Multi nutrient enriched diets restore cerebral perfusion and protect against neurodegeneration in a mouse model for Alzheimer's disease

Valerio Zerbi[1,2], Diane Jansen[1], Maximilian Wiesmann[1], Maartje Mutsaers[1], Pieter J Dederen[1], Ilse Arnoldussen[1], Andor Veltien[2], Sjaak Van Asten[2], Arend Heerschap[2], and Amanda J Kiliaan[1]

[1]*Anatomy, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands,* [2]*Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands*

Introduction The protective effects of long-chain omega-3 (n-3) fatty acids on the cardiovascular system have been demonstrated by several positive clinical trials[1]. Epidemiological studies reported that increased levels of n-3 fatty acids, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are associated with reduced risk of cognitive decline and AD[2]. In 2006 Morgan et al.[3], showed that DHA supplementation ameliorates endothelium-dependent vasodilation processes and protects from cerebral microcirculatory abnormalities, often seen in AD patients and in transgenic AD mouse models. More recently, a combination of n-3 fatty acids with other precursors and cofactors in membrane synthesis (such as uridine monophosphate (UMP), choline, EPA, phospholipids, B-vitamins and antioxidants) has been proposed for the dietary management of AD[4] and is hypothesized to synergistically enhance the formation of new neuronal membranes and synapses[5]. Furthering these findings, we now investigated the effects of multi nutrient enriched diets in the pathophysiology of AD, for which we evaluated cerebral blood flow (CBF) and hippocampal metabolite levels with MR imaging and spectroscopy (^{1}H MRS) in a mouse models for genetic AD.

Animals and diets We used 12-month-old double transgenic APPswe/PS1dE9 (n=25) male mice resembling familial AD (Jankowsky, 2004) and wild type littermates (WT) (C57BL6/J, n=43). From 2 months of age, mice were divided in groups and fed with 3 different diets: a standard Control diet, a diet enriched in DHA, EPA and UMP (*DHA+*), or a multi-nutrient diet containing DHA, EPA, UMP, choline, phospholipids, B-vitamins and antioxidants (*Fortasyn*).

Methods MR measurements were performed on a 11.7 T BioSpec Avance III small animal MR system (Bruker BioSpin, Ettlingen, Germany) equipped with actively shielded gradient set of 600 mT/m. We used a circular polarized resonator for transmission and an actively-decoupled mouse brain coil for receiving (Bruker BioSpin). The perfusion sequence was run under resting conditions using a flow-sensitive alternating inversion recovery (FAIR) technique[6]. Imaging parameters: TE=11.8ms; TR=13.75s; image matrix=128×128; spatial resolution=0.234×0.234×1 mm/pixel. Twenty-five images with increasing TIs (40 ms - 3000 ms) were obtained for T_1 calculation after selective inversion interleaved with non-selective inversion for a total scan time of 24 minutes per mouse. The perfusion was calculated using the relation: $\frac{CBF}{\lambda} = \frac{T_{1\,non-selective}}{T_{1\,blood}}\left(\frac{1}{T_{1\,selective}} - \frac{1}{T_{1\,non-selective}}\right)$. Regional perfusion was evaluated in the cerebral cortex, hippocampus, thalamus and corpus callosum. Metabolite levels were determined in the hippocampus with single voxel ^{1}H MRS (PRESS, TE=10.9ms, TR=2500ms, averages=800) and quantified with LCModel. Brain tissue was analyzed immunohistochemically for microglial cells (CD68) and amyloid-β plaques, and biochemically for fatty acids components and several inflammatory factors.

Results In cerebral cortex, APP/PS1 mice on control and DHA+ diet showed a lower perfusion than their wild type littermates on the equivalent diets (p<0.001 and p=0.010). In transgenic mice, the Fortasyn diet group showed higher CBF compared with the control diet group (p=0.050). In hippocampus and in corpus callosum, APP/PS1 mice on control diet showed a significant decreased CBF compared to wild type (p=0.024 and p=0.006). Transgenic mice on control and on Fortasyn diet had lower blood supply in thalamus than wild type (p<0.001 and p=0.011), but the Fortasyn and the DHA+ diet group incremented the CBF towards APP/PS1 mice on control diet (p=0.05)(**Figure 1 and 2**). Data from ^{1}H MRS showed a slight decrease of N-acetyl-aspartate levels in the hippocampus of the APP/PS1 mice on control diet (p=0.070) and a decrease of myo-inositol in the transgenic mice on Fortasyn diet (p=0.070) (not shown).

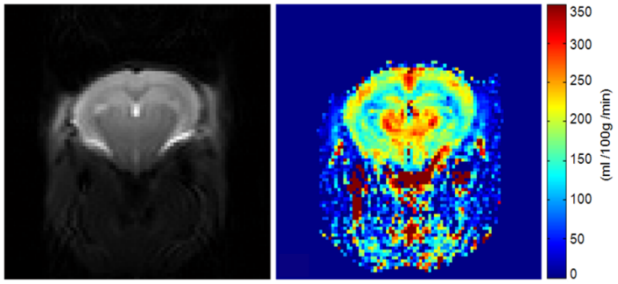

Figure 1. *Cerebral blood flow map obtained with FAIR pASL technique with spin-echo EPI.*

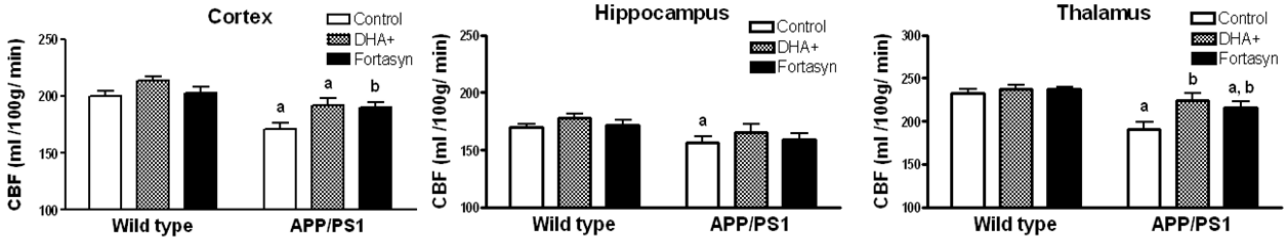

Figure 2. *Cerebral blood flow (CBF) was measured in APP/PS1 and wild type mice on three different diets (Control, DHA+EPA+UMP, Fortasyn) in the cortex, hippocampus and thalamus regions. a: p<0,05 compared with WT on same diet. b: p<0,05 compared with APP/PS1 on control diet*

Discussion and conclusion In the present study we successfully obtained high-resolution CBF maps of the mouse brain with FAIR-ASL MRI at 11.7T, and the results in terms of absolute CBF values showed a good agreement with previously published autoradiographic measurements[7]. Our results clearly showed that at 12 months of age, APP/PS1 mice on control diet have decreased brain perfusion as compared to wild type littermates in all of the measured ROIs. Our data revealed a positive effect of the multi nutrient enriched Fortasyn diet in maintaining cortical brain perfusion at physiological levels. Changes in metabolite levels detected by ^{1}H MRS suggest neuroprotection by DHA+ and Fortasyn supplementation. Taken together, these results strongly suggest that DHA, EPA and UMP in combination with phospholipids, B-vitamins and antioxidants, i.e. precursors and cofactors in membrane synthesis, have the potential to significantly delay the occurrence of brain perfusion failure and possibly slow AD pathology development.

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The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 211696.