

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 ($A\beta$) 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% caloric 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 $A\beta$ 1–40, $A\beta$ 1–42 and phospho-tau in the hippocampus compared to the control diet group, whereas $A\beta$ and phospho-tau levels were not decreased in 3xTgAD mice in the IF group. IF may therefore protect neurons against adverse effects of $A\beta$ 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 $A\beta$ and tau pathologies.

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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 ($A\beta$), a 40–42 amino acid proteolytic cleavage product of the amyloid precursor protein (APP). $A\beta$ 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 $A\beta$ 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 $A\beta$ 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 A β 1–40 and A β 1–42. 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 A β 1–40 and A β 1–42 levels

Hippocampal tissue was homogenized in a buffer containing 100 mM PIPES, 500 mM NaCl, 0.2% Triton X-100, 0.1% NaN₃, 2% BSA, 0.5 mM sodium vanadate, 2 mM EDTA, 200 μ M PMSF and a cocktail of protease inhibitors (10.4 mM AEBSF, 8 μ M 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 \times 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 A β 1–40 and A β 1–42 in the samples were measured using ELISA with antibodies that specifically recognize full-length A β 1–40 or A β 1–42 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

(Sigma). Membranes were then washed and incubated with secondary antibodies for 1 h at room temperature. Protein bands were visualized using a chemiluminescence detection kit (Amersham Biosciences). Band intensities were quantified by densitometric analysis, and values for total tau and phospho-tau were normalized to levels of actin and total tau, respectively.

Statistical analysis

In the acquisition trials of the Morris water maze the following parameters were recorded: the escape latency (time required to reach the platform from the releasing point in seconds), the path length (distance traveled by the mouse from the release point to reach platform in centimeters) and the swim speed (cm/s). Analysis of variance (ANOVA) was conducted on these data (using SYSTAT 8.0), with group as the between-subject factor and with repeated measures such as trial and day as within subject factors. Post hoc analysis (Scheffe test) was used to determine significance between the groups. For probe trial data time spent and distance traveled in quadrant 3 were recorded and analyzed by one way ANOVA and post hoc analysis by Scheffe test. Values for A β 1–40 and A β 1–42 levels were expressed as fmol/mg of hippocampal tissue and were analyzed by ANOVA followed by post hoc using LSD (Fischer's Least-Significant-Difference test).

Results

An age-related decrease in open-field activity in 3xTgAD mice is attenuated by CR and IF

3xTgAD mice that had been maintained on the AL diet for 7 or 14 months exhibited significantly less ambulatory activity compared to nonTg,AL control mice (Fig. 1a). There was no effect of diet on ambulatory counts in 3xTgAD mice maintained for 7 months on the diets. However, 3xTgAD mice maintained on CR or IF diets for 14 months exhibited significantly greater ambulation compared to 3xTgAD mice maintained on the AL diet for 14 months. Consistent with the ambulatory count data, 3xTgAD mice that had been maintained on the AL diet for 7 or 14 months exhibited significantly greater distance traveled in the open field compared to nonTg,AL control mice (Fig. 1b). There was no effect of diet on distance traveled in 3xTgAD mice maintained for 7 months on the diets. However, the distance traveled by 3xTgAD mice maintained on CR or IF diets for 14 months was significantly greater than that of 3xTgAD mice maintained on the AL diet for 14 months. These findings indicate that 3xTgAD mice exhibit reduced locomotion and exploratory behavior compared to non-transgenic control mice and that CR and IF diets enhance locomotion and exploratory behavior in the 3xTgAD mice.

Caloric restriction and intermittent fasting ameliorate age-related deficits in the Morris swim task

After 7 months on the diets, non-transgenic control mice, and 3xTgAD mice in all three diet groups, improved their performance in the hidden platform test as indicated by progressive decreases in goal latency times with increasing trials (Figs. 2a, b). Because there were no sex differences for any of the four groups at this age, data for males and females were combined. Two way ANOVA with repeated measures revealed a significant main effect of days

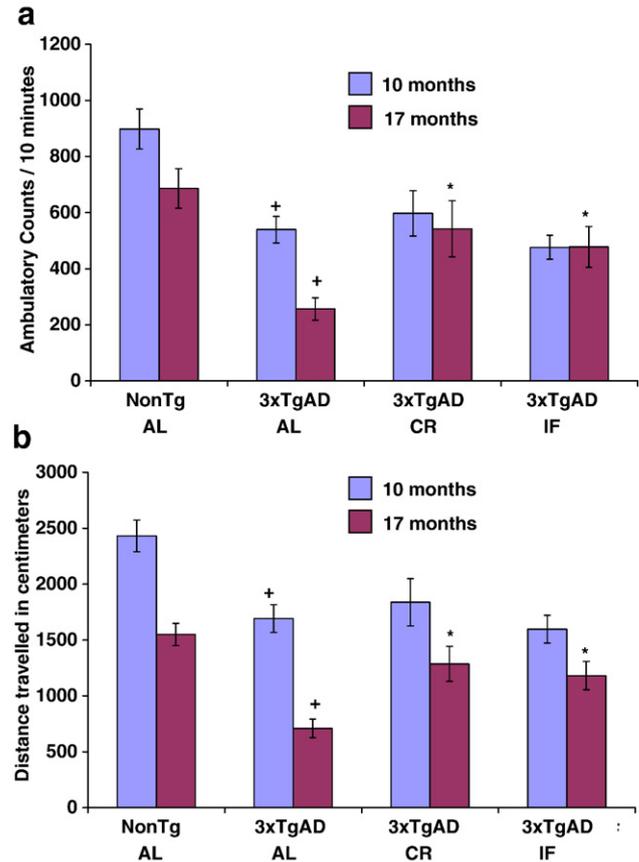


Fig. 1. Age-related decrease in open field activity is attenuated by CR and IF in 3xTgAD mice. Male and female mice of the indicated genotypes (non-transgenic and 3xTgAD) were maintained on the indicated diets (ad libitum, caloric restriction or intermittent fasting) for either 7 or 14 months. The open field activities (ambulatory counts and distance traveled) were quantified. Values are the mean and SEM ($n=17-20$ mice per group). $^+p<0.01$ compared to the corresponding non-transgenic AL value. $*p<0.02$ compared to the corresponding 3xTgAD AL value.

($p<0.001$). There were no significant differences in goal latency times among the four groups of mice. The distance traveled by the mice to reach the platform (path length) decreased across days ($F_{(6,511)}=18.553$, $p<0.0001$), and there was also a significant day \times group interaction ($F_{(21,511)}=2.152$, $p<0.002$) indicating differences among groups within each trial day. There was a significant difference between groups ($F_{(3,73)}=13.983$, $p<0.01$); post hoc analysis showed that the mice in the 3xTgAD,AL group swam longer path lengths as compared to the nonTg,AL group ($p<0.01$). 3xTgAD mice in the CR ($p<0.05$) and IF ($p<0.04$) groups swam significantly shorter path lengths as compared to the 3xTgAD,AL group indicating beneficial effects of the CR and IF diets in the 3xTgAD mice. Analysis of swim speed data in 10-month-old mice showed that there was a progressive increase in swim speed across days during the first 7 days of testing in all groups, and no significant differences in swim speeds among groups (Supplementary Fig. 1a).

On the platform removal in the probe test, there was a general tendency of the mice to swim preferentially in the target quadrant as opposed to the other quadrants (Table 1). ANOVA showed significant differences between the groups (group effect, $F_{(3,73)}=$

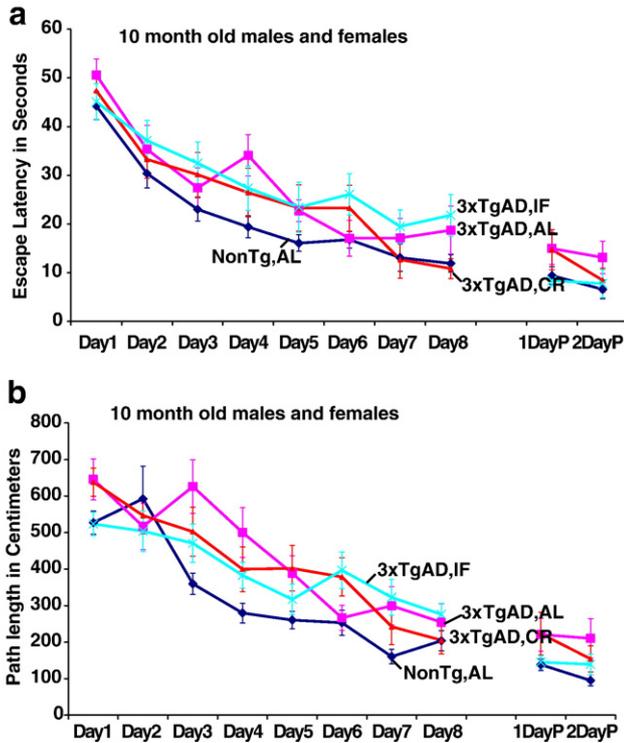


Fig. 2. Middle-age 3xTgAD mice exhibit modest deficits in performance in the water maze that is significantly ameliorated by caloric restriction. Male and female mice of the indicated genotypes (non-transgenic and 3xTgAD) were maintained on the indicated diets (AL, CR or IF) for 7 months. The goal latency and path length were measured in the hidden platform test. Values are the mean and SEM ($n=17-20$ mice per group). 1DayP and 2DayP, first and second days of the visible platform test.

3.532, $p<0.02$); post hoc analysis showed that 3xTgAD,CR mice spent significantly more time in target quadrant as compared to 3xTgAD,AL. Analysis of path length in the target quadrant revealed

significant differences between the groups (group effect, $F_{(3,73)}=3.423$, $p<0.03$); post hoc analysis showed that 3xTgAD,CR mice group traversed more distance in quadrant 3 compared to 3xTgAD,AL mice.

After 14 months on the diets, 3xTgAD male and female mice in all three diet groups improved their performance in the hidden platform test as indicated by a decrease in escape latencies across successive days (day effect, $F_{(7,413)}=34.674$, $p<0.0001$) with no day \times group interaction ($F_{(21,413)}=1.080$, $p>0.37$) (Figs. 3 and 4). However, there was a significant main effect of group ($F_{(3,59)}=8.273$, $p<0.0001$). Post hoc analysis showed that the nonTg,AL group had significantly faster escape latencies as compared to the 3xTgAD,AL group ($p<0.0001$). There was also a significant effect of sex in the 3xTgAD mice with female 3xTgAD,AL mice performing more poorly (longer escape latencies and path lengths) compared to male 3xTgAD,AL mice. Also, there were significant differences between the 3xTgAD,AL and the 3xTgAD,CR ($p<0.02$) and 3xTgAD,IF ($p<0.002$) groups (both males and females). The path length analysis showed that there was an overall decrease in the distance traveled to reach the platform from the start positions ($F_{(7,413)}=42.324$, $p<0.0001$), but there was no significant interaction between days and group (day \times group effect ($F_{(21,413)}=1.086$, $p<0.360$)). There was a significant group effect ($F_{(4,59)}=19.602$, $p<0.0001$). Post hoc analyses showed that the CR ($p<0.001$) and IF ($p<0.001$) groups (both males and females) had significantly shorter path lengths as compared to 3xTgAD,AL group. The swim speed data showed that there was a progressive increase in swim speed across trial days (day effect, $F_{(7,413)}=5.338$, $p<0.0001$), but there was no significant interaction between days and groups ($F_{(21,413)}=1.011$, $p>0.45$). However, there were significant differences between the groups (group effect, $F_{(3,59)}=2.705$, $p<0.05$); post hoc analysis showed that the 3xTgAD,IF male mice swam significantly faster than the 3xTgAD,AL male mice ($p<0.02$).

The removal of the platform from the target quadrant resulted in a general tendency to swim preferentially in the target quadrant as opposed to the other quadrants (groups effect, $F_{(3,59)}=5.881$, $p<0.001$). Further post hoc analysis revealed a significant difference in the time spent in the target quadrant between the

Table 1
Caloric restriction and intermittent fasting improve the performance of 3xTgAD mice in the water maze probe trial task

Groups	Percent of time spent in quadrant				Percent distance traveled in quadrant			
	Quad 1	Quad 2	Quad 3 target quad	Quad 4	Quad 1	Quad 2	Quad 3 target quad	Quad 4
<i>10 month-old mice</i>								
NonTg AL	19.8 \pm 2.0	23.51 \pm 3.1	37.5 \pm 2.3	19.4 \pm 1.5	19.8 \pm 1.8	19.7 \pm 1.4	39.0 \pm 2.2	21.5 \pm 1.6
3xTgAD AL	27.3 \pm 4.2	21.6 \pm 2.0	31.9 \pm 3.1*	19.2 \pm 2.0	27.1 \pm 4.3 [†]	20.5 \pm 1.9	31.7 \pm 3.3*	20.7 \pm 2.3
3xTgAD CR	17.3 \pm 2.0	18.7 \pm 2.3 [#]	41.2 \pm 3.4 ⁺	22.8 \pm 2.9 ⁺	18.9 \pm 2.2 ^{###}	17.3 \pm 1.9 ^{###}	41.1 \pm 3.6 ⁺	22.7 \pm 2.6
3xTgAD IF	21.9 \pm 2.5	18.3 \pm 2.7 [#]	29.6 \pm 3.1	30.2 \pm 3.7 ⁺	25.3 \pm 2.9	17.0 \pm 2.7	28.5 \pm 3.3	29.2 \pm 3.8 ⁺
<i>7 month-old mice</i>								
NonTg AL	26.8 \pm 2.2	18.1 \pm 1.6	33.4 \pm 2.3	21.0 \pm 2.1	25.0 \pm 2.0	23.0 \pm 3.4	32.0 \pm 2.4	20.6 \pm 1.6
3xTgAD AL	28.7 \pm 2.1	18.2 \pm 1.6	29.9 \pm 2.0*	23.2 \pm 2.2	28.0 \pm 2.1	18.1 \pm 1.7	30.41 \pm 2.2	23.4 \pm 1.9 [†]
3xTgAD CR	21.2 \pm 2.1	20.6 \pm 3.7	38.3 \pm 2.3 ⁺	19.9 \pm 2.9 [#]	22.4 \pm 2.1	20.4 \pm 3.4	37.4 \pm 2.7 ⁺	19.8 \pm 2.7 [#]
3xTgAD IF	24.7 \pm 4.1	17.0 \pm 2.1	41.1 \pm 5.2 ⁺	14.0 \pm 2.0 [#]	25.1 \pm 4.0	17.6 \pm 1.8	41.7 \pm 5.1 ⁺	15.6 \pm 2.7 [#]

Non-transgenic and 3xTgAD mice were maintained on the indicated diets (AL, ad-libitum; CR, caloric restriction; IF, intermittent fasting) beginning at 3 months of age. One cohort of mice was tested in the water maze at 10 months of age (upper) and the other at 17 months of age (lower). The percent time spent in each quadrant, and the percent distance traveled in each quadrant, were measured in the probe trial. Values are the mean and SEM ($n=16-20$ mice per group; values for males and females were combined). * $p<0.01$ compared to NonTg AL, [†] $p<0.01$ compared to NonTg AL, ⁺ $p<0.001$ compared to the corresponding 3xTgAD AL, [#] $p<0.05$ compared to the corresponding 3xTgAD AL value, ^{###} $p<0.001$ compared to the corresponding 3xTgAD AL value.

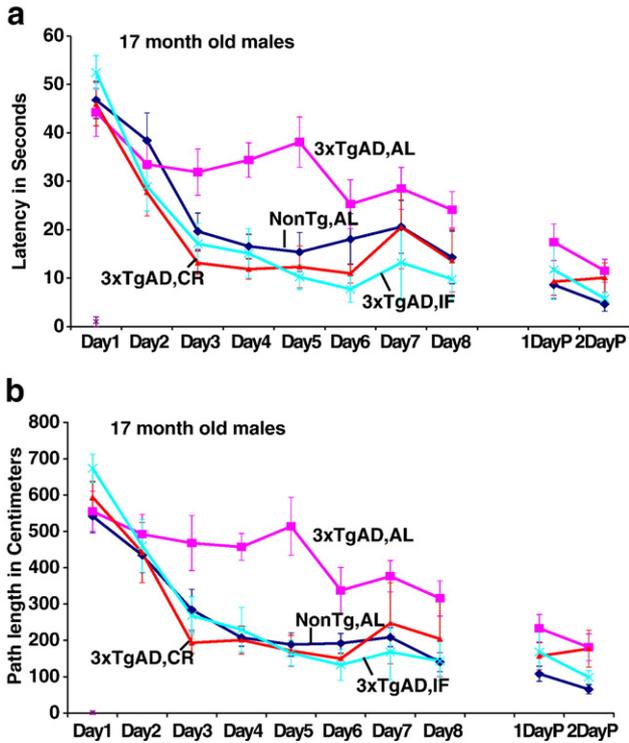


Fig. 3. Old male 3xTgAD mice exhibit a deficit in performance in the hidden platform test in the water maze that is ameliorated by caloric restriction and intermittent fasting. Male mice of the indicated genotypes (non-transgenic and 3xTgAD) were maintained on the indicated diets (ad libitum, caloric restriction or intermittent fasting) for 14 months. The goal latency (a) and path length (b) were measured in the hidden platform test. Values are the mean and SEM ($n=7-10$ mice per group). 1DayP and 2DayP, first and second days of the visible platform test.

groups. CR and IF 3xTgAD groups spent significantly more time in target quadrant as compared to 3xTgAD,AL group (Table 1). Analysis of distance traveled in the target quadrant showed a significant difference between the groups (group effect, $F_{(3,59)}=4.398, p<0.008$). The IF and CR 3xTgAD groups had significantly longer path lengths in the target quadrant as compared to the 3xTgAD,AL group (Table 1). The results of the probe trial tests suggest that old 3xTgAD mice exhibit impaired memory retention compared to non-transgenic control mice and that both the CR and IF diets ameliorate this age-related memory deficit.

Caloric restriction, but not intermittent fasting, decreases Aβ and phospho-tau levels in the hippocampus of 3xTgAD mice

Previous studies of mouse models of AD have established associations between the amount of Aβ present in the hippocampus and deficits in spatial learning (Chapman et al., 1999; Chen et al., 2000; Hock et al., 2003). It was also reported that CR can reduce the accumulation of Aβ in the brains of APP mutant mice (Patel et al., 2005; Wang et al., 2005). We therefore employed a highly sensitive ELISA method to quantify levels of Aβ1–40 and Aβ1–42 in hippocampal tissues from 3xTgAD mice that had been maintained for 14 months on AL, CR or IF diets. The concentrations of Aβ1–40 and Aβ1–42 were significantly lower in the hippocampus of mice in the CR group compared to the AL and IF groups (Fig. 5). One way ANOVA showed that there was a

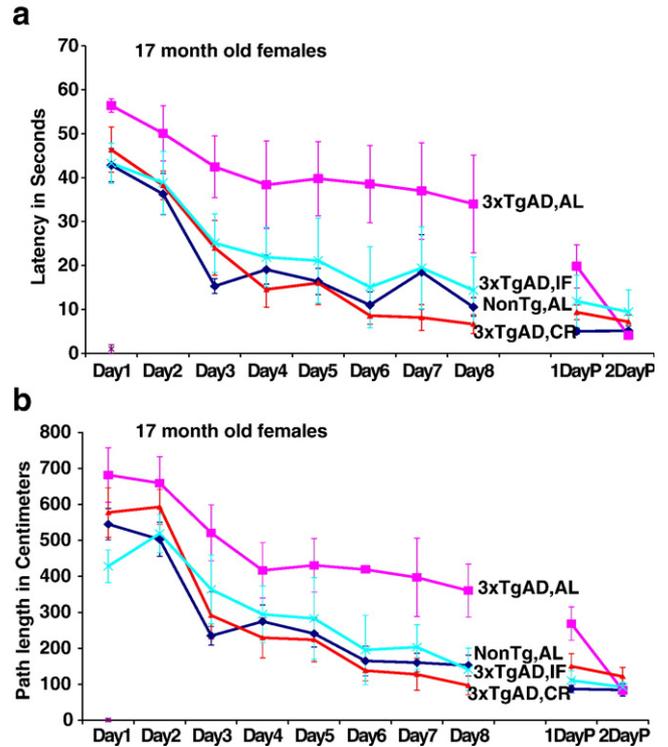


Fig. 4. Old female 3xTgAD mice exhibit a deficit in performance in the hidden platform test in the water maze that is ameliorated by caloric restriction and intermittent fasting. Seventeen-month-old female mice of the indicated genotypes (non-transgenic and 3xTgAD) were maintained on the indicated diets (ad libitum, caloric restriction or intermittent fasting) for 14 months. The goal latency (a) and path length (b) were measured in the hidden platform test. Values are the mean and SEM ($n=6-9$ mice per group). 1DayP and 2DayP, first and second days of the visible platform test.

significant difference between the groups (group effect, $F_{(2,32)}=3.161, p<0.05$); post hoc analysis showed that the CR group had significantly lower Aβ1–40 and Aβ1–42 levels as compared to the AL group (Aβ1–40, $p<0.05$; Aβ1–42, $p<0.03$). In contrast Aβ levels in the hippocampus of 3xTgAD,IF mice were not significantly different than Aβ levels in the hippocampus of 3xTgAD,AL mice.

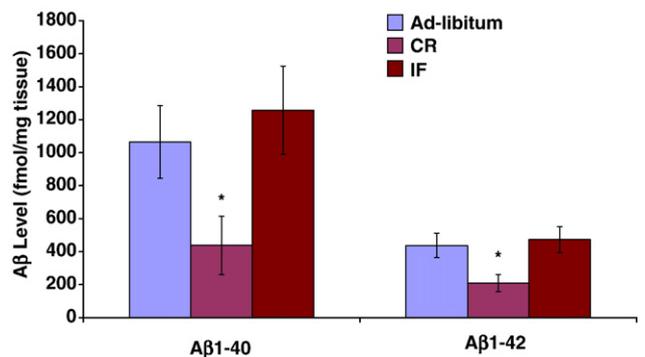


Fig. 5. Caloric restriction, but not intermittent fasting, reduces levels of Aβ1–40 and Aβ1–42 in the hippocampus of older 3xTgAD mice. Levels of Aβ1–40 and Aβ1–42 in hippocampal tissues from the indicated groups of mice were quantified by ELISA methods. Values are the mean and SEM ($n=13-19$). * $p<0.05$ compared to the AL and IF values.

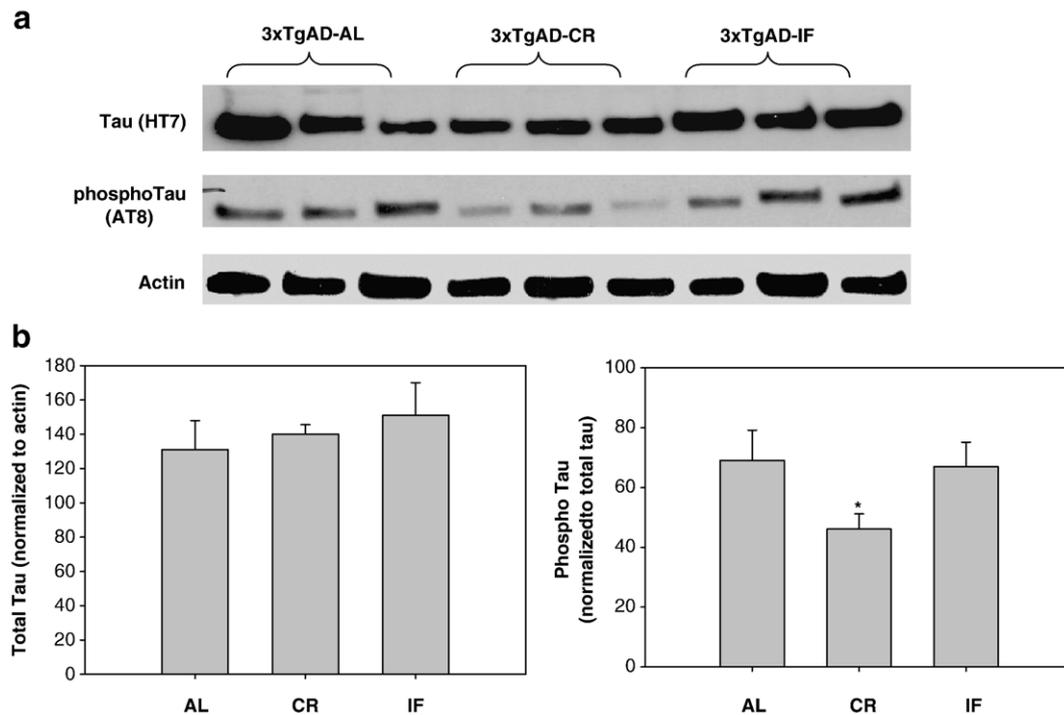


Fig. 6. Caloric restriction, but not intermittent fasting, suppresses tau pathology in the hippocampus of older 3xTgAD mice. Levels of total tau and phospho-tau were measured by immunoblot analysis using HT7 and AT8 antibodies, respectively, in hippocampal tissues from the indicated groups of mice. (a) Immunoblot probed with the indicated antibodies. (b) Results of densitometric analysis (values are the mean and SEM; $n=3-5$). * $p<0.05$ compared to the AL and IF values.

Levels of phosphorylated tau increase in an age-dependent manner in the hippocampus of 3xTgAD mice (Oddo et al., 2003a,b). To determine the effects of dietary energy restriction on the development of tau pathology, we measured levels of total tau and phospho-tau by immunoblot analysis in hippocampal tissue samples from 3xTgAD mice that had been maintained on AL, CR or IF diets for 14 months. Levels of total tau, measured using antibody HT7, were not different in samples from 3xTgAD mice on AL, CR and IF diets (Fig. 6). However, levels of phosphorylated tau, measured using antibody AT8, were significantly lower in samples from mice in group CR, compared to mice in either the AL or IF groups (Fig. 6).

Discussion

Our findings provide evidence that dietary energy restriction regimens can ameliorate learning and memory deficits in an animal model of AD. 3xTgAD mice fed ad libitum exhibited age-dependent impairment in performance in both acquisition (goal latency) and retention (probe trial) tasks in the Morris water maze compared to non-transgenic control mice. In contrast, 3xTgAD mice maintained on CR or IF diets for 14 months exhibited no deficits in water maze tasks, performing as well as non-transgenic mice. We found that levels of A β 1–40 and A β 1–42 were significantly lower in 3xTgAD mice on the CR diet compared to those on the ad libitum diet. The ability of CR to reduce A β levels in the 3xTgAD mice is consistent with two previous studies in which APP mutant mice on CR diets exhibited lower levels of A β in their brains (Patel et al., 2005; Wang et al., 2005). The mechanism whereby CR reduces A β levels is not known, but may involve reduced levels of oxidative stress because oxidative stress

enhances A β production and aggregation in mouse models (Pratico et al., 2001; McLellan et al., 2003), whereas CR reduces levels of oxidative stress in brain cells during normal aging (Sohal et al., 1994; Ugochukwu et al., 2006). Consistent with the latter mechanism, treatment of APP mutant mice with antioxidants reduces A β levels and ameliorates memory deficits (Lim et al., 2001; Quinn et al., 2006). On the other hand, diet-induced diabetes (which is known to increase oxidative stress) enhanced A β accumulation in a mouse model of AD (Ho et al., 2004).

Interestingly, we found that IF ameliorated behavioral deficits in the 3xTgAD mice, but did not affect levels of A β 1–40 or A β 1–42 in the hippocampus. Recent studies have documented beneficial effects of IF in animal models of Parkinson's disease (Duan and Mattson, 1999; Maswood et al., 2004), Huntington's disease (Duan et al., 2003) and stroke (Yu and Mattson, 1999). IF protects neurons against oxidative and excitotoxic and metabolic insults (Bruce-Keller et al., 1999). The neuroprotective mechanism of IF involves induction of a mild adaptive cellular stress response in which neurons upregulate the expression of neurotrophic factors and protein chaperones such as heat-shock protein 70 (Yu and Mattson, 1999; Lee et al., 2002). A β has been shown to impair synaptic function (Keller et al., 1997), and synapses from rats maintained on IF exhibit increased resistance to being damaged by A β (Guo and Mattson, 2000). It is therefore likely that IF preserves synaptic function in 3xTgAD mice even in the presence of levels of A β that impair synaptic function in mice fed ad libitum. One specific mechanism whereby IF may protect neurons and promote synaptic plasticity is by enhancing brain-derived neurotrophic factor (BDNF) signaling. Consistent with the latter possibility, mice maintained on IF exhibit increased levels of BDNF in multiple brain regions including the hippocampus (Duan et al., 2001; Lee et al., 2002), BDNF plays an important role in

hippocampal synaptic plasticity and learning and memory (Tyler et al., 2002; Lu, 2003) and BDNF can protect neurons from being damaged by various oxidative and metabolic insults (Spina et al., 1992; Duan et al., 2001).

Several dietary and behavioral factors have been suggested to modify the risk of AD. In addition to positive associations of high calorie intakes and diabetes with AD risk (Luchsinger et al., 2002; Launer, 2005), low levels of exercise (Laurin et al., 2001; Larson et al., 2006) and low levels of cognitive stimulation (Wilson et al., 2002) may increase the risk of AD. Studies in transgenic mouse models of AD have demonstrated ameliorative effects of exercise and environmental enrichment on amyloid pathology and cognitive function (Adlard et al., 2005; Jankowsky et al., 2005; Lazarov et al., 2005). Our findings provide direct evidence that long-term dietary energy restriction, either CR or IF, can ameliorate age-related impairments in learning and memory tasks. The differential effects of CR and IF on A β accumulation in the hippocampus suggest that dietary factors can suppress the disease process either upstream or downstream of A β pathology. The dissociation between A β and performance in the water maze task, evident in 3xTgAD,IF mice, also provides a possible explanation for the fact that some humans can tolerate a high A β load in their brains without cognitive impairment (Mufson et al., 1999; Giannakopoulos et al., 2003).

There are strong associations between tau pathology (tangles and levels of phosphorylated tau) and cognitive function in human subjects with AD or mild cognitive impairment (Nagy et al., 1995; Fellgiebel et al., 2004). However, there are human subjects with a relatively high amounts of tau pathology that perform well on cognitive tests. Strong evidence that tau pathology is not sufficient to cause cognitive decline comes from a study of transgenic mice expressing a repressible human tau variant; after suppression of the transgenic tau, memory function recovered (Santacruz et al., 2005). Studies of another mouse model of tauopathy have shown that some neurons degenerate before neurofibrillary lesions develop, whereas other neurons exhibit neurofibrillary pathology, but do not degenerate (Spires et al., 2006). Moreover, studies of transgenic mouse model with conditional overexpression of glycogen synthase kinase-3 (a kinase believed to contribute to hyperphosphorylation of tau in AD) have shown that tau hyperphosphorylation can be reversed (Engel et al., 2006). In the present study we found that 3xTgAD mice maintained on the IF diet exhibited levels of phosphorylated tau similar to 3xTgAD mice on the AL diet, and yet the IF diet improved performance of the mice in the behavioral tasks. Although we did not establish the mechanism by which hippocampal neurons in 3xTgAD mice on the IF diet are able to function well despite considerable tau pathology, we do know that the IF diet increases the resistance of neurons to a range of adverse conditions including oxidative and metabolic stress and excitotoxicity (Bruce-Keller et al., 1999; Yu and Mattson, 1999; Duan et al., 2001). Our findings in the 3xTgAD mice are consistent with the possibility that IF results in changes in neurons that increase their resistance to adverse effects of A β and tau.

Increasing evidence from studies of human populations suggests that overeating and diabetes increase the risk of AD (Ott et al., 1999; Janson et al., 2004; Luchsinger et al., 2004; Xu et al., 2004; Launer, 2005), whereas low calorie diets may reduce disease risk (Luchsinger et al., 2002; Gustafson et al., 2003). This influence of dietary energy intake appears to be exerted on fundamental aspects of the disease process, either accelerating or

retarding A β production and oxidative stress. A diabetogenic diet resulted in increased γ -secretase activity, production of A β 1–40 and A β 1–42 and amyloid plaque burden in APP mutant mice (Ho et al., 2004). IF and CR diets exert anti-diabetic effects in mice resulting in reduced levels of insulin and increased insulin sensitivity (Anson et al., 2003). It was reported that hyperinsulinemia results in increased levels of A β and inflammatory cytokines which have deleterious effects on memory (Craft, 2005). We found that a diabetogenic diet worsens performance of 3xTgAD mice in water maze tasks (V. Halagappa and M. P. Mattson, unpublished data) suggesting an adverse effect of high energy diets on synaptic plasticity and cognitive function. Collectively, the available data suggest that long-term reductions in energy intake during adult life may protect the brain against diseases of aging and should be considered, together with exercise and cognitive enrichment, as an approach for reducing the risk of AD.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nbd.2006.12.019.

References

- Adlard, P.A., Perreau, V.M., Pop, V., Cotman, C.W., 2005. Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *J. Neurosci.* 25, 4217–4221.
- Anson, R.M., Guo, Z., de Cabo, R., Iyun, T., Rios, M., Hagepanos, A., Ingram, D.K., Lane, M.A., Mattson, M.P., 2003. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6216–6220.
- Ashe, K.H., 2001. Learning and memory in transgenic mice modeling Alzheimer's disease. *Learn. Mem.* 8, 301–308.
- Billings, L.M., Oddo, S., Green, K.N., McLaugh, J.L., Laferla, F.M., 2005. Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron* 45, 675–688.
- Bruce-Keller, A.J., Umberger, G., McFall, R., Mattson, M.P., 1999. Food restriction reduces brain damage and improves behavioral outcome following excitotoxic and metabolic insults. *Ann. Neurol.* 45, 8–15.
- Chapman, P.F., White, G.L., Jones, M.W., Cooper-Blacketer, D., Marshall, V.J., Irizarry, M., Younkin, L., Good, M.A., Bliss, T.V., Hyman, B.T., Younkin, S.G., Hsiao, K.K., 1999. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat. Neurosci.* 2, 271–276.
- Chen, G., Chen, K.S., Knox, J., Inglis, J., Bernard, A., Martin, S.J., Justice, A., McConlogue, L., Games, D., Freedman, S.B., Morris, R.G., 2000. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* 408, 975–979.
- Craft, S., 2005. Insulin resistance syndrome and Alzheimer's disease: age- and obesity-related effects on memory, amyloid, and inflammation. *Neurobiol. Aging* 26, S65–S69.

- Duan, W., Mattson, M.P., 1999. Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *J. Neurosci. Res.* 57, 195–206.
- Duan, W., Guo, Z., Mattson, M.P., 2001. Brain-derived neurotrophic factor mediates an excitoprotective effect of dietary restriction in mice. *J. Neurochem.* 76, 619–626.
- Duan, W., Guo, Z., Jiang, H., Ware, M., Li, X.J., Mattson, M.P., 2003. Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression, and increases survival in huntingtin mutant mice. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2911–2916.
- Engel, T., Hernandez, F., Avila, J., Lucas, J.J., 2006. Full reversal of Alzheimer's disease-like phenotype in a mouse model with conditional overexpression of glycogen synthase kinase-3. *J. Neurosci.* 26, 5083–5090.
- Fellgiebel, A., Siessmeier, T., Schuerich, A., Winterer, G., Bartenstein, P., Schmidt, L.G., Muller, M.J., 2004. Association of elevated phospho-tau levels with Alzheimer-typical 18F-fluoro-2-deoxy-D-glucose positron emission tomography findings in patients with mild cognitive impairment. *Biol. Psychiatry* 56, 279–283.
- Giannakopoulos, P., Herrmann, F.R., Bussiere, T., Bouras, C., Kovari, E., Perl, D.P., Morrison, J.H., Gold, G., Hof, P.R., 2003. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology* 60, 1495–1500.
- Guo, Z.H., Mattson, M.P., 2000. 2-Deoxyglucose administration preserves glucose and glutamate transport and mitochondrial function in cortical synaptic terminals after exposure to amyloid beta-peptide and iron: evidence for a stress response. *Exp. Neurol.* 166, 173–179.
- Gustafson, D., Rothenberg, E., Blennow, K., Steen, B., Skoog, I., 2003. An 18-year follow-up of overweight and risk of Alzheimer disease. *Arch. Intern. Med.* 163, 1524–1528.
- Ho, L., Qin, W., Pompl, P.N., Xiang, Z., Wang, J., Zhao, Z., Peng, Y., Cambareri, G., Rocher, A., Mobbs, C.V., Hof, P.R., Pasinetti, G.M., 2004. Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. *FASEB J.* 18, 902–904.
- Hock, C., Konietzko, U., Streffer, J.R., Tracy, J., Signorell, A., Muller-Tillmanns, B., Lemke, U., Henke, K., Moritz, E., Garcia, E., Wollmer, M.A., Umbricht, D., de Quervain, D.J., Hofmann, M., Maddalena, A., Papassotiropoulos, A., Nitsch, R.M., 2003. Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. *Neuron* 38, 547–554.
- Horikoshi, Y., Sakaguchi, G., Becker, A.G., Gray, A.J., Duff, K., Aisen, P.S., Yamaguchi, H., Maeda, M., Kinoshita, N., Matsuoka, Y., 2004. Development of Abeta terminal end-specific antibodies and sensitive ELISA for Abeta variant. *Biochem. Biophys. Res. Commun.* 319, 733–737.
- Jacobsen, J.S., Wu, C.C., Redwine, J.M., Comery, T.A., Arias, R., Bowlby, M., Martone, R., Morrison, J.H., Pangalos, M.N., Reinhart, P.H., Bloom, F.E., 2006. Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 103, 5161–5166.
- Jankowsky, J.L., Melnikova, T., Fadale, D.J., Xu, G.M., Slunt, H.H., Gonzales, V., Younkin, L.H., Younkin, S.G., Borchelt, D.R., Savonenko, A.V., 2005. Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J. Neurosci.* 25, 5217–5224.
- Janson, J., Laedtke, T., Parisi, J.E., O'Brien, P., Petersen, R.C., Butler, P.C., 2004. Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes* 53, 474–481.
- Keller, J.N., Pang, Z., Geddes, J.W., Begley, J.G., Germeyer, A., Waeg, G., Mattson, M.P., 1997. Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. *J. Neurochem.* 69, 273–284.
- Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Karehot, J., Winblad, B., Helkala, E.L., Toumilehto, J., Soininen, H., Nissinen, A., 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch. Neurol.* 62, 1556–1560.
- Kobayashi, D.T., Chen, K.S., 2005. Behavioral phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease. *Genes Brain Behav.* 4, 173–196.
- Larson, E.B., Wang, L., Bowen, J.D., McCormick, W.C., Teri, L., Crane, P., Kukull, W., 2006. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Ann. Intern. Med.* 144, 73–81.
- Launer, L.J., 2005. Diabetes and brain aging: epidemiologic evidence. *Curr. Diabetes Rep.* 5, 59–63.
- Laurin, D., Verreault, R., Lindsay, J., MacPherson, K., Rockwood, K., 2001. Physical activity and risk of cognitive impairment and dementia in elderly persons. *Arch. Neurol.* 58, 498–504.
- Lazarov, O., Robinson, J., Tang, Y.P., Hairston, I.S., Korade-Mirmics, Z., Lee, V.M., Hersh, L.B., Sapolsky, R.M., Mirmics, K., Sisodia, S.S., 2005. Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell* 120, 701–713.
- Lee, J., Duan, W., Mattson, M.P., 2002. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J. Neurochem.* 82, 1367–1375.
- Lim, G.P., Chu, T., Yang, F., Beech, W., Frautschy, S.A., Cole, G.M., 2001. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J. Neurosci.* 21, 8370–8377.
- Luchsinger, J.A., Tang, M.X., Shea, S., Mayeux, R., 2002. Caloric intake and the risk of Alzheimer disease. *Arch. Neurol.* 59, 1258–1263.
- Luchsinger, J.A., Tang, M.X., Shea, S., Mayeux, R., 2004. Hyperinsulinemia and risk of Alzheimer disease. *Neurology* 63, 1187–1192.
- Lu, B., 2003. BDNF and activity-dependent synaptic modulation. *Learn. Mem.* 10, 86–98.
- Lyketsos, C.G., Lopez, O., Jones, B., Fitzpatrick, A.L., Breitner, J., DeKosky, S., 2002. Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. *JAMA* 288, 1475–1483.
- Maswood, N., Young, J., Tilmont, E., Zhang, Z., Gash, D.M., Gerhardt, G.A., Grondin, R., Roth, G.S., Mattison, J., Lane, M.A., Carson, R.E., Cohen, R.M., Mouton, P.R., Quigley, C., Mattson, M.P., Ingram, D.K., 2004. Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* 101, 18171–18176.
- Mattson, M.P., 2004. Pathways towards and away from Alzheimer's disease. *Nature* 430, 631–639.
- Mattson, M.P., 2005. Energy intake, meal frequency, and health: a neurobiological perspective. *Annu. Rev. Nutr.* 25, 237–260.
- McLellan, M.E., Kajdasz, S.T., Hyman, B.T., Bacskai, B.J., 2003. In vivo imaging of reactive oxygen species specifically associated with thioflavine S-positive amyloid plaques by multiphoton microscopy. *J. Neurosci.* 23, 2212–2217.
- Morgan, D., Diamond, D.M., Gottschall, P.E., Ugen, K.E., Dickey, C., Hardy, J., Duff, K., Jantzen, P., DiCarlo, G., Wilcock, D., Connor, K., Hatcher, J., Hope, C., Gordon, M., Arendash, G.W., 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408, 982–985.
- Mufson, E.J., Chen, E.Y., Cochran, E.J., Beckett, L.A., Bennett, D.A., Kordower, J.H., 1999. Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment. *Exp. Neurol.* 158, 469–490.
- Nagy, Z., Esiri, M.M., Jobst, K.A., Morris, J.H., King, E.M., McDonald, B., Litchfield, S., Smith, A., Barnettson, L., Smith, A.D., 1995. Relative roles of plaques and tangles in the dementia of Alzheimer's disease: correlations using three sets of neuropathological criteria. *Dementia* 6, 21–31.
- Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kaye, R., Metherate, R., Mattson, M.P., Akbari, Y., LaFerla, F.M., 2003a.

- Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 39, 409–421.
- Oddo, S., Caccamo, A., Kitazawa, M., Tseng, B.P., LaFerla, F.M., 2003b. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol. Aging* 24, 1063–1070.
- Ott, A., Stolk, R.P., vanHarskamp, F., Pols, H.A., Hofman, A., Breteler, M.M., 1999. Diabetes mellitus and the risk of dementia: the Rotterdam study. *Neurology* 53, 1937–1942.
- Patel, N.V., Gordon, M.N., Connor, K.E., Good, R.A., Engelman, R.W., Mason, J., Morgan, D.G., Morgan, T.E., Finch, C.E., 2005. Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. *Neurobiol. Aging* 26, 995–1000.
- Pratico, D., Uryu, K., Leight, S., Trojanowski, J.Q., Lee, V.M., 2001. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J. Neurosci.* 21, 4183–4187.
- Quinn, J.F., Bussiere, J.R., Hammond, R.S., Montine, T.J., Henson, E., Jones, R.E., Stackman, R.W., 2006. Chronic dietary alpha-lipoic acid reduces deficits in hippocampal memory of aged Tg2576 mice. *Neurobiol. Aging* (Jan 28; electronic publication ahead of print).
- Santacruz, K., Lewis, J., Spire, T., Paulson, J., Kotilinek, L., Ingelsson, M., Guimaraes, A., DeTure, M., Ramsden, M., McGowan, E., Forster, C., Yue, M., Orme, J., Janus, C., Mariash, A., Kuskowski, M., Hyman, B., Hutton, M., Ashe, K., 2005. Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 309, 476–481.
- Sohal, R.S., Agarwal, S., Candas, M., Forster, M.J., Lal, H., 1994. Effect of age and caloric restriction on DNA oxidative damage in different tissues of C57BL/6 mice. *Mech. Ageing Dev.* 76, 215–224.
- Spina, M.B., Squinto, S.P., Miller, J., Lindsay, R.M., Hyman, C., 1992. Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and *N*-methyl-4-phenylpyridinium ion toxicity: involvement of the glutathione system. *J. Neurochem.* 59, 99–106.
- Spires, T.L., Orme, J.D., SantaCruz, K., Pitstick, R., Carlson, G.A., Ashe, K.H., Hyman, B.T., 2006. Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse model of tauopathy. *Am. J. Pathol.* 168, 1598–1607.
- Steffens, D.C., Otey, E., Alexopoulos, G.S., Butters, M.A., Cuthbert, B., Ganguli, M., Geda, Y.E., Hendrie, H.C., Krishnan, R.R., Kumar, A., Lopez, O.L., Lyketsos, C.G., Mast, B.T., Morris, J.C., Norton, M.C., Peavy, G.M., Petersen, R.C., Reynolds, C.F., Salloway, S., Welsh-Bohmer, K.A., Yesavage, J., 2006. Perspectives on depression, mild cognitive impairment, and cognitive decline. *Arch. Gen. Psychiatry* 63, 130–138.
- Tyler, W.J., Alonso, M., Bramham, C.R., Pozzo-Miller, L.D., 2002. From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn. Mem.* 9, 224–237.
- Ugochukwu, N.H., Mukes, J.D., Figgers, C.L., 2006. Ameliorative effects of dietary caloric restriction on oxidative stress and inflammation in the brain of streptozotocin-induced diabetic rats. *Clin Chim Acta.* (Feb 28; electronic publication ahead of print).
- Wang, J., Ho, L., Qin, W., Rocher, A.B., Seror, I., Humala, N., Maniar, K., Dolios, G., Wang, R., Hof, P.R., Pasinetti, G.M., 2005. Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer's disease. *FASEB J.* 19, 659–661.
- Wilson, R.S., De Leon, C.F., Barnes, L.L., Schneider, J.A., Bienias, J.L., Evans, D.A., Bennett, D.A., 2002. Participation in cognitively stimulating activities and risk of incident Alzheimer disease. *JAMA* 287, 742–748.
- Xu, W.L., Qiu, C.X., Wahlin, A., Winblad, B., Fratiglioni, L., 2004. Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study. *Neurology* 63, 1181–1186.
- Yu, Z.F., Mattson, M.P., 1999. Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. *J. Neurosci. Res.* 57, 830–839.