Age-related differences in metabolites in the posterior cingulated cortex and hippocampus of normal ageing brain: a ^{1}H-MRS study

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Introduction:
Ageing is a diverse process, associated with a progressive, yet variable, decline of cognitive abilities [1]. A loss of neurons and a decreased brain volume have been associated with normal ageing, however, neuronal density would remain constant with age [2,3]. The question is whether there is a decline in the number of neurons, a reduced neuronal density or an impaired metabolic activity. The effect of age on metabolism has been studied in several ^{1}H-MRS studies with a large variability in results due to different inclusion criteria (study size, age range, medical history), regions of interest, data acquisition (field strength, single voxel vs multiple voxel studies, MR sequence, TR, TE) and data processing (relative vs absolute quantification, correction factors) [4].
In neurodegenerative disorders such as Alzheimer's disease (AD) and mild cognitive impairment (MCI), metabolic abnormalities have been observed in the posterior cingulated cortex (PCC) and the hippocampus (HC) [5]. In this regard, we found it necessary to define normality and assess age-related metabolic differences of N-acetylaspartate (NAA), total creatine (tCr), choline (Cho) and myo-inositol (Ins) in these specific brain areas in a large group of normal ageing subjects.

Materials and Methods:
Subjects Ninety healthy subjects (42 women and 48 men aged 18-76 years, mean ± SD, 48.4 ± 16.8 years) were studied with 1H-MRS, a study called NOMARED (NOrmal age-related MAgnetic REsonance Database). All subjects underwent a clinical medical and neurological examination and a structured psychiatric interview, to exclude (a history of) neurological and psychiatric disorders. To exclude cognitive impairment, volunteers underwent thorough neuropsychological testing: Auditory Verbal Learning Test (AVLT), Visual Association Test (VAT), … For inclusion in the study, test results on the AVLT and VAT had to be within the normal range. The study was approved by the local ethics committee and all subjects gave written informed consent. **^{1}H-MRS** Measurements were performed on a 3 T Siemens scanner. Spectra were acquired using a STEAM pulse sequence (TR/TE/TM = 1500/20/10 ms) and a PRESS pulse sequence (TR/TE = 1500/30 ms), in the left HC and the PCC respectively. The number of averages was 128. Weak water suppression was used in order to use the residual water signal as an internal reference for relative quantification. In the PCC of 57 subjects, the CSF-fraction was determined by acquiring a series of unsuppressed water spectra at different TEs and absolute quantification was performed. Spectra were processed using the scanner software (syngoMR B15, NUMARIS 4). **Statistics** Analysis of covariance (ANCOVA) was performed, with age as a covariate, in order to determine the main effects of age and gender as well as the interaction effect of age and gender on metabolite ratios and absolute metabolite concentrations. Results were considered to be significant at $P < 0.05$.

Results:
Table 1 shows the ANCOVA results of metabolite ratios. A significant correlation was found between age and the CSF-content ($R = 0.65$, $P = 0.000$) in the PCC. Metabolite ratios Ins/tCr and Ins/H_2O were found significantly increased with age in the PCC ($P < 0.05$ and $P < 0.001$, respectively), and in the HC ($P < 0.001$ for both). An increased tCr/H_2O was only observed in the PCC ($P < 0.001$). Following absolute quantification, significantly increased concentrations of Ins and tCr in the PCC confirmed the relative findings ($P < 0.001$ for both). Increased Ins/tCr and Ins/H_2O was found in the HC of female subjects, supporting a gender and gender-by-age interaction effect ($P < 0.05$). Gender and gender-by-age interaction effects were not found in other ratios and concentrations.

Discussion:
Age-related increases of tCr and Ins were found in the PCC, whereas this held only true for Ins in the HC, indicating possible gliosis in the ageing brain. Gliosis is the proliferation of astrocytes in damaged tissue of the central nervous system. These observations are in agreement with the fact that both brain regions are involved in the development of MCI and AD, both associated with ageing [5]. No age-dependent NAA decreases were found in the PCC and the HC. Also, the CSF fraction remains relatively constant within the age group of 18-50 years but increases significantly in individuals older than 60 years. The results in these specific brain regions are important when comparing normal ageing with age-related pathologies such as MCI and AD. These results also indicate that particular metabolic shifts are not always markers for disease, but rather markers for a normal evolution in metabolism with age.

References:
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Table 1. *P*-values for ANCOVA results of metabolite ratios.

	PCC			HC		
	age	**gender**	**age*gender**	**age**	**gender**	**age*gender**
NAA/tCr	0.068	0.142	0.284	0.158	0.219	0.345
Cho/tCr	**0.019**	0.935	0.916	0.643	0.229	0.186
Ins/tCr	**0.019**	0.272	0.144	**0.004**	0.832	0.497
NAA/H_2O	0.289	**0.009**	**0.029**	0.063	0.123	0.191
tCr/H_2O	**0.001**	0.170	0.198	0.723	0.685	0.726
Cho/H_2O	0.864	0.965	0.909	0.620	0.779	1.000
Ins/H_2O	**0.000**	0.953	0.943	**0.000**	0.985	0.365