Cerebral blood volume and metabolite levels in mouse models for Alzheimer (APP/PS1) and atherosclerosis (ApoE4 and ApoE knockout): genotype differences and early effects of DHA and cholesterol containing diets

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Introduction Research into the development of Alzheimer's disease (AD) provides increasing evidence that a combination of genetic and vascular factors and lifestyle strongly influences the development of cognitive impairment and neural degeneration. Results from trials and epidemiological studies suggest that docosahexaenoic acid (DHA) and cholesterol intake may affect the course of AD, possibly by influencing cerebral circulation and metabolism[1,2]. To investigate the effects of diets enriched with DHA or cholesterol in the pathophysiology of AD, we evaluated the cerebral blood volume (CBV) and the hippocampal metabolites concentration with MR imaging and spectroscopy (^{1}H MRS) in three different mouse models for genetic AD and vascular risk factors in AD.

Animals and diets We compared 12-month-old wild type (WT) male mice (C57BL6/J, n=30) with a transgenic mouse model resembling familial AD by carrying the Swedish family-specific Amyloid Precursor Protein (APP) aberration and mutated PreSenilin 1 (PS1) (APPswe/PS1dE9, n=21) and two strains resembling vascular risk factors in sporadic AD by carrying human ApolipoproteinE4 alleles (ApoE4, n=24), and ApoE knockout mice (ApoE-/-, n=23). From 2 months of age, mice were divided in groups and fed with 3 different diets: a standard diet, a cholesterol-rich diet, or a multi-nutrient diet containing precursors and cofactors in membrane synthesis, such as docosahexanoic acid (DHA), phospholipids, UMP, choline, B-vitamins and antioxidants (Fortasyn™ Connect).

Methods MR measurements were performed at 7T (ClinScan, Bruker, Germany). CBV was determined by acquiring Gradient Echo FLASH images before and after i.v. injection of USPIO (Sinerem®, Guerbet, France, 130µg Fe/mouse). Changes in the transverse relaxation rates ΔR_2^* after injection of USPIO provides an index proportional to the blood volume of total vasculature[3]. Image parameters: 3 Echo Times (range 5-9ms), TR=500ms, pulse angle=10°, spatial resolution=0.16×0.16×0.8mm, Averages=4. The resulting relative CBV images were analyzed in several region of interests (ROIs) with histograms to extract parameters associated with macro- and microvasculature CBV (**Fig. 1** and **2**). Metabolite levels were determined in the hippocampus with single voxel ^{1}H MRS (STEAM, TE=15ms, TR=1500ms, averages=1024). Morris Water Maze (MWM), reverse MWM and Open Field test were used to assess spatial learning, memory and behavior. Brain tissue was analyzed immunohistochemically and biochemically for inflammatory markers and amyloid-β.

Results We found significant decreased CBV in hippocampus (p=0.021) and cortical regions (p=0.017) in APP/PS1 mice, especially in the small capillaries. ApoE knockout mice also showed decreased CBV in prefrontal cortex (p=0.083), but in contrast this mostly occurred in the macrovascular fraction (p=0.008). CBV in ApoE4 mice was not different from WT. In the hippocampus of APP/PS1 mice *myo*-inositol content is increased (p=0.018) (**Fig. 3a**) and NAA tends to be decreased (p=0.079). However, multi-nutrient Fortasyn diet tends to increase the NAA level compared with standard diet in APP/PS1 and wild type mice (p=0.083) (**Fig. 3b**). Increased taurine level was found in ApoE4 mice compared either with wild type (p=0.015) and ApoE knockout mice (p=0.009). Dysfunctions in memory and behavior were found in all three mouse strains studied.

Discussion and conclusion Comparably with AD patients[4], we found a decreased CBV of macro- and microvasculature, neuronal degeneration (decreased NAA) and increased *myo*-inositol as measure of inflammation and also cognitive impairment in APP/PS1 mice. A decreased CBV in the macrovasculature was found in ApoE knockout mice which may indicate atherosclerosis. A decreased neurodegeneration was detected *in vivo* at 12 months of age in APP/PS1 mice: the multi-nutrient Fortasyn diet increased the NAA concentration in the AD mouse model, but not in the atherosclerosis mouse models. This work is part of an ongoing longitudinal study and more severe differential effects of specific diets are expected at older age [1].

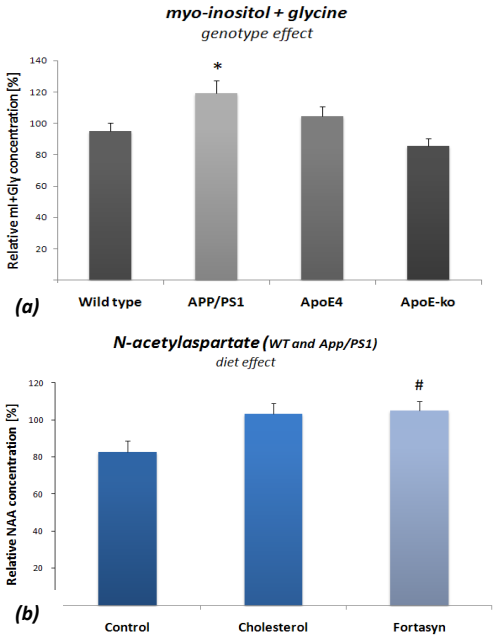

Fig. 3 *(a) Hippocampal ^{1}H MRS showed high myo-inositol and glycine concentration in APP/PS1 mice. (b) Fortasyn diet increase N-acetylaspartate concentration either in wild-type and APP/PS1*

References

1) CR Hooijmans et al., *Changes in cerebral blood volume and amyloid pathology in aged Alzheimer APP/PS1 mice on a docosahexaenoic acid (DHA) diet or cholesterol enriched Typical Western Diet (TWD).* Neurobiol Dis. (2007).
2) R. Takechi et al., *Dietary fats, cerebrovasculature integrity and Alzheimer's disease risk.* Progress in Lipid Research 49 (2010)
3) JL. Boxerman et al., *MR Contrast due to Intravascular Magnetic Susceptibility Perturbations.* Magn Reson Med. (1995)
4) Minati et al., *MR spectroscopy, Functional MRI, and Diffusion-Tensor Imaging in the Aging brain: a Conceptual Review.* J. of Geriatric and Neurology. (2007)

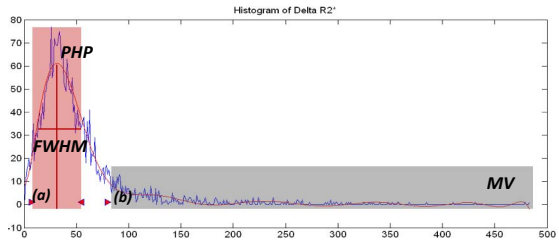

Fig. 1 Frequency-intensity histogram of ΔR_2^* map. Peak height position (PHP), Full Width Half Maximum (FWHM) and maximum value (MV) of the curve were calculated and values were assigned for (a) ΔR_2^* range of microvasculature and for (b) ΔR_2^* range of macrovasculature

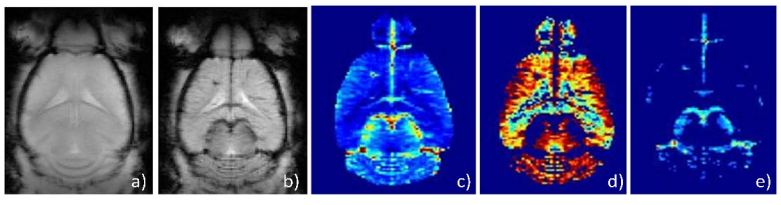

Fig. 2 (a) T_2^*-weighted image before USPIO injection (b) T_2^*-weighted image after USPIO injection (c) ΔR_2^* map (d) ΔR_2^* map of pixels belonging to microvasculature (e) ΔR_2^* map of pixels belonging to macrovasculature