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Multinutrient diets improve cerebral perfusion and neuroprotection in a murine model of Alzheimer's disease

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ABSTRACT

Nutritional intervention may retard the development of Alzheimer's disease (AD). In this study we tested the effects of 2 multi-nutrient diets in an AD mouse model ($APP_{swe}/PS1_{dE9}$). One diet contained membrane precursors such as omega-3 fatty acids and uridine monophosphate (DEU), whereas another diet contained cofactors for membrane synthesis as well (Fortasyn); the diets were developed to enhance synaptic membranes synthesis, and contain components that may improve vascular health. We measured cerebral blood flow (CBF) and water diffusivity with ultra-high-field magnetic resonance imaging, as alterations in these parameters correlate with clinical symptoms of the disease. $APP_{swe}/PS1_{dE9}$ mice on control diet showed decreased CBF and changes in brain water diffusion, in accordance with findings of hypoperfusion, axonal disconnection and neuronal loss in patients with AD. Both multinutrient diets were able to increase cortical CBF in $APP_{swe}/PS1_{dE9}$ mice and Fortasyn reduced water diffusivity, particularly in the dentate gyrus and in cortical regions. We suggest that a specific diet intervention has the potential to slow AD progression, by simultaneously improving cerebrovascular health and enhancing neuroprotective mechanisms.

1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia worldwide. The disease is clinically characterized by cognitive impairment, memory loss, anomia, apraxia, anosognosia, and alteration of personality. Pathologic hallmarks of AD include the presence of neurofibrillary tangles, amyloid-beta (Aβ) plaque formation, and neurodegeneration (Sennvik et al., 2000). The development of AD also involves several associated processes all of which are described in AD pathophysiology, such as: synaptic loss, chronic brain inflammation, white matter degeneration, mitochondrial dysfunction, and cerebrovascular defects. After many years of extensive research, the idea that a single event can trigger AD pathogenesis is fading, replaced by the belief that all these events combined lead to a progressive damage of neuronal function, loss of synaptic integrity, and cognitive decline. The failure of several anti-AD drugs in phase 3 clinical trials has been related to the fact that these medicines targeted a single trait of the disease, already in a stage when neuronal damage had become too widespread (Kozauer and Katz, 2013); this emerging idea emphasizes the importance of combining new therapeutic approaches that act in parallel on multiple AD risk factors in an early stage of the disease (Iqbal and Grundke-Iqbal, 2010). One such approach currently available is the use of nutrients and dietary modifications.

Aside from aging, many risk factors of AD, including atherosclerosis and hypercholesterolemia, seem to be related to the development of a less healthy lifestyle in the Western population. The consumption of high cholesterol and high caloric food typical of the western diet, resulting in overweight with its associated disorders, is proposed as 1 of the main causes of the increased AD prevalence, largely because of their negative effect on the vascular system (Canevari and Clark, 2007; Casserly and Topol, 2004; Luchsinger et al., 2005; Puglielli et al., 2003; Wolozin et al., 2006). A beneficial impact on AD onset and development has been suggested via healthy dietary behavior instead. Among a long list of nutrients that are considered potential candidates for interventions to delay AD, omega-3 (n-3) long-chain polyunsaturated

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fatty acids (LCPUFA) from fish oil are the most studied. With the discovery of the therapeutic effects of n-3 LCPUFA supplementation against cardiovascular pathologies (Lee et al., 2008), the idea arose about influencing AD development by attenuating vascular risk factors. Furthermore, high levels of n-3 LCPUFA replace omega-6 (n-6) fatty acids and cholesterol from cell membranes leading to increased fluidity of the membrane, increased number of receptors, enhanced receptor binding and affinity, and improved neurotransmission and signaling (Bourre et al., 1989), which is important for optimal cognitive functioning (Bourre et al., 1991; Farkas et al., 2002; Fontani et al., 2005). Several epidemiologic studies associated a reduced risk of developing dementia, including AD, with an increased dietary intake of n-3 LCPUFA from fish oil, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Barberger-Gateau et al., 2007; Beydoun et al., 2008; Eskelinen et al., 2008; Kalmijn et al., 1997; Schaefer et al., 2006). However, evidence from clinical randomized controlled trials failed to confirm the promising findings of their efficacy as AD treatment (Cunnane et al., 2009; Quinn et al., 2010; Yurko-Mauro et al., 2010).

One of the reasons behind these inconclusive results may lie in the concomitant presence of multiple phenotypic traits in AD. While n-3 LCPUFA alone improve the functionality of the neural and vascular system and have a beneficial effect on cardiovascular disease prevention, this might not be sufficient to avert neurodegenerative processes and synaptic loss as occurring in AD. Several preclinical studies showed that specific combinations of nutrients, rather than a single component, can synergistically act to express better therapeutic outcomes; animals fed with a combination of uridine monophosphate (UMP), DHA, and choline, which are key nutritional precursors to form phosphatidyl-choline (Kennedy and Weiss, 1956), but not DHA alone, showed increased levels of brain phospholipids, dendritic spines, and neurite outgrowth, with beneficial effects on cognition (Cansev and Wurtman, 2007; Sakamoto et al., 2007; Wurtman et al., 2006). Recently, a novel combination diet that also includes other precursors and cofactors in membrane synthesis (such as phosphatidylcholine, B-vitamins, and antioxidants), called Fortasyn, has been proposed for the dietary management of AD (Kamphuis and Scheltens, 2010). To date, 2 randomized controlled clinical trials have shown improvements in the delayed verbal recall task and better cognitive performance in patients with mild AD supplemented with a Fortasyn-containing diet (Cummings, 2012; Scheltens et al., 2010, 2012). The mechanisms by which these dietary nutrients influence the pathophysiology of AD still need to be elucidated, and more studies are necessary to confirm their efficacy. In this study, we tested the hypothesis that the multi-nutrient Fortasyn diet, and a diet consisting of DHA, EPA, and UMP without additional nutrients, have a positive effect in delaying AD pathology progress, both by improving cerebral perfusion and by protecting against neuronal degeneration. With this aim, we evaluated cerebral blood flow (CBF) and gray and white matter integrity with magnetic resonance imaging at ultra-high field in a 12-month-old double transgenic mouse model for genetic AD ($APP_{swe}/PS1_{dE9}$).

2. Methods

2.1. Animals

The $APP_{swe}/PS1_{dE9}$ founder mice were originally obtained from Johns Hopkins University, Baltimore, MD, USA (Borchelt et al., 1997), and a colony was established at the Central Animal Facility at Radboud University Nijmegen Medical Center, the Netherlands. In short, mice were created by co-injection of chimeric mouse/human APPswe (mouse APP695 harboring a human Aβ domain and mutations K595N and M596L linked to Swedish familial AD pedigrees) and human PS1-dE9 (deletion of exon 9) vectors controlled by independent mouse prion protein promotor elements. The 2 transfected genes co-integrate and co-segregate as a single locus (Jankowsky et al., 2001). This line (line 85) was originally maintained on a hybrid background by backcrossing to C3HeJ × C57BL6/J F1 mice (so-called pseudo F2 stage). For the present work, the breeder mice were backcrossed to C57BL6/J for 13 generations to obtain mice for the present study. To reduce the variability of the data, only male mice were used for the experiment. Throughout the experiments, the animals were housed in a controlled environment, with room temperature at 21 °C, and an artificial 12:12 hours light:dark cycle (lights on at 7 AM). Food and water were available *ad libitum*. The experiments were performed according to Dutch federal regulations for animal protection and were approved by the Veterinary Authority of Radboud University Nijmegen Medical Center.

2.2. Diets

From 2 months of age, male transgenic mice (n = 27) and wild-type littermates (n = 43) were assigned to 3 different diet groups until the end of the experiment (Table 1). One group of mice was used as a control group and received a standard normal rodent chow with 5% fat percentage obtained from a combination of corn oil (2.2%), soy oil (1.9%), and coconut oil (0.9%). In the second group the fat composition in the food was replaced by fish oil (3.0%), corn oil (1.9%), and coconut oil (0.1%). In addition, UMP was added (1%). This diet will be called DEU (DHA + EPA + UMP) throughout the article. The third group received the same DEU diet with additional nutrients such as choline, phospholipids, vitamins B6, B12, C, and E, folic acid, and selenium. This multi-nutrient diet will be called Fortasyn. The composition of the diets is shown in Table 1. All 3 diets were isocaloric. All diets were manufactured and pelleted by Research Diet Services (Wijk bij Duurstede, The Netherlands) and stored at −20 °C until use.

2.3. Magnetic resonance imaging protocol

Magnetic resonance imaging (MRI) measurements were performed at the age of 12 months on an 11.7 T BioSpec Avance III small animal MR system (Bruker BioSpin, Ettlingen, Germany) equipped with an actively shielded gradient set of 600 mT/m and operated by a Paravision 5.1 software platform. We used a circular polarized volume resonator for signal transmission and an actively decoupled

Table 1
Oil sources and additives in the experimental diets

5% Fat percentage	Dietary groups		
	Control [g/100 g]	DEU [g/100 g]	Fortasyn [g/100 g]
Oils			
Soya oil	1.9	-	-
Coconut oil	0.9	0.1	0.1
Corn oil	2.2	1.9	1.9
Fish oil	-	3.0	3.0
Additives			
Pyridoxine-HLC	-	-	0.00033
Folic acid (90%)	-	-	0.00067
Cyanocobalamin	-	-	0.00350
Ascorbic acid	-	-	0.16
Dl-α-tocopheryl acetate	-	-	0.465
Uridine monophosphate (UMP)	-	1.0	1.0
Choline chloride	-	-	0.402
Soya lecithin	-	-	0.412
Sodium selenite	-	-	0.00023