with age and between the brain structures. (Insert Supplementary Tables S1 and S2 here).

The results of the linear regression analysis for age are shown in Tables 1 to 3 and Fig. 2, and the results of Pearson's correlation tests between age-related regional NAA concentration, NAAgm, and the relative GM volume are given in Table 4.

In Table 1 are presented the correlation coefficients, R, and *p* values for associations of age with regional metabolite concentrations in native brain tissue (containing both GM and WM), linewidths, and the FVBT and FVCSF. Significant ($p < 0.0009$) decreases with age were found for [NAA] in all lobes with R varying from −0.58 to −0.70, for [Glx] in RFL and LPL (R = −0.36 to −0.40), with a tendency of age-related decrease that were not significant ($0.009 < p < 0.01$) for [Glx] in LTL and LOL (R = −0.30 to −0.35). An indication of increases with age that did not reach significance were found for [tCr] in two regions (LPL and Cbl, R = 0.31 to 0.33), and for [mI] in 4 regions (RFL, LTL, RPL, and LPL, R = 0.29 to 0.31), while [Cho] did not show age-correlations. The spectral LW increased significantly with age in 3 regions (ROL, LOL, and Cbl, R = 0.39), and with a trend to increased values in RFL and LTL (R = 0.29 to 0.35). Meanwhile, the FVBT decreased significantly with age while FVCSF increased with age significantly ($p < 0.0015$) in most of the brain regions, indicating clearly age-related loss of brain tissue volume with increased CSF volume.

In Table 2 are shown the correlation coefficients, R, and *p* values for [Cho], [tCr], [Glx], and [mI] in extrapolated pure GM and pure WM, respectively. The gray matter [Cho], [tCr], and [mI] did not change significantly, while gray matter [Glx] decreased with age significantly ($p < 0.0006$) in RFL (R = −0.39). For white matter metabolites, significant increases with age ($p < 0.0006$) were found for [Cho] in ROL (R = 0.37), [tCr] in RTL, RPL, and LPL (R = 0.38 to 0.40), and [mI] in RPL (R = 0.41); A trend to increased values with age that did not reach significance ($0.0006 < p < 0.01$) were found for [Cho] in RFL, RPL and LPL (R = 0.30 ~ 0.34), and [mI] in RFL and RTL (R = 0.30 ~0.33). The while white matter [Glx] did not change significantly.

In Table 3 are shown the correlation coefficients, R, and *p* values for lobar [NAA] in extrapolated pure GM and pure WM, and the FVGM and FVWM, where, only results for statistically significant changing parameters are shown (the intercept, slope and age-dependent rates of the variations calculated as percentage per decade relative to the values at 20 years old). Similar to [NAA] in native brain tissue (Table 1) the gray matter [NAA] decreased with age significantly ($p < 0.0006$) in all lobes (R = −0.37 to −0.73), with a mean rate of −3.8% per decade, and the white matter [NAA] decreased with age only in three regions (RFL, LFL, and LTL, R = −0.39 to −0.50), while the FVGM decreased significantly

($p < 0.0015$) ($R = -0.56$ to -0.71, mean rate −2.7% per decade) and the FVWM increased slowly but significantly ($R = 0.38$ to 0.58, mean rate 1.6% per decade) with age in all lobes, indicating that the age-related loss of gray matter volume was the largest contribution to the changes in brain tissue volume. In Figure 2 the values of lobar [NAA] in extrapolated pure GM and WM, and the fractional tissue volume of GM, WM, and CSF are plotted as functions of age, where linear fits with 95% confidence intervals and prediction intervals were also drawn in statistically significant cases.

As shown in Table 4, significant positive correlations ($p < 0.003$) between age-related lobar [NAA] and the FVGM were found for 7 lobes, with the exception of LPL ($R = 0.35$ to 0.51), and between [NAAgm] and the FVGM in 3 lobes (RFL, LFL, and LPL, $R = 0.33$ to 0.47). In Fig. 3 the values of frontal lobar [NAA] in native brain tissue (Fig.3a) and in GM (Fig. 3b) are plotted against age and FVGM in three dimensions, to show the correlations between these variables.

Discussion

This study has determined age-related lobar and cerebellar concentrations of five metabolites and the corresponding fractional volumes of the brain tissue and CSF in normal aging human brain. The results for regional [NAA], [Cho], [tCr], [Glx], and [mI] distributions are consistent with those reported by studies that used conventional MRS acquisition techniques (Deelchand et al., 2015; Guerrini et al., 2009; Hennig et al., 1992; Jacobs et al., 2001; Pouwels et al., 1999; Pouwels and Frahm, 1998). These results may provide reference data for studies on patients with brain disorders, with appropriate consideration for scanner and pulse sequence differences. A recent study, however, showed that the volumetric EPSI sequence and the data processing used here can be implemented across multivendor MR scanners and resulted in comparable numerical results (Sabati et al., 2015).

Comparing the results obtained in native brain tissue (Table 1) with those in GM and WM (Tables 2 and 3), it was found that the largest age-dependent decrease of lobar [NAA] occurred in GM, with a smaller decrease in WM, while the age-dependent increases of lobar [tCr] and [mI] occurred only in WM. Although lobar [Cho] did not show age-dependence in native brain tissue it did increase significantly with age in WM in ROL, and with a trend to increased values in WM of RFL, RPL, and LPL. The results of age-dependent changes in cerebral [Glx] were less tissue-specific: while in native brain the lobar [Glx] decreased with age significantly in two lobes (RFL, and LPL) and with a tendency in LTL and LOL, the [Glx] in WM did not show significant age-dependence, and in GM decreased in RFL. In a few cases, significant correlations were observed in native tissue but not for either GM or WM separately in that region (comparing Table 1 and 2), which may suggest that either the native tissue correlation was significant by chance (a type 1 error, probably as shown by the Glx in native LPL) or the GM or WM correlation was subject to a type 2 error (the correlation for either GM or WM was stronger than the p values suggested).

Age-related cerebral metabolite changes in healthy subjects have been reported previously , although mainly in small brain areas (Haga et al., 2009). Consistent with the present findings, decreased [NAA] with age was frequently reported, whereas the alterations of

[Cho] and [tCr] in relation to age are less consistent and few reports dealt with the associations of [Glx] and [mI] with age (Brooks et al., 2001; Chang et al., 1996; Haga et al., 2009; Maudsley et al., 2009; Maudsley et al., 2012; Raininko and Mattsson, 2010; Sailasuta et al., 2008; Tunc-Skarka et al., 2013). Our observations of increased cerebral [Cho] and [tCr] in WM with age are consistent with those reported by Maudsley et al. (Maudsley et al., 2009; Maudsley et al., 2012), with additional observations of increased [mI] in WM that is consistent with those reported by Tunc-Skark et al. (Tunc-Skarka et al., 2013). Interestingly, studies on aging WM in rhesus monkeys (Bowley et al., 2010; Sandell and Peters, 2003) reported that cellular aging in WM are correlated with a number of microstructural changes, such as reduction in the number of myelinated nerve fibers, increased occurrence of degenerating axons with less compact myelin sheaths showing segmental demyelination followed by ongoing, albeit inadequate, reparative processes. Therefore, our observations of increased [Cho], indicating altered cellular membrane turn-over, and increased [mI], indicating altered gliosis, in WM suggest that similar cellular aging with microstructural alterations also exist in humans, while the observed increase of [tCr] in white matter may indicate a compensatory alteration of the energy supply and neuroprotection (Rae and Broer, 2015). It is worth noting that except for NAA the correlations with age found for Cho, tCr, Glx and mI were mainly weak (with most correlation coefficients, R, around 0.3), which means that factors related to age might account for a statistically significant but relatively small contribution to the variance in these metabolites.

Few studies have reported on the effect of aging on metabolites in the cerebellum, with inconsistent results (Maudsley et al., 2009; Maudsley et al., 2012). In cerebellum, [NAA], [Cho], [Glx] and [mI] did not show age-related changes, while [tCr] increased significantly with age, which indicates that cerebellar aging is different from cerebral neuronal decline, with altered cerebellar energy metabolism. Although the reason behind this finding remains unclear, the observation of increased cerebellar [tCr] in the elderly is consistent with the observations of increased cerebellar [tCr] in patients with degenerative ataxias (Guerrini et al., 2009). A tendency of increased cerebellar [tCr] was also reported by Maudsley et al (Maudsley et al., 2012). Further studies on cerebellum are necessary to verify the results. The observed positive correlations of the spectral LW to age in RFL, LTL, ROL, LOL, and in cerebellum are consistent with the findings of region-dependent increased brain iron concentrations (Mitsumori et al., 2009), shorter metabolite T2 relaxation times in older subjects (Kirov et al., 2008; Marjanska et al., 2013), and similar observations by Maudsley et al (Maudsley et al., 2012).

This study found changes of brain tissue volumes with age, with increased CSF volume and decreased tissue volume in all brain regions, and that the age-related loss of the GM volume was the largest contribution to the changes in brain tissue volume, which is consistent with previous structural and functional studies (Allen et al., 2005; Good et al., 2001; Hedden et al., 2014; Seeley et al., 2009). Because NAA is localized within neurons and involved in synaptic processes (Miller, 1991) it has been suggested that the decrease of brain NAA could be due to either a reduction in GM volume or neuronal function (Brooks et al., 2001; Chao et al., 2010); however, to our knowledge no previous studies have examined the association between age-related NAA concentration and the GM volume. The most interesting findings of the present study were the simultaneous measurement of aging-resulted changes in brain

tissue volume and in brain metabolite concentrations, which revealed positive correlations between age-related cerebral [NAA] and FVGM, weaker but also significant positive correlations between extrapolated [NAAgm] and FVGM (Table 4).

The facts that both cerebral [NAA] in native tissue and FVGM decreased with age indicates that age-related decrease of cerebral [NAA] is associated with a reduction of gray matter volume, which is also consistent with the positive correlations between lobar [NAA] and FVGM (Table 4, left). The weaker but still significant positive correlations between extrapolated [NAAgm] and FVGM (Table 4, right) suggest that an independent reduction in neuronal metabolic activity or neuronal density with age may have additional contributions, because [NAAgm] is the concentration in an idealized "pure" gm voxel and, hence, should be independent of the total volume of GM. In WM, the age-related increase of glial cell volume, as indicated by observations of increased [mI], may partially make up the loss of neuronal density (Leuba and Kraftsik, 1994), which could be reason for age-related decrease of lobar WM [NAA] (Table 3).

Similar to lobar [NAA], but to a much lesser degree, lobar [Glx] also exhibited age-related reductions in several brain regions (Table 1). This observation may indicate positive correlations of lobar [Glx] with the gray matter volume, although a correlation test was not carried out due to inconsistent distribution of the changes in brain tissues. Glutamate, which is the major component of the Glx signal, is the principal excitatory neurotransmitter in brain that is rapidly synthesized from glucose in neural tissues (Shen, 2013). Therefore, the observations of lobar [Glx] decreased with age and possible positive correlations between [Glx] and FVGM may again indicate age-related decrease of neuronal metabolic activity or neuronal density.

Limitations of this study include that the tissue free-water content was not measured, which would impact the measured metabolite concentrations, since this was used as internal reference for the signal normalization under the assumption that water content was the same for all GM and WM across different brain regions, and the same values for all subjects (Maudsley et al., 2006; Maudsley et al., 2009). As a result, changes with age, which has been previously suggested (Brooks et al., 2001; Christiansen et al., 1994), and differences with brain region were not considered. The results of Neeb et al. (Neeb et al., 2006) indicate that between the third and eighth decades of life the changes of brain water content with age are relatively small and less than the variability between the subjects, and that the decrease of brain water content with age may only be a factor for grey matter in males over ~55 years old and is less than 5% at the age of 70 years. A related limitation is that the signal normalization did not account for possible differences in water or metabolite relaxation rates between subjects or with age. Additional limitations of this study include that the possible effect of handedness was not considered, which was necessitated by the limited sample size (only n=6 left-handed subjects). In addition, the aging effects were estimated over large brain structures without consideration of specific smaller brain structures. Also subjects younger than 20 years or older than 70 years were not studied due to difficulty in volunteer recruitment.

In conclusion, this study provides direct *in vivo* evidence that physiological neuronal decline in aging human brain is characterized by a reduction in GM volume and neuronal density - a predominant reason for reduced brain NAA content, in combination with cellular aging in WM by altered cell membrane turn-over, gliosis and energy metabolism – which is an indication of microstructural alterations in WM. Aging also results in altered cerebellar energy metabolism with loss of cerebellar tissue volume. Furthermore, the obtained data can be used as a reference for future studies on patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support

This work was partially supported by Deutsche Forschungsgemeinschaft and by NIH grant R01 EB016064 (A.A.M.).

Glossary

wbMRSI	whole brain ^{1}H-MR spectroscopic imaging
EPSI	spin-echo planar spectroscopic imaging
FVCSF	fractional volume of the CSF
FVTB	fractional volume of gray matter
FVW	fractional volume of white matter
LFT	left frontal lobe
RFL	right frontal lobe
LTL	left temporal lobe
RTL	right temporal lobe
LPL	left parietal lobe
RPL	right parietal lobe
LOL	left occipital lobe
ROL	right occipital lobe
Cbl	cerebellum

References

Allen JS, Bruss J, Brown CK, Damasio H. Normal neuroanatomical variation due to age: the major lobes and a parcellation of the temporal region. Neurobiol Aging. 2005; 26:1245–1260. discussion 1279-1282. [PubMed: 16046030]

Barker, PB.; Bizzi, A.; De Stefano, N.; Gullapalli, R. Clinical MR Spectroscopy: Techniques and Applications. Cambridge University Press; 2009. D.M., L.D.

Bowley MP, Cabral H, Rosene DL, Peters A. Age changes in myelinated nerve fibers of the cingulate bundle and corpus callosum in the rhesus monkey. J Comp Neurol. 2010; 518:3046–3064. [PubMed: 20533359]

Brooks JC, Roberts N, Kemp GJ, Gosney MA, Lye M, Whitehouse GH. A proton magnetic resonance spectroscopy study of age-related changes in frontal lobe metabolite concentrations. Cereb Cortex. 2001; 11:598–605. [PubMed: 11415962]

Chang L, Ernst T, Poland RE, Jenden DJ. In vivo proton magnetic resonance spectroscopy of the normal aging human brain. Life Sci. 1996; 58:2049–2056. [PubMed: 8637436]

Chao LL, Mueller SG, Buckley ST, Peek K, Raptentsetseng S, Elman J, Yaffe K, Miller BL, Kramer JH, Madison C, Mungas D, Schuff N, Weiner MW. Evidence of neurodegeneration in brains of older adults who do not yet fulfill MCI criteria. Neurobiol Aging. 2010; 31:368–377. [PubMed: 18550226]

Christiansen P, Toft PB, Gideon P, Danielsen ER, Ring P, Henriksen O. MR- visible water content in human brain: a proton MRS study. Magn Reson Imaging. 1994; 12:1237–1244. [PubMed: 7854029]

Collins DL, Zijdenbos AP, Kollokian V, Sled JG, Kabani NJ, Holmes CJ, Evans AC. Design and construction of a realistic digital brain phantom. IEEE Trans Med Imaging. 1998; 17:463–468. [PubMed: 9735909]

Deelchand DK, Adanyeguh IM, Emir UE, Nguyen TM, Valabregue R, Henry PG, Mochel F, Oz G. Two-site reproducibility of cerebellar and brainstem neurochemical profiles with short-echo, single-voxel MRS at 3T. Magn Reson Med. 2015; 73:1718–1725. [PubMed: 24948590]

Ding XQ, Maudsley AA, Sabati M, Sheriff S, Dellani PR, Lanfermann H. Reproducibility and reliability of short-TE whole-brain MR spectroscopic imaging of human brain at 3T. Magn Reson Med. 2015; 73:921–928. [PubMed: 24677384]

Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage. 2001; 14:21–36. [PubMed: 11525331]

Grachev ID, Apkarian AV. Aging alters regional multichemical profile of the human brain: an in vivo 1H-MRS study of young versus middle-aged subjects. J Neurochem. 2001; 76:582–593. [PubMed: 11208921]

Guerrini L, Belli G, Mazzoni L, Foresti S, Ginestroni A, Nave R, Diciotti S, Mascalchi M. Impact of cerebrospinal fluid contamination on brain metabolites evaluation with 1H-MR spectroscopy: a single voxel study of the cerebellar vermis in patients with degenerative ataxias. J Magn Reson Imaging. 2009; 30:11–17. [PubMed: 19557841]

Haga KK, Khor YP, Farrall A, Wardlaw JM. A systematic review of brain metabolite changes, measured with 1H magnetic resonance spectroscopy, in healthy aging. Neurobiol Aging. 2009; 30:353–363. [PubMed: 17719145]

Hedden T, Schultz AP, Rieckmann A, Mormino EC, Johnson KA, Sperling RA, Buckner RL. Multiple Brain Markers are Linked to Age-Related Variation in Cognition. Cereb Cortex. 2014

Hennig J, Pfister H, Ernst T, Ott D. Direct absolute quantification of metabolites in the human brain with in vivo localized proton spectroscopy. NMR Biomed. 1992; 5:193–199. [PubMed: 1449955]

Jacobs MA, Horska A, Zijl PC, Barker PB. Quantitative proton MR spectroscopic imaging of normal human cerebellum and brain stem. Magn Reson Med. 2001; 46:699–705. [PubMed: 11590646]

Kalbe E, Kessler J, Calabrese P, Smith R, Passmore AP, Brand M, Bullock R. DemTect: a new, sensitive cognitive screening test to support the diagnosis of mild cognitive impairment and early dementia. Int J Geriatr Psychiatry. 2004; 19:136–143. [PubMed: 14758579]

Kirov II, Fleysher L, Fleysher R, Patil V, Liu S, Gonen O. Age dependence of regional proton metabolites T2 relaxation times in the human brain at 3 T. Magn Reson Med. 2008; 60:790–795. [PubMed: 18816831]

Leuba G, Kraftsik R. Visual cortex in Alzheimer's disease: occurrence of neuronal death and glial proliferation, and correlation with pathological hallmarks. Neurobiol Aging. 1994; 15:29–43. [PubMed: 8159261]

Lustig C, Snyder AZ, Bhakta M, O'Brien KC, McAvoy M, Raichle ME, Morris JC, Buckner RL. Functional deactivations: change with age and dementia of the Alzheimer type. Proc Natl Acad Sci U S A. 2003; 100:14504–14509. [PubMed: 14608034]

Marjanska M, Emir UE, Deelchand DK, Terpstra M. Faster metabolite (1)H transverse relaxation in the elder human brain. PLoS One. 2013; 8:e77572. [PubMed: 24098589]

Maudsley AA, Darkazanli A, Alger JR, Hall LO, Schuff N, Studholme C, Yu Y, Ebel A, Frew A, Goldgof D, Gu Y, Pagare R, Rousseau F, Sivasankaran K, Soher BJ, Weber P, Young K, Zhu X. Comprehensive processing, display and analysis for in vivo MR spectroscopic imaging. NMR Biomed. 2006; 19:492–503. [PubMed: 16763967]

Maudsley AA, Domenig C, Govind V, Darkazanli A, Studholme C, Arheart K, Bloomer C. Mapping of brain metabolite distributions by volumetric proton MR spectroscopic imaging (MRSI). Magn Reson Med. 2009; 61:548–559. [PubMed: 19111009]

Maudsley AA, Govind V, Arheart KL. Associations of age, gender and body mass with 1H MR-observed brain metabolites and tissue distributions. NMR Biomed. 2012; 25:580–593. [PubMed: 21858879]

Miller BL. A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. NMR Biomed. 1991; 4:47–52. [PubMed: 1650241]

Mitsumori F, Watanabe H, Takaya N. Estimation of brain iron concentration in vivo using a linear relationship between regional iron and apparent transverse relaxation rate of the tissue water at 4.7T. Magn Reson Med. 2009; 62:1326–1330. [PubMed: 19780172]

Neeb H, Zilles K, Shah NJ. Fully-automated detection of cerebral water content changes: study of age- and gender-related H2O patterns with quantitative MRI. Neuroimage. 2006; 29:910–922. [PubMed: 16303316]

Pouwels PJ, Brockmann K, Kruse B, Wilken B, Wick M, Hanefeld F, Frahm J. Regional age dependence of human brain metabolites from infancy to adulthood as detected by quantitative localized proton MRS. Pediatr Res. 1999; 46:474–485. [PubMed: 10509371]

Pouwels PJ, Frahm J. Regional metabolite concentrations in human brain as determined by quantitative localized proton MRS. Magn Reson Med. 1998; 39:53–60. [PubMed: 9438437]

Rae CD, Broer S. Creatine as a booster for human brain function. How might it work? Neurochem Int. 2015; 89:249–259. [PubMed: 26297632]

Raininko R, Mattsson P. Metabolite concentrations in supraventricular white matter from teenage to early old age: A short echo time 1H magnetic resonance spectroscopy (MRS) study. Acta Radiol. 2010; 51:309–315. [PubMed: 20170295]

Romero-Garcia R, Atienza M, Cantero JL. Predictors of coupling between structural and functional cortical networks in normal aging. Hum Brain Mapp. 2014; 35:2724–2740. [PubMed: 24027166]

Sabati M, Sheriff S, Gu M, Wei J, Zhu H, Barker PB, Spielman DM, Alger JR, Maudsley AA. Multivendor implementation and comparison of volumetric whole-brain echo-planar MR spectroscopic imaging. Magn Reson Med. 2015; 74:1209–1220. [PubMed: 25354190]

Sailasuta N, Ernst T, Chang L. Regional variations and the effects of age and gender on glutamate concentrations in the human brain. Magn Reson Imaging. 2008; 26:667–675. [PubMed: 17692491]

Sandell JH, Peters A. Disrupted myelin and axon loss in the anterior commissure of the aged rhesus monkey. J Comp Neurol. 2003; 466:14–30. [PubMed: 14515238]

Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD. Neurodegenerative diseases target large-scale human brain networks. Neuron. 2009; 62:42–52. [PubMed: 19376066]

Shen J. Modeling the glutamate-glutamine neurotransmitter cycle. Front Neuroenergetics. 2013; 5:1. [PubMed: 23372548]

Steer RA, Clark DA, Beck AT, Ranieri WF. Common and specific dimensions of self-reported anxiety and depression: the BDI-II versus the BDI-IA. Behav Res Ther. 1999; 37:183–190. [PubMed: 9990749]

Tunc-Skarka N, Meier S, Demirakca T, Sack M, Weber-Fahr W, Brusniak W, Wolf I, Matthaus F, Schulze TG, Diener C, Ende G. Effects of normal aging and SCN1A risk-gene expression on brain metabolites: evidence for an association between SCN1A and myo-inositol. NMR Biomed. 2013

Highlights

We found in vivo evidence that 1) Physiological neuronal decline in aging human brain is presented by reduction of gray matter volume and neuronal density, which is a predominant reason for reduced brain NAA content; 2) Aging resulted in altered cell membrane turnover, gliosis, and energy metabolism with microstructural alterations in white matter.

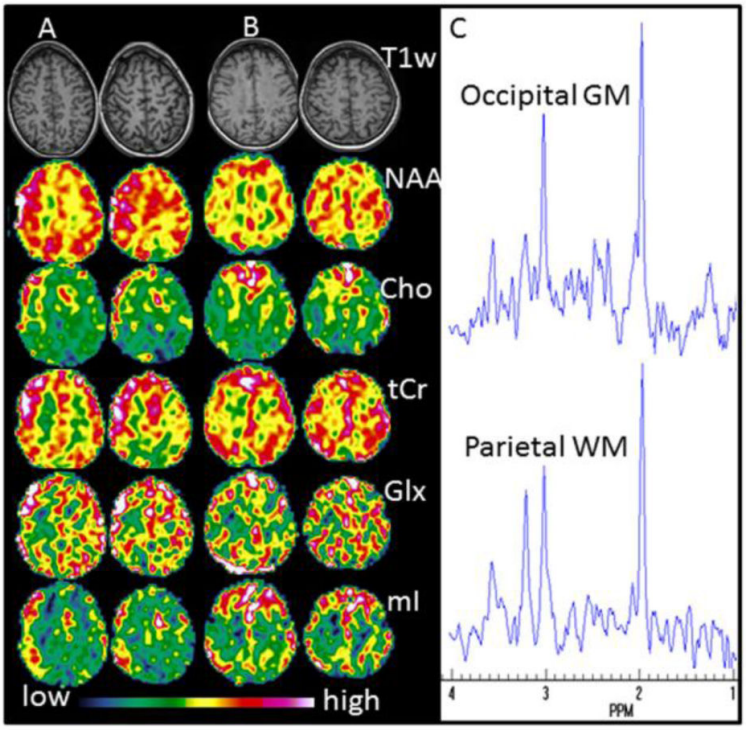

Figure 1.

Example individual images of NAA, Cho, tCr, Glx, and mI and the corresponding T1-weighted images (T1w) at two axial sections around the level of centrum semiovale obtained from a female of 25 years old (Fig.1A) and a female of 70 years old (Fig.1B), and two single voxel spectra obtained from the younger subject in occipital gray matter and parietal white matter (Fig. 1C). Hyperintensitive areas due to excessive linewidth were excluded in the lobar calculations.

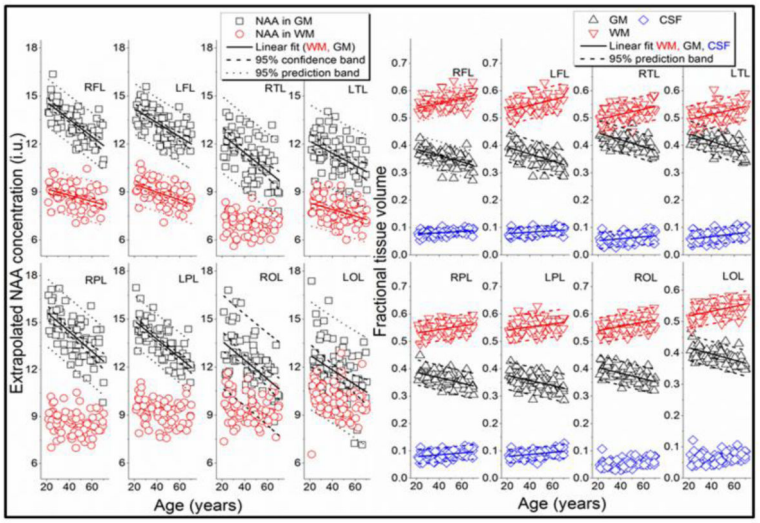

Fig.2

Figure 2.

Cerebral lobar NAA concentrations and fractional tissue volumes of gray matter, white matter, and CSF plotted as functions of age, including linear fits with 95% prediction interval and 95% confidence interval (only for NAA) for statistically significant cases ($p < 0.01$).

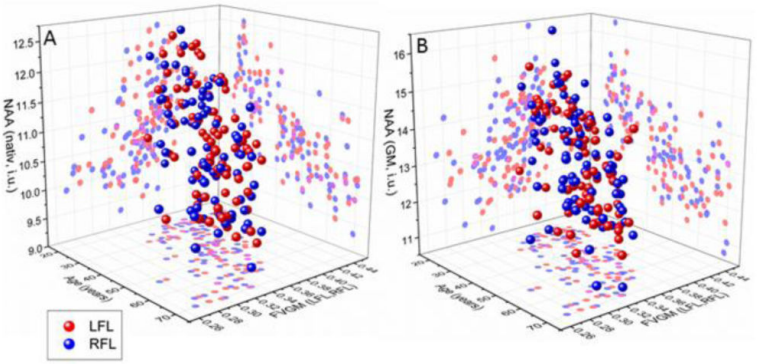

Figure 3.

Frontal lobar [NAA] in native brain tissue (Fig.3A) and GM (Fig. 3B) plotted against age and FVGM showing the correlations between these variables.

Table 1

Results* of linear regression analyses of brain regional NAA, Cho, tCr, mI, and Glx concentrations in native brain tissue containing both gray and white matter, linewidth as well as the fractional volumes of brain tissue and CSF versus age

	NAA		Cho		tCr		Glx		mI		LW		FVBT[2]		FVCSF[3]	
Brain[1] region	R	*p*	R	*p*	*R*	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*
RFL	**−0.69**	***<0.0009***	0.16	*0.155*	0.10	*0.360*	**−0.40**	***<0.0009***	**0.31**	*0.005*	**0.29**	*0.008*	**−0.43**	***<0.0015***	**0.44**	***<0.0015***
LFL	**−0.70**	***<0.0009***	0.10	*0.390*	0.04	*0.728*	−0.27	*0.015*	0.24	*0.029*	0.28	*0.013*	**−0.44**	***<0.0015***	**0.44**	***<0.0015***
RTL	**−0.59**	***<0.0009***	0.02	*0.865*	0.15	*0.181*	−0.27	*0.014*	0.25	*0.024*	0.21	*0.062*	**−0.42**	***<0.0015***	**0.43**	***<0.0015***
LTL	**−0.63**	***<0.0009***	0.09	*0.412*	0.19	*0.095*	**−0.35**	*0.002*	**0.29**	*0.008*	**0.35**	*0.002*	**−0.50**	***<0.0015***	**0.51**	***<0.0015***
RPL	**−0.62**	***<0.0009***	0.19	*0.086*	0.21	*0.060*	−0.27	*0.015*	**0.31**	*0.006*	0.22	*0.053*	**−0.43**	***<0.0015***	**0.43**	***<0.0015***
LPL	**−0.64**	***<0.0009***	0.24	*0.030*	**0.33**	*0.003*	**−0.36**	***<0.0009***	**0.30**	*0.007*	0.20	*0.078*	**−0.48**	***<0.0015***	**0.48**	***<0.0015***
ROL	**−0.63**	***<0.0009***	0.15	*0.194*	0.11	*0.325*	−0.12	*0.267*	0.10	*0.364*	**0.39**	***<0.0009***	−0.27	*0.017*	**0.29**	*0.008*
LOL	**−0.58**	***<0.0009***	0.02	*0.872*	0.22	*0.048*	**−0.30**	*0.006*	0.04	*0.753*	**0.39**	***<0.0009***	−0.26	*0.020*	0.27	*0.014*
Cbl	−0.12	*0.271*	−0.02	*0.845*	**0.31**	*0.005*	0.06	*0.583*	0.22	*0.050*	**0.39**	***<0.0009***	**−0.51**	***<0.0015***	**0.52**	***<0.0015***

*Bonferroni corrections for multiple correlation tests were made. For 5 metabolites and the spectral linewidth the results are statistically significant if $p < 0.0009$, and not significant but with a tendency if $0.0009 < p < 0.01$, while for FVTB, FVCSF the results are statistically significant if $p < 0.0015$, and not significant but with a tendency if $0.0015 < p < 0.01$.

[1]Definition of the brain regions: left and right frontal lobe (LFL/RFL), left and right temporal lobe (LTL/RTL), left and right parietal lobe (LPL/RPL), left and right occipital lobe (LOL/ROL), and cerebellum (Cbl)..

[2]FVBT = fractional volume of brain tissue.

[3]FVCSF = fractional volume of cerebrospinal fluid.

Table 2

Results* of linear regression analyses of extrapolated Cho, tCr, mI, and Glx concentrations in gray matter and white matter versus age

	Cho		tCr		Glx		mI	
Regional tissue[1]	**R**	***p***	**R**	***p***	**R**	***p***	**R**	***p***
RFLGM	−0.13	*0.247*	0.10	*0.391*	**−0.39**	***<0.0006***	0.27	*0.015*
LFLGM	−0.11	*0.320*	0.07	*0.531*	−0.05	*0.644*	0.15	*0.195*
RTLGM	−0.19	*0.096*	0.03	*0.774*	−0.09	*0.407*	0.06	*0.601*
LTLGM	−0.27	*0.016*	0.24	*0.033*	−0.10	*0.398*	0.20	*0.077*
RPLGM	−0.23	*0.040*	0.09	*0.440*	−0.01	*0.909*	0.15	*0.188*
LPLGM	−0.15	*0.192*	0.18	*0.116*	−0.17	*0.123*	0.14	*0.200*
ROLGM	−0.28	*0.012*	−0.02	*0.840*	0.17	*0.119*	0.16	*0.163*
LOLGM	−0.09	*0.409*	0.09	*0.402*	0.08	*0.463*	0.09	*0.408*
RFLWM	**0.30**	*0.006*	0.20	*0.073*	0.03	*0.792*	**0.30**	*0.007*
LFLWM	0.25	*0.025*	0.15	*0.184*	−0.13	*0.263*	0.27	*0.015*
RTLWM	0.17	*0.140*	**0.39**	***<0.0006***	−0.02	*0.875*	**0.33**	*0.003*
LTLWM	0.26	*0.019*	0.16	*0.163*	−0.17	*0.140*	0.28	*0.011*
RPLWM	**0.34**	*0.002*	**0.38**	***<0.0006***	−0.14	*0.211*	**0.41**	***<0.0006***
LPLWM	**0.32**	*0.003*	**0.40**	***<0.0006***	−0.15	*0.178*	**0.35**	*0.002*
ROLWM	**0.37**	***<0.0006***	0.25	*0.027*	−0.24	*0.034*	0.04	*0.697*
LOLWM	0.11	*0.312*	0.27	*0.017*	−0.28	*0.011*	0.01	*0.930*

*Bonferroni corrections for multiple correlation tests were made. For 5 metabolite in 8 lobar regions of extrapolated pure GM and pure WM the results are statistically significant if $p < 0.0006$, and not significant but with a tendency if $0.0006 < p < 0.01$.

[1]For definition of the brain regions see footnote in Table 1.

Table 3

Results* of linear regression analyses of extrapolated NAA concentrations in gray matter and white matter, as well as the fractional volumes of gray matter (FVGM) and white matter (FVWM) versus age

Regional tissue[1]	NAA							Tissue fractional volume						
	R	*p*	Intercept		Slope		Variations[3]	R	*p*	Intercept		Slope		Variations[2]
			Value	SD	Value	SD	% per decde			Value	SD	Value	SD	% per decade
RFLGM	**−0.72**	***<0.0006***	14.6	0.3	−0.054	0.006	**−3.70**	**−0.64**	***<0.0015***	0.398	0.009	−0.001	0.000	**−2.94**
LFLGM	**−0.62**	***<0.0006***	14.2	0.3	−0.044	0.006	**−3.09**	**−0.66**	***<0.0015***	0.407	0.009	−0.001	0.000	**−3.19**
RTLGM	**−0.57**	***<0.0006***	12.6	0.4	−0.057	0.009	**−4.52**	**−0.66**	***<0.0015***	0.458	0.008	−0.001	0.000	**−2.71**
LTLGM	**−0.44**	***<0.0006***	12.2	0.4	−0.038	0.009	**−3.13**	**−0.71**	***<0.0015***	0.457	0.008	−0.001	0.000	**−3.04**
RPLGM	**−0.64**	***<0.0006***	15.7	0.4	−0.063	0.008	**−4.00**	**−0.62**	***<0.0015***	0.397	0.008	−0.001	0.000	**−2.49**
LPLGM	**−0.73**	***<0.0006***	14.9	0.3	−0.058	0.006	**−3.88**	**−0.56**	***<0.0015***	0.385	0.009	−0.001	0.000	**−2.39**
ROLGM	**−0.51**	***<0.0006***	13.6	0.5	−0.059	0.011	**−4.36**	**−0.62**	***<0.0015***	0.415	0.008	−0.001	0.000	**−2.46**
LOLGM	**−0.37**	***<0.0006***	12.7	0.6	−0.046	0.013	**−3.61**	**−0.66**	***<0.0015***	0.426	0.007	−0.001	0.000	**−2.32**
Mean							−3.79							−2.69
RFLWM	**−0.40**	***<0.0006***	9.2	0.2	−0.019	0.005	**−2.18**	**0.57**	***<0.0015***	0.526	0.009	0.001	0.000	**1.79**
LFLWM	**−0.50**	***<0.0006***	9.5	0.2	−0.024	0.005	**−2.71**	**0.58**	***<0.0015***	0.514	0.009	0.001	0.000	**2.04**
RTLWM	−0.08	*0.475*						**0.50**	***<0.0015***	0.486	0.009	0.001	0.000	**1.91**
LTLWM	**−0.39**	***<0.0006***	8.3	0.3	−0.021	0.006	**−2.63**	**0.51**	***<0.0015***	0.485	0.009	0.001	0.000	**1.96**
RPLWM	−0.17	*0.135*						**0.48**	***<0.0015***	0.523	0.007	0.001	0.000	**1.27**
LPLWM	−0.25	*0.027*						**0.38**	***<0.0015***	0.536	0.009	0.001	0.000	**1.03**
ROLWM	−0.16	*0.162*						**0.50**	***<0.0015***	0.531	0.008	0.001	0.000	**1.49**
LOLWM	−0.19	*0.088*						**0.54**	***<0.0015***	0.511	0.007	0.001	0.000	**1.47**
Mean														1.62

*Bonferroni corrections for multiple correlation tests were made. For 5 metabolite in 8 lobar regions of extrapolated pure GM and pure WM the results are statistically significant if $p < 0.0006$, and not significant but with a tendency if $0.0006 < p < 0.01$. For fractional tissue volume of GM and WM the results are statistically significant if $p < 0.0015$, and not significant but with a tendency if $0.0015 < p < 0.01$

[1]For definition of the brain regions see footnote in Table 1.

[2]Values at 20 years of age in i.u.

[3]In ratio to the values at 20 years of age, in unit of “percent per decade”.

Table 4

Pearson's linear correlation coefficients* of the regional NAA concentrations(native NAA) as well as extrapolated gray matter NAA (NAAgm) with fractional volume of the gray matter

Brain[1] region	native NAA to FVGM		NAAgm to FVGM	
	R	***p***	**R**	***p***
RFL	**0.50**	< ***0.003***	**0.47**	< ***0.003***
LFL	**0.51**	< ***0.003***	**0.33**	< ***0.003***
RTL	**0.36**	< ***0.003***	0.25	*0.027*
LTL	**0.51**	< ***0.003***	0.30	*0.006*
RPL	**0.35**	< ***0.003***	0.31	*0.004*
LPL	0.27	*0.016*	**0.38**	< ***0.003***
ROL	**0.43**	< ***0.003***	0.31	*0.004*
LOL	**0.38**	< ***0.003***	0.23	*0.037*

*Bonferroni corrections for multiple correlation tests were made. The results are statistically significant if $p < 0.003$.

[1]For definition of the brain regions see footnote in Table 1.