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Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive decline in cognitive function associated with the neuropathological hallmarks amyloid β-peptide (Aβ) plaques and neurofibrillary tangles. Because aging is the major risk factor for AD, and dietary energy restriction can retard aging processes in the brain, we tested the hypothesis that two different energy restriction regimens, 40% calorie restriction (CR) and intermittent fasting (IF) can protect against cognitive decline in the triple-transgenic mouse model of AD (3xTgAD mice). Groups of 3xTgAD mice were maintained on an ad libitum control diet, or CR or IF diets, beginning at 3 months of age. Half of the mice in each diet group were subjected to behavioral testing (Morris swim task and open field apparatus) at 10 months of age and the other half at 17 months of age. At 10 months 3xTgAD mice on the control diet exhibited reduced exploratory activity compared to non-transgenic mice and to 3xTgAD mice on CR and IF diets. Overall, there were no major differences in performance in the water maze among genotypes or diets in 10-month-old mice. In 17-month-old 3xTgAD mice the CR and IF groups exhibited higher levels of exploratory behavior, and performed better in both the goal latency and probe trials of the swim task, compared to 3xTgAD mice on the control diet. 3xTgAD mice in the CR group showed lower levels of Aβ1–40, Aβ1–42 and phospho-tau in the hippocampus compared to the control diet group, whereas Aβ and phospho-tau levels were not decreased in 3xTgAD mice in the IF group. IF may therefore protect neurons against adverse effects of Aβ and tau pathologies on synaptic function. We conclude that CR and IF dietary regimens can ameliorate age-related deficits in cognitive function by mechanisms that may or may not be related to Aβ and tau pathologies.

Keywords: Amyloid; Caloric restriction; Hippocampus; Learning and memory; Synaptic plasticity

Introduction

Alzheimer's disease (AD) is characterized by progressive impairment of memory accompanied by psychiatric disturbances (Lyketsos et al., 2002; Mattson, 2004; Steffens et al., 2006). The behavioral abnormalities in AD result from dysfunction and death of neurons in brain regions involved in cognition and mood such as the hippocampus, entorhinal cortex, basal forebrain, and frontal and parietal lobes. These brain regions suffer degeneration of synapses and neurons associated with abnormal accumulation of extracellular deposits of amyloid β-peptide (Aβ), a 40–42 amino acid proteolytic cleavage product of the amyloid precursor protein (APP). Aβ may cause synaptic dysfunction and degeneration of neurons by inducing membrane-associated oxidative stress, resulting in disruption of cellular ion homeostasis (Mattson, 2004). Transgenic mouse models that express a familial AD (FAD) APP mutation alone or in combination with an FAD presenilin-1 mutation exhibit progressive Aβ deposition and variable levels of synaptic dysfunction and cognitive impairment depending upon the particular model (Morgan et al., 2000; Ashe, 2001; Jankowsky et al., 2005; Kobayashi and Chen, 2005; Jacobsen et al., 2006). We recently generated a novel triple mutant mouse model of AD (3xTgAD mice) in which the mice express FAD APP and presenilin-1 mutations together with a tau mutation (Oddo et al., 2003a). The 3xTgAD mice exhibit age-dependent Aβ deposition and tau pathology in the hippocampus and cerebral cortex which are associated with impaired synaptic plasticity (Oddo et al., 2003a,b) and deficits in spatial learning tasks (Billings et al., 2005).

Previous studies have shown that caloric restriction (CR) and intermittent fasting (IF) diets are neuroprotective and improve functional outcome in animal models of stroke, Parkinson's and Huntington's diseases (reviewed in Mattson, 2005). The animal studies suggest that CR and IF may benefit the brain by reducing levels of oxidative stress and by enhancing cellular stress resistance

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mechanisms. Data from studies of human populations and animal models suggest that reduced food intake may also protect against AD. For example, a prospective epidemiological study of a large cohort in New York City provided evidence that individuals with a low calorie intake have a reduced risk of developing AD (Luchsinger et al., 2002). Another study showed that obesity at midlife increases the risk of AD (Kivipelto et al., 2005). Moreover, diseases caused by excessive calorie intake (diabetes and cardiovascular disease) are associated with increased risk of AD (Launer, 2005). CR was recently reported to reduce the development of amyloid pathology in the hippocampus and cerebral cortex of transgenic mice overexpressing FAD APP mutations (Patel et al., 2005; Wang et al., 2005), suggesting that CR can suppress a key pathogenic process in AD. However, the effects of CR and IF diets on the development of cognitive dysfunction in AD are unknown. In the present study we determined if long-term CR and/or IF could ameliorate age-related behavioral impairments in 3xTgAD mice.

Materials and methods

Animals and experimental design

Male and female non-transgenic C57BL/6 mice and 3xTgAD mice (Oddo et al., 2003a) were housed in cages (4–5 five per cage) and maintained under a 12 h light and dark cycle. These mice were in a colony that had been exposed to mouse hepatitis virus, but the mice were not shedding virus at the time of analysis. At 3 months of age mice were divided into 4 groups of 20 males and 20 females per group assigned to the following dietary regimens: non-transgenic ad libitum (nonTg,AL), 3xTgAD ad libitum (3xTgAD,AL), 3xTgAD 40% calorie restriction (3xTgAD, CR), 3xTgAD intermittent fasting (3xTgAD,IF). All mice ate standard mouse food pellets (AIN-93G; catalog #101845 from Dyets, Inc., Bethlehem, PA). Mice on the CR diet were provided an amount of food equal to 60% of that consumed by mice in the 3xTgAD,AL group (40% caloric restriction). Mice on the IF diet were deprived of food for 24 h every other day. Half of the mice in each diet group were subjected to behavioral testing after 7 months on the diets, and the other half were tested after 14 months on the diets. One week after behavioral testing the mice were euthanized and the hippocampus was dissected and frozen for analyses of Aβ1–40 and Aβ1–42. During the different dietary regimens body weights were measured weekly; the mice in the 3xTgAD,AL group gained less weight than the nonTg,AL mice such that the 3xTgAD mice weighed 5–10 g less than the non-transgenic mice at the end of the study. Similar to a previous study of C57BL/6 mice (Anson et al., 2003), the 3xTgAD mice (both males and females) in the CR group maintained significantly lower body weights (approximately 3–7 g lower) than 3xTgAD mice on either AL or IF diets. Over the course of the 14-month diet interventions, some mice in each group died prior to behavioral analyses resulting in numbers that were different among the groups.

Open field activity

Spontaneous locomotor activity in the open field was assessed using an apparatus equipped with infrared light sensitive photocells. The apparatus was placed in a darkened, ventilated and quiet testing room with other behavioral testing apparatus. Each animal was placed in the apparatus and ambulatory counts and total distance traveled were recorded over a period of 10 min.

Water maze test

A circular tank (diameter 100 cm, height 50 cm, painted white) was filled with water (22±1 °C) to a depth of 30 cm; the water was rendered opaque by the addition of non-toxic water paint (Palmer Paints Products Inc, Michigan, USA). Spatial visual clues were provided in the form of different shaped objects on the walls of each quadrant. A circular, white escape platform (diameter 10 cm) was submerged approximately 1 cm below the surface of the water, 10 cm off the edge of the tank at a position designated as quadrant 3 (target quadrant). A video camera was mounted on the ceiling in the center of the pool. The swimming path length was monitored with a Videomex tracking system and data were collected using Videomex Water Maze Software (Columbus Instruments, Ohio, USA) and stored on disk for future analysis.

Acquisition trials (4 trials per day for 8 days) were started by placing the mouse in the pool facing the wall of the tank from different randomly chosen start positions, and the time required to find the invisible platform was recorded. A trial lasted until the mouse found the platform or until 60 s had elapsed. If the mouse did not find the platform within 60 s, it was guided to the platform and placed on it for 60 s. After the completion of the fourth trial on each day, the mouse was dried and returned to its home cage. Twenty four hours after the final acquisition trial, the platform was removed from the pool and a probe trial lasting 60 s was performed; the time spent and path length in the target quadrant were recorded. In the probe trial the mouse was started facing the wall of the tank from the position opposite to the removed platform. Twenty four hours after the probe trial, mice were trained for visible platform test (4 trials per day for 2 days) to confirm that each mouse had no visual deficits.

Quantification of Aβ1–40 and Aβ1–42 levels

Hippocampal tissue was homogenized in a buffer containing 100 mM PIPES, 500 mM NaCl, 0.2% Triton X-100, 0.1% NaN_3, 2% BSA, 0.5 mM sodium vanadate, 2 mM EDTA, 200 μM PMSF and a cocktail of protease inhibitors (10.4 mM AEBSF, 8 μM aprotinin, 0.2 mM leupeptin, 0.4 mM bestatin, 0.15 mM pepstatin A and 0.14 mM E-64). Samples were homogenized on ice at a power level of 4 and pulses at 1 s intervals for 30 s. Samples were allowed to sit on ice for 1 h, centrifuged at 12,000×*g* for 15 min (4 °C) and supernatants were collected and used for ELISA. Protein concentrations were determined using a BCA kit (Pierce). The concentrations of Aβ1–40 and Aβ1–42 in the samples were measured using ELISA with antibodies that specifically recognize full-length Aβ1–40 or Aβ1–42 using methods described previously (Horikoshi et al., 2004).

Immunoblot analysis

The protein concentration in hippocampal tissue samples was determined by the BCA protein assay kit (Pierce, USA). Fifty micrograms of protein was separated by SDS–PAGE (8–12%) and transferred to nitrocellulose membranes. The membranes were blocked in 5% nonfat milk for 1 h at room temperature, followed by an overnight incubation at 4 °C with antibodies against total tau (HT7; Innogenetics), phospho-tau (AT8; Innogenetics) or β-actin