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Whole-food diet worsened cognitive dysfunction in an Alzheimer's disease mouse model☆

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ABSTRACT

Food combinations have been associated with lower incidence of Alzheimer's disease. We hypothesized that a combination whole-food diet containing freeze-dried fish, vegetables, and fruits would improve cognitive function in TgCRND8 mice by modulating brain insulin signaling and neuroinflammation. Cognitive function was assessed by a comprehensive battery of tasks adapted to the Morris water maze. Unexpectedly, a "Diet × Transgene" interaction was observed in which transgenic animals fed the whole-food diet exhibited even worse cognitive function than their transgenic counterparts fed the control diet on tests of spatial memory ($p < 0.01$) and strategic rule learning ($p = 0.034$). These behavioral deficits coincided with higher hippocampal gene expression of tumor necrosis factor-α ($p = 0.013$). There were no differences in cortical amyloid-β peptide species according to diet. These results indicate that a dietary profile identified from epidemiologic studies exacerbated cognitive dysfunction and neuroinflammation in a mouse model of familial Alzheimer's disease. We suggest that normally adaptive cellular responses to dietary phytochemicals were impaired by amyloid-beta deposition leading to increased oxidative stress, neuroinflammation, and behavioral deficits.

1. Introduction

The availability of transgenic animal models of Alzheimer's disease (AD) has permitted investigations into the impact of nutrients and other compounds isolated from foods on disease-related neuropathology, particularly amyloid-beta peptide (Aβ), and behavioral deficits. Among the most studied compounds are docosahexaenoic acid (DHA), antioxidant vitamins, and certain dietary phytochemicals which have been shown to be beneficial in many (Calon et al., 2004; Frautschy et al., 2001; Lee et al., 2012b; Murakami et al., 2011; Rezai-Zadeh et al., 2008; Wang et al., 2008), but not all cases (Arendash et al., 2007). These beneficial findings have been attributed to control of oxidative stress and, despite concerns over lack of direct in vivo evidence (Orr et al., 2013), neuroinflammation. However, it is becoming increasingly evident that dietary compounds, especially DHA and phytochemicals, exert a pleiotropic effect by modulating cell signaling pathways that impact on additional mechanisms like synaptic plasticity and the enzymatic processing of Aβ (Davinelli et al., 2012; Frautschy and Cole, 2010; Grimm et al., 2011; Zhao et al., 2011). Many of these cell signaling changes involve regulators of cellular energy homeostasis and growth that are also commonly modulated by insulin. Interestingly, impaired neuronal insulin signaling is a prominent feature of AD that has been linked to disease severity, Aβ deposition, and degree of cognitive dysfunction (Bomfim et al., 2012; Craft et al., 2013; de la Monte, 2012; Talbot et al., 2012).

Despite the diversity of potential mechanisms and promising results in animal models, clinical trials have found limited or no benefits of single nutrients like antioxidant vitamins (Galasko et al., 2012; Sano et al., 1997) and DHA (Freund-Levi et al., 2006; Quinn et al., 2010) in AD. This mismatch between basic and clinical studies has led some to speculate that background diet may be an important determinant of intervention success such that single nutrient supplementation is unlikely to benefit those with replete diets, or conversely, that supplementation with a single nutrient

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is unlikely to overcome the negative impacts of overall low diet quality (Morris, 2012). The recognition that whole foods provide a wide array of compounds that may interact to produce synergistic effects has led to the interest in the role that food combinations or diet quality may play in preventing AD with some positive results in the epidemiologic literature (Gu et al., 2010; Scarmeas et al., 2006).

The present study is the first in a systematic investigation of the impact of a combined whole-food diet (WFD) on cognitive function and Aβ deposition in a transgenic mouse model of AD. Given the evidence surrounding their efficacy from epidemiologic and basic studies, food sources of DHA, antioxidant vitamins, and phytochemicals—namely freeze-dried fish, fruits, and vegetables—were targeted. Because these dietary compounds have been shown to influence brain insulin signaling and neuroinflammation, cerebral gene expression of these pathways was also determined. The following tests of learning and memory were administered to measure cognitive functions known to be affected in AD: (1) spatial memory (Morris, 1984) in which distal environmental cues are used to find a submerged platform. This test is sensitive to impairment within the hippocampus, a subcortical structure that is widely implicated in the memory loss reliably seen early in AD; (2) nonmatching-to-sample (NMTS) which requires that animals differentiate between sample and test stimuli and select one according to a learned rule. NMTS and similar rule-learning tasks incorporate conditional and working memory components that are critical for many types of problem solving under the control of the prefrontal cortex (Moscovitch and Winocur, 1995). These abilities, along with the integrity of the frontal lobes, are increasingly compromised in AD as the disease progresses; and (3) brightness discrimination learning in which mice must discriminate between black and white stimuli to find the platform. This task is believed to depend on the caudate nucleus and related striatal structures (McDonald et al., 1999), a brain system that is affected in the later stages of AD. Our working hypothesis was that the WFD containing fish, vegetables, and fruits would beneficially influence cognitive performance and Aβ deposition through modulation of brain insulin signaling and neuroinflammation.

2. Methods

2.1. Mice and diets

TgCRND8 mice (Chishti et al., 2001) overexpressing mutations in the human *APP* gene (KM670/671NL, V717F) and maintained on a mixed C3H/C57 outbred background were obtained courtesy of the Tanz Centre for Research in Neurodegenerative Diseases. Animals were housed 3–4 per cage (L: 29 cm, W: 18 cm, H: 12 cm) in a facility with controlled temperature (21 °C), humidity (40%), and light cycle (12 hour light and/or dark). Mice had *ad libitium* access to either the WFD or control diets from weaning (aged 3 weeks) until sacrifice at 7 months of age (Harlan Teklad, Madison, WI, USA; see Supplementary Table 1 for detailed composition). The WFD contained skinless freeze-dried Atlantic salmon (prepared by Guelph Food Technology Centre, Guelph, Ontario, Canada) and a proprietary mixture of powdered, freeze-dried vegetables and fruits (BerryGreen, New Chapter, Brattleboro, VT, USA). The 3 most abundant ingredients of this mixture were spinach, blueberries, and cruciferous vegetables (kale, cabbage, broccoli, and brussel sprouts). The total fat content and fatty acid profile of the freeze-dried salmon was determined by flame-ionized gas chromatography as described previously (Reza-Lopez et al., 2009). The total phenolic content and oxygen radical absorbance capacity expressed in millimoles of Trolox equivalents (mmolTE) of the fruit and vegetable mixture were determined by an independent lab (Brunswick Laboratories, Southborough, MA, USA). Based on these analyses, the WFD provided 2.46 mg docosahexaenoic acid (0.246% wt/wt), 1.10 mg total phenolics, and 0.018 mmolTE per gram of diet. The control diet was formulated to have the same energy density (3.8 kcal/g), macronutrient composition (17% fat, 64% carbohydrate, and 19% protein per kcal of diet), and fiber content as the WFD. Corn oil acted as the main source of dietary fat.

At 4 months of age, mice were transferred to Trent University for cognitive testing. Testing commenced after a 2-week acclimatization period and lasted for an additional 2.5 months. Animals were sacrificed at 7 months of age by pentobarbital overdose. Following rapid excision, brains were dissected on a cold surface in PBS, flash frozen in liquid nitrogen, and stored at −80 °C.

2.2. Cognitive testing

The spatial memory, NMTS, and brightness discrimination tasks were administered in a circular pool (130 cm diameter and approximately 30 cm high), located in the center of a room (360 cm × 360 cm). The pool was filled with water rendered opaque by diluted nontoxic white tempera paint, to a depth of 18 cm and maintained at room temperature (21 °C). An inverted flower pot (15 cm high by 10 cm in diameter) with a white surface, situated a few centimeters below the surface of the water, served as a platform on which the mice could climb to escape the water. A heat lamp near the pool provided a warm area where mice waited between trials. Throughout testing, the water was cleaned after each trial and changed every 2–3 days.

For the spatial memory and the NMTS tasks, the pool was divided into 6 zones of equal size. Swimming patterns of mice were monitored by an overhead video camera connected to a recorder and data processing system. The system enabled computation of the time required to find and climb on the platform and the time spent in the platform zone. Records were kept of the animals' swimming routes that were used to count errors. For the brightness discrimination task, the pool was fitted with a T-maze whose walls extended 10 cm above the water surface. The stem of the "T" was 27 cm long. The horizontal arm was 65 cm long with slats along the walls into which black or white panels were inserted. The submerged platform was located at the end of the panel designated as the positive arm.

These tasks are commonly used in our laboratory to assess the effects of various types of brain dysfunction on cognitive performance in mice (Winocur et al., 2006) and rats (Winocur et al., 2013). All testing was conducted by a single experimenter who was blind to the treatment history.

2.2.1. Spatial memory

Initially, mice received 2 days of orientation training, consisting of 5 trials/day in which mice were placed individually in the pool and allowed to swim to and climb on the platform, which was visible a few centimeters above the surface of the liquid. The location in which the mice were placed in the pool and the location of the platform were varied from trial to trial. A trial continued until the mouse mounted the platform with all 4 paws or until 120 seconds elapsed. The mouse was allowed to remain on the platform for 10 seconds; if it failed to find the platform in the allotted time, it was manually guided to the platform where it was allowed to remain for 10 seconds. The mouse was then removed and placed in a clean cage under the heat lamp to await the next trial. The mice were run in squads of 4–5, allowing for an interval of 2–3 minutes between trials.

Spatial memory testing began on day 3. The platform was now below the surface of the water and always located in the center of the north-east zone of the pool. For each trial, the mouse was placed in the water at the edge of the pool, facing the wall, at a different location. The starting locations were determined by a semi-random