Multi-nutrient diet increases cerebral blood flow and functional connectivity in apoE4 and wildtype mice

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Target audience Researchers interested in the role of dietary intake and of apoE genotype in AD research. Researchers involved in the development of resting-state fMRI in mice.

Purpose The cholesterol-transporter apo-lipoprotein ε (apoE) genotype is associated with the risk of developing Alzheimer's disease (AD). Recently, brain functional connectivity (FC) in apoE4 carriers has been investigated by means of resting-state fMRI, showing a marked differentiation in several functional networks compared to carriers of other apoE isoforms[1]. It has been suggested that an increased susceptibility to vascular brain damages of apoE4 carriers and a lower efficiency of the apoE4 protein in repairing and remodelling damaged synapses may determine a gradual loss of neuronal connectivity[2]. Specific diets containing fish oil have been proposed to prevent the effects of the apoE4 genotype in AD. Particularly, a combination of omega-3 fatty acids with precursors and cofactors in membrane synthesis, named Fortasyn Connect, has been developed to enhance the formation of neuronal membranes and synapses and contain components that may improve vascular health[3].
The purpose of the present study is to test whether such a specific multi-nutrient diet has beneficial effects against the apoE4 genotype in term of restoring functional connectivity and brain perfusion towards normal physiological levels. With this aim, we integrated different MRI techniques and immunohistochemical staining in a cross-sectional study using a rodent model.

Methods We used 12 and 18-month-old apoE4 mice (n=35) created by targeting the murine APOE gene for replacement with the human APOE4 alleles. We compared the results with wild-type mice (C57BL6/J, n=37). Starting at 2 months of age, mice were fed with a standard diet or a specific Fortasyn-containing diet providing fish oil, phospholipids, uridine monophosphate, choline, B-vitamins, and antioxidants. To study genotype and aging related differences in brain function and structure, resting state functional MRI (rsfMRI) and cerebral blood flow (CBF) were measured in each cohort on a 11.7T BioSpec Avance III animal MR system (Bruker BioSpin, Ettlingen, Germany).
Blood oxygenation level dependent (BOLD) data were acquired with a spin echo echo-planar imaging sequence (TE= 16.9ms, TR = 1.7s, voxel resolution = 0.19×0.26×0.5 mm, repetitions = 600). After in-plane smoothing (0.4×0.4mm) and temporal high-pass filtering (cut-off 0.01Hz), brains were normalized into a study-specific template, and 17 ROI were selected, based on a previous study of mouse brain functional connectivity[4]. Z-transformed total and partial correlation matrices between average time course fMRI data within ROIs were calculated using FSL. CBF was measured using flow-sensitive alternating inversion recovery (FAIR) and analyzed as previously described[5]. During acquisition, mice were kept under low-dose Isofluran, known to largely preserve time-series characteristics and brain connectivity in rodents[6]. In the same animals, the amount of post-synapses were assessed by immunohistochemistry.

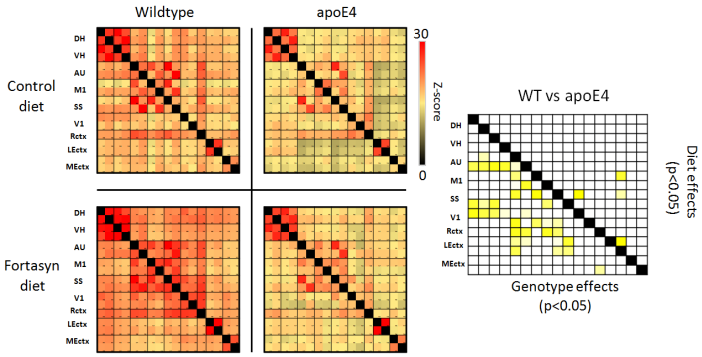

Figure 1. Correlation matrices from 12-month-old animals revealed a reduced FC in apoE4 mice in several hippocampal and cortical regions. In some areas, Fortasyn-containing diet was able to increase FC in both genotypes. DH=dorsal hippocampus; VH=ventral hippocampus; AU=auditory cortex; M1=motor cortex; SS=Somatosensory cortex; V1=visual cortex; Rctx=Retrosplenial cortex; LEctx=lateral entorhinal cortex; MEctx=medial

Results BOLD correlations maps in all animals displayed distinct FC distribution among brain areas, with stronger correlations between hippocampal regions and between cortical regions, also inter- and intra-hemispheres. Overall, apoE4 animals showed a marked reduction in FC compared to wildtype between several ROI at both ages (fig 1). At 12 months of age, mice on Fortasyn-containing diet showed an increased FC between the SS, the V1, the LEctx and the M1 (fig 1). At 18 months of age, the Fortasyn diet increased the connectivity between VH and LEctx.

The CBF data revealed a reduced brain perfusion in apoE4 mice compared to wildtype at 18months of age, in cortex and thalamic regions (fig 2). Wildtype and apoE4 mice on Fortasyn diet exhibited increased CBF in the same areas, at both ages. Immunohistochemistry (PSD95) showed a reduced post-synaptic density in 18-month-old apoE4 mice in the inner and outer molecular layer of the hippocampus. Mice on Fortasyn also revealed an increased amount of PSD95 in SS (p=0.005), stratum lucidum of CA3 (p=0.004) and inner molecular layer of the dentate gyrus (p=0.016) (not shown).

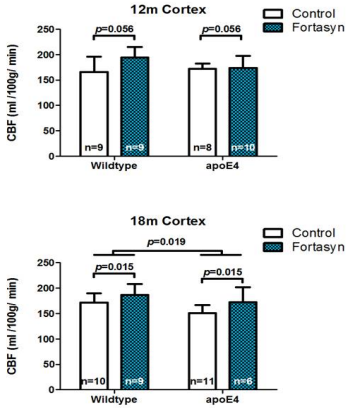

Figure 2. Cortical CBF is reduced in 18-month-old apoE4 mice. Increased CBF is found in mice on Fortasyn diet, in both genotypes and in both age groups.

Discussion and conclusion In aging apoE4 and wildtype mice we successfully quantified FC and CBF by MR at 11.7T and post-synaptic density. The results from the apoE4 mice are consistent with findings in human studies on apoE-ε4 carriers, showing overall a reduced perfusion and impaired FC, particularly evident at older age. As changes in FC occurred before vascular deficits, this study demonstrates that resting-state fMRI may represent a novel tool for identifying early AD-like changes in translational research. Interestingly, we provide new evidence that the Fortasyn diet is associated to: 1) enhanced CBF; 2) increased neural connectivity; 3) increased number of post-synapses. We suggest that this increased connectivity may be the result of a better preservation of synapses, as hypothesized for the effect of the specific diet formulation. Taken together these results point to a positive effect of Fortasyn diet on brain function, potentially translating to both normal human subjects and carriers of AD's vascular disease risk factors.

References [1] Fleisher et al., 2009; [2] Zlokovic, 2011; [3] van Wijk et al., 2013; [4] Yonckers et al., 2011; [5] Zerbi et al., 2013 ; [6] Liang Z et al., 2012. *This research received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 211696*