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Research article

Lifelong vitamin E intake retards age-associated decline of spatial learning ability in apoE-deficient mice

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Abstract

The potential for lifelong vitamin E supplementation to delay age-associated cognitive decline was tested in apoE-deficient and wild-type C57BL/6 mice. Beginning at eight weeks of age, the mice were maintained on a control diet or diets supplemented with DL-α-tocopheryl acetate yielding approximate daily intakes of either 20 or 200 mg/kg body weight. When 6 or 18 months of age, cognitive functioning of the mice was assessed using swim maze and discriminated avoidance testing procedures. For the mice maintained on control diets, the age-related declines in swim maze performance were relatively larger in apoE-deficient mice when compared with wild-type. On the other hand, age-associated declines in learning and working memory for discriminated avoidance were similar in the two genotypes. The 200-mg/kg dose of vitamin E prevented the accelerated decline in spatial learning apparent in 18-month-old apoE-deficient mice, but had no equivalent effect on performance declines attributable to normal aging in the wild-type mice. Vitamin E supplementation failed to prevent age-related impairments in learning and memory for discriminated avoidance observed in both the wild-type and apoE-deficient mice. The current findings are consistent with the hypothesis that apoE deficiency confers an accelerated, though probably selective, loss of brain function with age. This loss of function would appear to involve pathogenic oxidative mechanisms that can be prevented or offset by antioxidant supplementation.

Introduction

Apolipoprotein E genotype has been associated with late-onset, familial and sporadic Alzheimer's disease (AD) and with an earlier progression of age-associated cognitive slowing in healthy individuals (Corder et al. 1993; Hyman et al. 1996; Reed et al. 1994; Roses 1996; Saunders et al. 1993; Yaffe et al. 2000). This susceptibility gene is found in three allelic variants: APOE2, APOE3 and APOE4. Inheritance of the APOE4 allele confers a gene dose-related increase in risk of clinical expression and earlier onset of AD (Corder et al. 1993; Saunders et al. 1993; Strittmatter et al. 1993) whereas APOE2 is associated with smaller risk and later onset (Corder et al. 1994).

In accordance with the link between APOE genotype and neurodegenerative disease, apoE appears to play an important role in a number of biological processes within the central nervous system (CNS). ApoE is thought to be particularly important in cellular maintenance and repair, and the allelic variants of apoE are associated with different degrees of neuroprotection and capacity for recovery from

CNS injury in a manner that accords with their degree of association with AD (Huang et al. 2004; Mahley and Rall 2000). ApoE-deficient mice, when compared to wild-type mice, exhibit age-dependent synaptic and dendritic neuropathologies (Masliah et al. 1995; Buttini et al. 1999), neurochemical alterations (Fisher et al. 1998; Gordon et al. 1995), inclusion body pathology (Veurink et al. 2003), age-dependent cognitive deficits (Gordon et al. 1995, 1996; Masliah et al. 1997; Oitzl et al. 1997; Gozes 2004; Veinbergs et al. 1999) and increased susceptibility to excitotoxicity (Buttini et al. 1999). APOE4 transgenic mice also exhibit cognitive deficits (Raber et al. 2000) and neuropathology (Buttini et al. 1999, 2000) when compared with APOE3 transgenic animals.

A variety of mechanisms are thought to be involved in the ability of apoE4 to affect expression and progress of neurodegenerative processes. One possibility is that apoE may influence the degree of oxidative stress/damage within the CNS. *In vitro* studies have indicated that apoE yields isoform-dependent protection against oxidative insult (Miyata and Smith 1996; Lee et al. 2004) and can influence nitric oxide production by microglia in an isoform-dependent manner (Colton et al. 2002, 2004). Moreover, when compared with wild-type mice, apoE-deficient mice exhibit increased protein and lipid oxidative damage in several brain regions, decreased endogenous antioxidants, and increased sensitivity to oxidative stress (Shea et al. 2002; Veinbergs et al. 2000; Choi et al. 2004; Matthews and Beal 1996; Ramassamy et al. 2001; Montine et al. 1999; Reich et al. 2001).

If the cognitive and neurological alterations associated with apoE deficiency involve an increase in oxidative stress and associated molecular damage, then experimental augmentation of antioxidant defense in animals lacking apoE could be expected to prevent the consequential decline in neuronal function as the animals age. The current studies addressed this hypothesis by maintaining apoE-deficient and wild-type mice on diets supplemented with different concentrations of the antioxidant vitamin E, and comparing the degrees of subsequent age-related cognitive impairment among the groups. Cognitive function in these groups of mice was assessed according to two different paradigms involving different dimensions of learning and memory performance. A spatial swim maze task was employed to measure ability of the mice to learn and remember the location of a hidden platform. It has been shown that performance on this task is dependent on cortical and hippocampal functions, and is negatively correlated with concentrations of protein oxidative damage (Forster et al. 1996; Nicolle et al. 2001). A discriminated avoidance test (Forster and Lal 1992) required the mice to rapidly identify and remember the correct arm of a T-maze, and to respond preemptively in order to avoid shock. This test was used to assess simple and reversal learning, and to test working memory capacity of the mice.

Materials and methods

Animals

The study began with 147 male, 4-week-old C57BL/6J mice and 162 homozygous for the *Apoe*^{tm1Unc} mutation (apoE-deficient mice) backcrossed 10 times to C57BL/6J mice, obtained from The Jackson Laboratory (Piedrahita et al. 1992). The Jackson Laboratory estimated that the N10 generation contained 99.91% of the genome from the C57BL/6J. Controls were C57BL/6J mice from the Jackson Laboratory production colony. Upon arrival at the University of North Texas Health Science Center (UNTHSC) vivarium, the mice were housed individually in $28 \times 19 \times 12.5$ cm solid bottom polycarbonate cages with wire tops, modified into two mouse units by insertion of a stainless steel divider. The colony room was maintained at 23 ± 1 °C/40% humidity, under a 12-h light–dark cycle with lights on at 0700 h. The mice had access to food and water, *ad libitum* at all times except during testing periods. Sentinels from the colony room tested serum negative for common pathogens during routine screening (Charles River Laboratories) performed at 3-month intervals throughout the study.

Vitamin E supplementation

Three weeks following their arrival in the UNTHSC vivarium, separate groups of 48–54 mice of each genotype, approximately eight weeks of age, were assigned to receive the control diet (NIH-31 open formula) or one of two DL-α-tocopheryl acetate (Vitamin E)-supplemented diets containing 10 (10×) or 100 (100×) times the concentration of vitamin E in

the control diet. The adjusted concentrations of the three diets compounded by Harlan Teklad (Madison, WI) equaled 16.5 (control), 165 or 1,650 mg DL-α-tocopheryl acetate per kg of food. Dietary intake was recorded in subsets of each genotype/diet group from two to five months of age (n = 11–14) and from 12 to 18 months of age (n = 22–29), in order to estimate the daily doses of DL-α-tocopheryl acetate and to determine if supplementation had any effect on food intake. Body weights of the mice were recorded weekly throughout the experiment. Concentrations of α-tocopherol following 100× supplementation with this test diet were increased ~3-fold in plasma and by 50–60% in the cerebral cortex of both young and old groups of C57BL6 mice after 12 weeks of dietary supplementation (Sumien et al. 2003, 2004). The supplementation was maintained throughout the experiment.

Each mouse received approximately six weeks of behavioral testing; two weeks for spatial discrimination in a swim maze followed by approximately four weeks of discriminated avoidance testing using a T-maze, beginning when they were either 6 or 18 months of age. Twenty-four mice from each of the six genotype/supplementation groups were tested when six months of age (after four months of supplementation) whereas the remaining mice were maintained in the colony until 18 months of age, at which time the survivors received the same test battery.

Spatial swim task

The apparatus used for this study consisted of a circular tank [110 cm (dia) × 60 cm deep], filled to a depth of 34 cm with 24 ± 2 °C tap water, colored opaque white (Forster et al. 1996). A 10-cm square platform was placed in the tank 1 cm below the surface of the water. The test consisted of a pretraining phase, in which the mice learned the simple response components of swimming and standing on the hidden platform, and a place discrimination acquisition phase in which the mice learned to locate the platform using spatial cues. Performance of the mice in the latter phase was recorded via a computerized tracking system (San Diego Instruments model # SA-3).

During the pretraining phase, a clear acrylic corridor (76 × 11 × 41 cm) leading directly to the platform was placed in the tank and a black curtain was draped around the tank to remove any visual cues. The mouse was placed at one end of the acrylic alley and allowed to swim to the end with the hidden platform. The mouse remained on the platform for 10 s and then was placed into a holding cage for an intertrial interval (ITI) of 3 min. This phase consisted of four sessions of five trials delivered over two days. The latency to reach the platform in seconds was recorded for each trial.

In the acquisition phase, each mouse was placed into the open tank (curtain and alley removed) from one of three starting positions and allowed to swim until it found the hidden platform up to a maximum time of 90 s. After 10 s on the platform, the mouse was removed and placed into a holding cage for an ITI of 10 min. Each acquisition session consisted of five trials with the platform in the same location (about 40 cm from the edge of the tank). This phase consisted of eight sessions, with two sessions conducted each day, separated by at least 3 h. The average length of the path taken to locate the platform over each of the five trials was the measure of spatial performance recorded during each session.

A sixth trial (a probe trial) was performed during acquisition sessions 4 and 7 in which the platform was lowered at the start of the trial and then raised after a period of 40 s. The performance measured on each probe trial was the proportion of time spent within a 20-cm diameter annulus from where the platform had been lowered, representing approximately 3% of the total area of the water surface. Greater than chance performance on this measure was considered to reflect the strength and accuracy of the spatial memory.

Discriminated avoidance task

A discriminated avoidance task described previously (Forster and Lal 1992; Forster et al. 1995; McDonald et al. 2005) was used to assess learning for simple avoidance and simple discrimination, discrimination reversal, learning of a response strategy, and delayed memory performance. The apparatus consisted of an acrylic T-shaped maze with compartments in the stem and each goal arm separated by doors. The maze rested on a grid floor wired to deliver 0.27 mA scrambled shock to the feet.

The discriminated avoidance task involved a series of daily test sessions (one per day for up to 30 days)

in which the mouse could avoid shock to the feet by running to a correct goal arm of the maze within 5 s from opening of the start door. Information as to which arm would be correct on a given session was presented during the first trial (an *information trial*) during which the mouse received shock in the incorrect arm of the maze and was permitted to escape to the correct arm. On the subsequent trials of each session, the mouse could avoid shock by running to the correct arm. In previous studies, both young and old mice learned the simple discrimination (choosing the correct arm) and avoidance (running to the correct goal within 5 s) components of the task on the first session, but required up to 30 or more sessions before they were able to efficiently use information presented only on the first trial of each session (a working memory requirement) to avoid all subsequent shocks (Forster and Lal 1992).

In the current study, each session consisted of an information trial (as described above) followed by a series of training trials that continued until the mouse made a correct avoidance on four of the last five (or a maximum of 25 trials had been reached). After the start door opened on each trial, shock was initiated 5 s later if the mouse had not entered the correct goal arm, or immediately if the mouse entered the incorrect goal arm. Shock was continued for a maximum of 60 s or until the correct goal arm was entered. After 10 s in the correct goal arm, the mouse was removed and placed into a holding cage for an ITI of 1 min. The daily training sessions continued until, during four consecutive sessions, the mouse had made a correct avoidance on the first trial following the information trial (designated as the *test trial*) and on at least three of the next four trials. Attainment of this criterion suggested that the mouse had acquired a stable response strategy (learning set) of remembering and acting on information presented in the first trial of each session.

After the learning set criterion had been met, an additional session was conducted in which the memory demand was increased by interposing a 7-min delay (as opposed to the 1-min ITI) between the information trial and the test trial. If the mouse failed to make a correct arm choice on the test trial of that session, the mouse received additional training sessions under the 1-min delay until the learning set criterion had been reconfirmed, followed by an additional test under the 7-min delay. This process was repeated until the mouse could make a correct arm choice after 7 min, or a maximum of 30 sessions had been conducted.

Four different components of performance in the discriminated avoidance task were analyzed in the current study: (a) Ability of the mice to learn the simple discrimination and avoidance components of the task was assessed by the number of trials to reach the four of five correct avoidance criterion on the first training session. (b) Ability of the mice to reverse their initial training bias was assessed in terms of trials to criterion on the second session, in which the correct choice was always opposite from the original training. (c) Learning of the response strategy (learning set) was assessed in terms of the total number of sessions required to reach the criterion. (d) Delayed memory performance was assessed in terms of the number of sessions required to meet the criterion for performance after the 7-min delay.

Data analysis

Data for each behavioral measure were considered in factorial analyses of variance with Genotype, Diet (vitamin E dose), and Age as between groups factors, and Sessions as a within groups factor where applicable. The effects of Diet on age-related functional declines were tested within the Age × Diet interaction of separate analyses of variance on each genotype, using planned individual comparisons between young and old age groups. For analysis of survival data, Kaplan–Meier survival distributions were calculated and a log-rank χ^2 statistic was used to compare survival of the genotypes under each diet.

Results

Body weight

When compared with C57BL/6J control mice, the apoE-deficient mice showed more rapid weight gain as a function of age and greater peak body weight (Figure 1). This overall pattern resulted in a significant main effect of Genotype, as well as a Genotype × Age interaction (all $P < 0.001$). The vitamin E supplementation failed to significantly affect weight of the genotypes across ages, as indicated by the absence of a main effect or any interactions involving Diet (all $P > 0.141$).

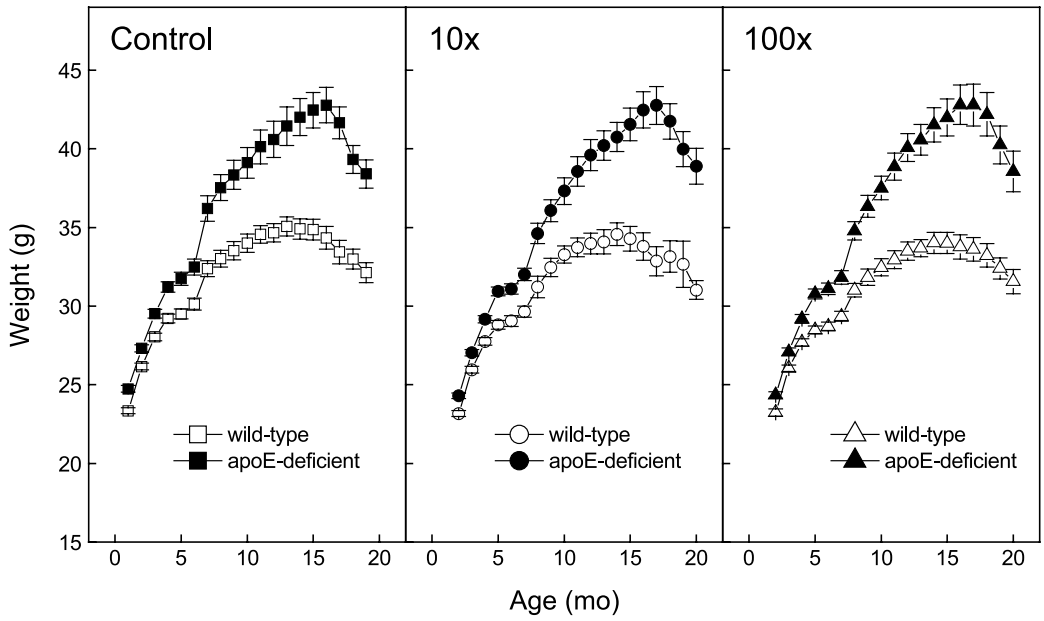

Figure 1. Effect of apolipoprotein E genotype and vitamin E supplementation (panels left to right) on body weight of mice as a function of age. Body weights were recorded at weekly intervals and averaged over 4-week periods. Each value represents the mean ± SE of 23–54 mice. (Approximately one half of the mice were euthanized between six and eight months following their behavioral testing, whereas survivors remained on study until 18 months).

Food intake

Dosage of α-tocopheryl acetate was calculated at different points across the life span based on the food intake and body weight of the mice (data not shown). Throughout the study, α-tocopheryl acetate intake (per kg body weight/day) for each genotype remained proportionately 10 and 100 times that of mice on the control diet. In both genotypes, intake of α-tocopheryl acetate was similar and tended to decline from 2 to 12 months ($P < 0.001$), primarily due to increases in weight without significant change in food intake. Intake of α-tocopheryl acetate/kg body weight was 30% higher in apoE-deficient mice when compared with the wild-type mice by 18 months ($P < 0.001$), primarily due to the relatively greater weight loss in apoE-deficient animals after 15 months of age. On a per mouse basis, the effect of genotype on α-tocopheryl acetate intake was negligible.

Survival

The relationship between the vitamin E dose and survival of each genotype is depicted in Figure 2. ApoE-deficient mice had significantly higher mortality overall

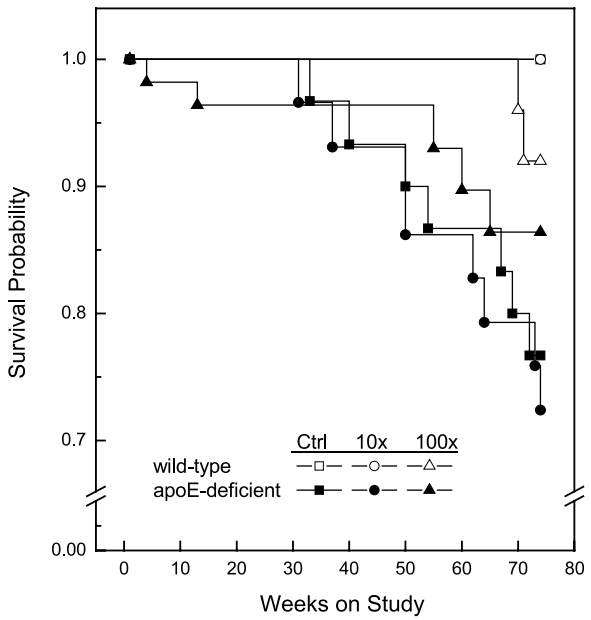

Figure 2. Effect of apolipoprotein E genotype and vitamin E supplementation on Kaplan–Meier probability of survival as a function of age. (Note broken ordinate scale).

than wild-type controls, $\chi^2(1, n = 308) = 13.664$, $P < 0.001$. The vitamin E-supplemented diets did not have a significant effect on survival for either wild-type or apoE-deficient mice, although there was a trend in the direction of increased survival in the apoE-deficient mice supplemented with 100 × vitamin E.

Spatial swim maze performance

Pretraining

Over the four sessions of pretraining in the straight alley, all age and treatment groups showed a progressive decrease in the latency to reach the platform. The 18-month-old groups showed longer latencies to reach the platform on the initial pretraining session when compared with the young groups, but none of the age, genotype or diet groups differed in latency by the fourth pretraining session. A four-way analysis of variance confirmed a significant Session x Age interaction ($P < 0.001$), but failed to indicate significant main effects or interactions involving Diet or Genotype ($P > 0.078$).

Path length

The ability of the mice to learn the spatial discrimination task was considered in analyses of variance on path length over sessions 1–4, whereas maximum spatial performance accuracy was assessed in analyses of sessions 5 through 8. The mice of all ages and genotypes learned to swim to the hidden platform, as indicated by their progressively shorter path lengths over the first four testing sessions. However, as suggested in Figure 3, both the initial level of performance and rate of improvement over these sessions varied as a function of both age and genotype. Without supplementation, spatial performance of both

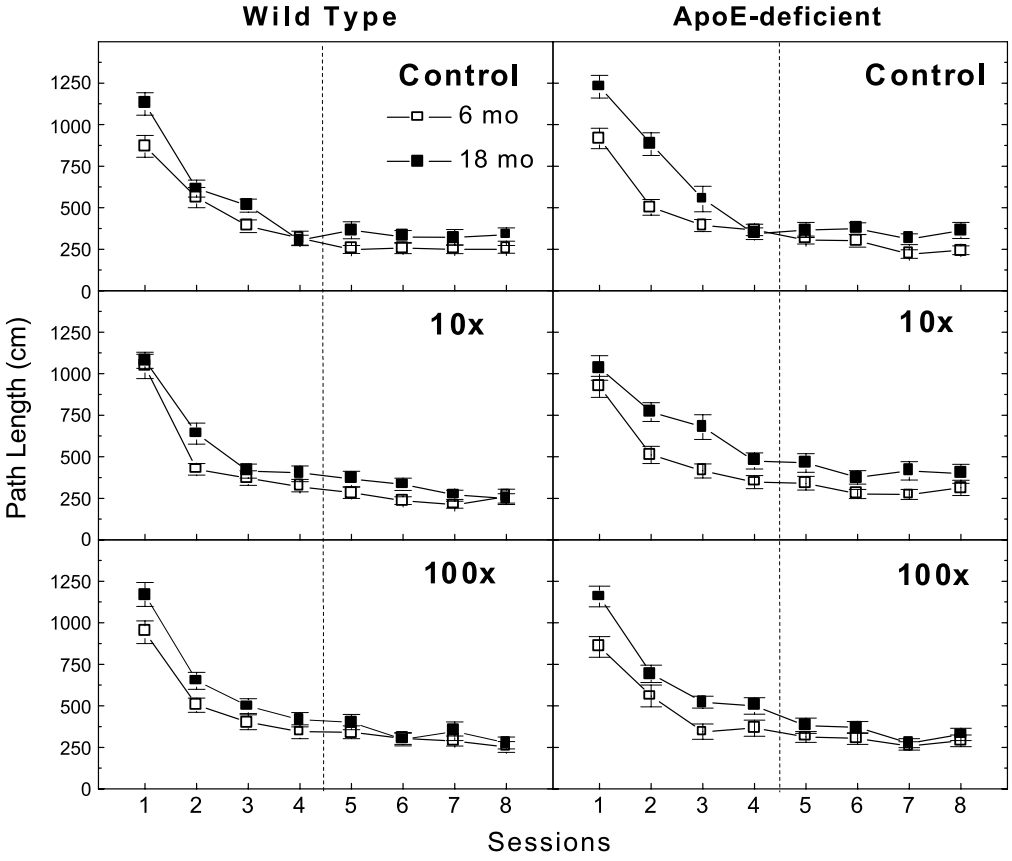

Figure 3. Learning of spatial discrimination as measured by distance traveled to reach a hidden platform (Path Length) within a swim maze for wild-type (left panel) and apoE-deficient mice (right panel). The panels from top to bottom show the effect of age under each diet condition. Each value represents the mean average path length ± SE for the five trials conducted within each session.

wild-type and apoE-deficient mice showed a decrement by 18 months of age, although this decrement was larger in the apoE-deficient mice (Figure 3, top right). Relative to diet-matched 6-month-olds, the spatial performance of 18-month-old apoE-deficient mice, but not wild-type mice, tended to improve with vitamin E supplementation. This effect was most evident for the 18-month-old apoE-deficient mice supplemented with the high (100×) dose of vitamin E (Figure 3, lower right panel). There was no apparent effect of diet or genotype on path length among the 6-month-old groups. In support of these general observations, a four-way analysis of variance conducted on path length for sessions 1–4 indicated significant effects of Age, Genotype and their two-way interaction (all $P < 0.021$), as well as a significant three-way interaction of Age, Diet, and Sessions ($P = 0.012$). Separate three-way analyses within each genotype suggested that the age-related effect of diet was attributable to the effect of vitamin E in the ApoE-deficient mice as opposed to the wild-type. Analysis for apoE-deficient mice indicated a significant three-way interaction of Sessions, Diet and Age ($P < 0.017$), whereas the analysis for wild-type failed to indicate significant main effects or interactions involving the Diet factor (all $P > 0.142$).

Eighteen-month-old, non-supplemented controls of both genotypes showed longer average path length over sessions 5 through 8 when compared with their young counterparts. This effect of age was also evident in mice maintained on the 10× vitamin E diet, but was diminished or absent in mice on the 100× diet. Analyses of variance within each genotype confirmed a significant effect of Age (all $P < 0.002$), but failed to suggest main effects or interactions involving Diet (all $P > 0.283$). When a separate analysis of variance was performed for each diet, with Genotype, Age and Sessions as the factors, the effect of Age was significant for mice receiving the control or 10× diets (all $P < 0.002$) but not for mice supplemented with the 100× diet ($P = 0.106$), confirming the observation from Figure 3.

Probe trial performance

The performance of the mice on probe trials conducted at the end of sessions 4 and 7 are shown in Figure 4 for wild-type and Figure 5 for apoE-deficient mice. All of the groups showed greater than chance percentage of time in the 20-cm annulus during both probe trials, suggesting a spatial bias of the mice for the platform position. Regardless of diet, young mice of both genotypes also showed an increase in % annulus time from session 4 to session 7. However, this increase was absent or reversed in 18-month-olds of both genotypes on the control diet, accounting for the greater % annulus time in the 6- *versus* 18-month-olds observed for session 7. The 100× vitamin E diet resulted in an improvement in probe trial performance of the 18-month-old apoE-deficient mice during this session, to a level nearly equivalent to that of diet-matched 6-month-olds. There was a similar, though smaller, trend to this effect for the 18-month-old wild-type mice maintained on either the 10× or 100× diet. When the session 7 data for % annulus time of the wild-type mice were analyzed using a two-way analysis of variance, only a significant main effect of Age ($P = 0.001$) was evident. On the other hand, analysis of session 7 for the apoE-deficient mice revealed a significant main effect of Age and a significant Age × Diet interaction ($P = 0.034$). Individual comparisons within the Age × Diet interactions confirmed significant age-related decreases in annulus time for apoE-deficient mice maintained on the control or 10× diet (all $P < 0.031$), but not for mice on the 100× diet ($P = 0.682$).

Discriminated avoidance learning and memory

The four components of performance analyzed in the discriminated avoidance task are shown in Figure 6

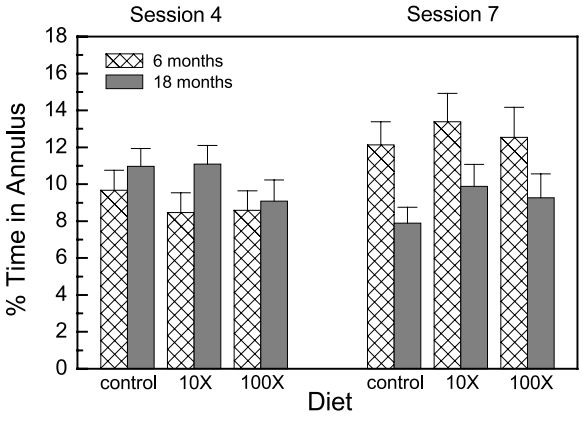

Figure 4. Performance of wild-type C57BL/6 mice on a probe trial conducted after the fourth (left) or seventh session (right) of spatial swim maze training as a function of age and vitamin E diet. Probe trial performance is expressed as the percentage of probe trial time ± SE spent in a 20-cm annulus over the center of the platform position after it had been lowered below the surface for a period of 40 s.

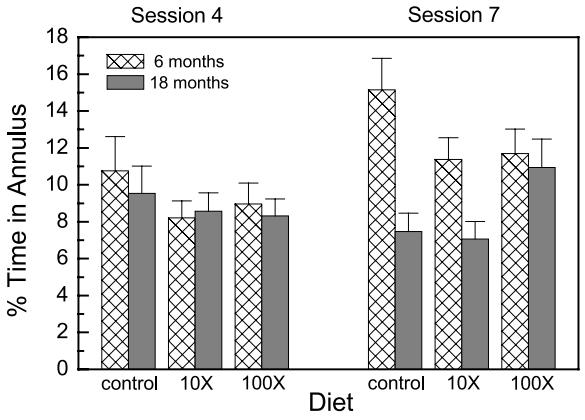

Figure 5. Performance of apoE-deficient C57BL/6 mice on a probe trial conducted after the fourth (left) or seventh session (right) of spatial swim maze training as a function of age and vitamin E diet. Probe trial performance is expressed as the percentage of probe trial time (± SE) spent in a 20-cm diameter annulus over the center of the platform position after it had been lowered below the surface for a period of 40 s.

for each genotype, diet and age group: Initial learning (A), learning of the first reversal (B), learning an efficient response strategy (learning set) (C) and delayed memory performance (D). Overall, the 18-month-old mice showed impaired performance in each component of the discriminated avoidance test, with little indication of a qualitative difference related to genotype. Additionally, there was no indication that vitamin E supplementation affected performance of either genotype. Analyses of variance on initial learning and learning set each revealed a significant main effect of age (all $P < 0.001$) and additionally, a main effect of genotype (all $P < 0.001$) due to the somewhat better performance in both age groups of the apoE-deficient mice when compared to the wild-type. However, the Genotype × Age interactions for these measures were nonsignificant (all $P > 0.309$) as were all effects/interactions involving diet (all $P > 0.145$). Separate analyses revealed significant main

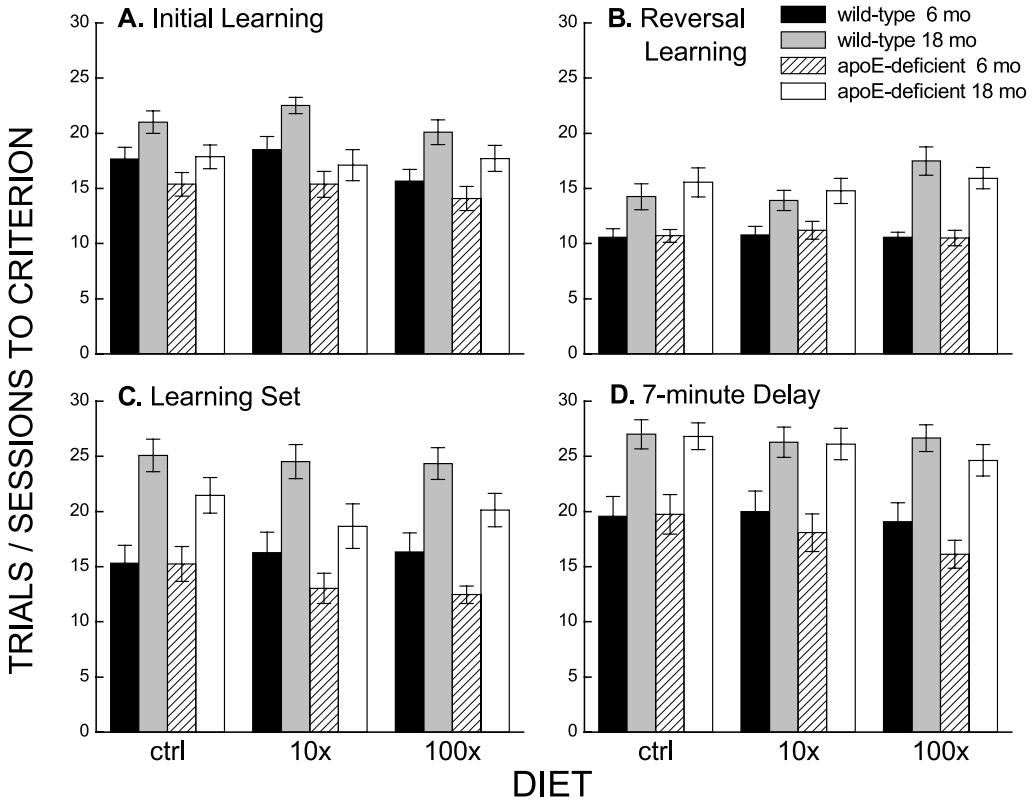

Figure 6. Effect of age, genotype and diet on performance components (± SE) of the discriminated avoidance task: (A) The mean number of trials to reach the avoidance learning criterion during the initial testing session (Initial Learning). (B) The mean number of trials to reach the avoidance learning criterion during the second session in which the mice learned to turn in a direction opposite that trained on the previous day (Reversal Learning). (C) The mean number of sessions required for learning of a stable response strategy (Learning Set) requiring working memory. (D) The mean number of sessions required for efficient performance after a 7-min delay.

effects of age within each genotype (all $P < 0.007$), confirming the observation that, overall, a decline in performance with age occurred in both genotypes. Analyses of variance on reversal learning (Figure 6B) and performance involving 7-min delay (Figure 6D) revealed only significant main effects of age (all $P < 0.001$), whereas all other effects and interactions were non-significant (all $P > 0.096$).

Discussion

The main findings of this study were: (1) In C57BL/6 mice, apoE deficiency was associated with an accelerated mortality rate and a cognitive deficit involving impaired spatial maze learning; (2) ApoE deficiency did not affect the age-related impairment in simple avoidance learning or working memory in these mice; (3) lifelong dietary supplementation of the antioxidant vitamin E ameliorated the accelerated age-associated deficits in spatial memory associated with apoE deficiency, but failed to affect age-impaired avoidance learning or working memory.

The present results clearly indicate that wild-type C57BL/6 mice, when fed control diets, exhibit a deleterious effect of age in several different measures of cognitive performance, in accordance with findings of previous studies (Forster et al. 1996; Forster and Lal 1992; Sumien et al. 2004). These effects involved a slower initial learning of spatial discrimination in older mice, as well as a decreased maximum efficiency in spatial navigation after initial learning. In the discriminated active avoidance task, older mice also showed an impaired initial learning, as well as a decreased ability to learn a new response once the initial response had been learned. Furthermore, the older mice required more training before adopting an efficient response strategy requiring working memory and, once learned, showed more difficulty performing under conditions of increased memory demand. When age-matched apoE-deficient mice were compared with wild-type for these age-sensitive aspects of cognitive performance, they showed markedly greater age-related impairments in initial learning of spatial discrimination, but did not differ from wild-type in any of the other dimensions of age-related cognitive decline that were tested. While the observation of accelerated deficits in spatial swim maze performance of apoE-deficient mice is consistent with previous literature (Gordon et al. 1995, 1996; Masliah et al. 1997; Oitzl et al. 1997; Veinbergs et al. 1999, 2000; Grootendorst et al. 2001), the current studies suggest that the accelerated decline does not extend to several other dimensions of learning and memory performance. Thus, brain processes targeted by apoE deficiency could be relatively selective when compared with those involved in normal brain aging.

The observed increase in mortality of apoE-deficient mice may be related to atherosclerosis, which could also affect the observed changes in cognitive performance secondary to vascular disease in the brain or perhaps deficits of motor function from peripheral vascular disease. The increased mortality could also represent accelerated aging in various organ systems independent of cognitive abilities, suggesting the possibility that impairments in learning and memory could be a result of sensory or motor deficits in this genotype. This explanation is unlikely based on the results of a previous study in which apo-E deficiency did not affect the age-associated losses detected in a variety of tests for sensory and psychomotor function (McDonald et al. 1997). Furthermore, there was no effect of genotype or diet on path-independent swim speed during pretraining or learning phases of the water maze test in the current study, suggesting that obvious changes in motor performance were not likely responsible for deficits in spatial performance. Although the visual capacity of the apoE-deficient mice was not addressed in the current study, a previous investigation failed to indicate a difference in visible platform performance attributable to apo-E deficiency (Veinbergs et al. 2000).

To the extent that apoE can influence oxidant production (Colton et al. 2002) and antioxidant defenses (Miyata and Smith 1996; Lomnitski et al. 1999), increased oxidative stress represents one possible mechanism for the accelerated cognitive impairment in apoE-deficient mice. This hypothesis is consistent with reported increases in markers of oxidative stress/damage in the brains of apoE-deficient mice relative to age-matched controls, including increases in products of lipid peroxidation (Veinbergs et al. 2000; Montine et al. 1999; Pratico et al. 1998), increased 3-nitrotyrosine (Matthews and Beal 1996), and increased carbonylation of protein (Choi et al. 2004). Moreover, the association of apoE deficiency with spatial maze performance, a behavior thought to be dependent on hippocampal function, is consistent with the regional pattern of protein oxidation reported

by Choi et al. (2004), who observed a one-fold increase in total protein oxidation primarily in the hippocampus of young apoE-deficient mice when compared with wild-type. It is noteworthy that two of the proteins showing increased carbonylation in the hippocampus of the apoE-deficient mice, creatine kinase and dihydropyrimidase-related protein 2, are also targets of oxidative damage in patients with Alzheimer's disease (Castegna et al. 2002a, b).

The responsiveness of apoE-deficient mice to dietary supplementation with vitamin E, observed in the current and previous studies (Veinbergs et al. 2000), adds additional support for the involvement of oxidative stress/damage in the neurological alterations and cognitive impairment in apoE-deficient mice. In the current studies, vitamin E supplementation appeared to prevent the accelerated decline in learning of the spatial discrimination in the swim task in these mice, but seemed to be less effective in preventing deficits attributable to normal aging in either of the genotypes. In the swim maze task, vitamin E supplementation did not clearly affect the age-related deficits in initial learning exhibited by the wild-type mice. Moreover, while vitamin E supplementation improved performance of the aged apoE-deficient mice, it did not improve performance beyond that of the age-matched, wild-type controls. In the discriminated avoidance task, vitamin E supplementation clearly did not affect age-related cognitive deficits in either genotype.

Whereas the current findings suggest that neurological deficits associated with apoE deficiency are responsive to vitamin E supplementation, they provide no direct evidence that prevention of these deficits involves an attenuation of oxidative damage. In a previous study, it was observed that vitamin E supplementation implemented in aged wild-type (C57BL/6) mice failed to reverse deficits in cognitive or psychomotor function, and did not attenuate age-associated protein or lipid oxidative damage (Sumien et al. 2004). The modest effect of long-term vitamin E on behavior of wild-type mice in the current studies may well reflect a similar failure to affect accumulation of oxidative damage. Veinbergs et al. (2000) reported that vitamin E supplementation attenuated lipid oxidation in apoE-deficient mice, but not in wild-type animals, a finding that is consistent with the current behavioral results.

The modest effect of vitamin E on cognitive decline in wild-type mice is somewhat inconsistent with previous literature suggesting beneficial effects following long-term supplementation (Joseph et al. 1998). It is unlikely that insufficient dosage was responsible for the modest effects in wild-type mice, given that the 200 mg/kg/day dose used was relatively high. Based on a dose response study reported in the literature (Martin et al. 1999), this dose was nearly two times the amount needed to achieve maximal increases in brain α-tocopherol concentration. In previous studies of both young and old C57BL/6 mice, supplementation at this level yielded a 30% increase in the whole brain synaptosomal α-tocopherol content (Lass et al. 1999), as well as a 50%–60% increase in homogenates of cerebral cortex (Sumien et al. 2004). It seems possible that the effect of vitamin E supplementation was more easily observed in the apoE-deficient mice when compared with the wild-type, based upon a more advanced progression of spatial performance decline in those mice at the ages tested in the current study. Thus, the effectiveness of vitamin E supplementation in wild-type mice may not be fully evident until these mice reach more advanced ages. It is worth noting in this regard, that there was a consistent trend toward beneficial effects of vitamin E supplementation on spatial performance in the wild-type mice.

In summary, the current findings are consistent with the hypothesis that apoE deficiency confers an accelerated, though probably selective, loss of brain function with age. This loss of function would appear to involve pathogenic oxidative mechanisms that can be offset by lifelong supplementation with vitamin E.

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References

Buttini M, Orth M, Bellosta S, Akeefe H, Pitas RE and Wyss-Coray T et al. (1999) Expression of human apolipoprotein E3 or E4 in the brains of Apoe−/− mice: isoform-specific effects on neurodegeneration. J Neurosci 19: 4867–4880

Buttini M, Akeefe H, Lin C, Mahley RW, Pitas RE and Wyss-Coray T et al. (2000) Dominant negative effects of apolipopro-

tein E4 revealed in transgenic models of neurodegenerative disease. Neuroscience 97: 207–210

Castegna A, Aksenov M, Aksenova M, Thongboonkerd V, Klein JB and Pierce WM et al. (2002a) Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part I. Creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. Free Radic Biol Med 33: 562–571

Castegna A, Aksenov M, Thongboonkerd V, Klein JB, Pierce WM and Booze R et al. (2002b) Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II. Dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. J Neurochem 82: 1524–1532

Choi J, Forster MJ, McDonald SR, Weintraub ST, Carroll CA and Gracy RW (2004) Proteomic identification of specific oxidized proteins in ApoE-knockout mice: relevance to Alzheimer's disease. Free Radic Biol Med 36: 1155–1162

Colton CA, Brown CM, Cook D, Needham LK, Xu Q and Czapiga M et al. (2002) APOE and the regulation of microglial nitric oxide production: a link between genetic risk and oxidative stress. Neurobiol Aging 23: 777–785

Colton CA, Needham LK, Brown C, Cook D, Rasheed K and Burke JR et al. (2004) APOE genotype-specific differences in human and mouse macrophage nitric oxide production. J Neuroimmunol 147: 62–67

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC and Small GW et al. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261: 921–923

Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE and Gaskell PC Jr et al. (1994) Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 7: 180–184

Fisher A, Brandeis R, Chapman S, Pittel Z and Michaelson DM (1998) M1 muscarinic agonist treatment reverses cognitive and cholinergic impairments of apolipoprotein E-deficient mice. J Neurochem 70: 1991–1997

Forster MJ and Lal H (1992) Within-subject behavioral analysis of recent memory in aging mice. Behav Pharmacol 3: 337–349

Forster MJ, Prather PL, Patel SR and Lal H (1995) The benzodiazepine receptor inverse agonist RO 15-3505 reverses recent memory deficits in aged mice. Pharmacol Biochem Behav 51: 557–560

Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H and Sohal RS (1996) Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Proc Natl Acad Sci USA 93: 4765–4769

Gordon I, Grauer E, Genis I, Sehayek E and Michaelson DM (1995) Memory deficits and cholinergic impairments in apolipoprotein E-deficient mice. Neurosci Lett 199: 1–4

Gordon I, Genis I, Grauer E, Sehayek E and Michaelson DM (1996) Biochemical and cognitive studies of apolipoprotein-E-deficient mice. Mol Chem Neuropathol 28: 97–103

Gozes I (2004) Apolipoprotein E knockout mice as a model of behavioral dysfunction. J Mol Neurosci 23: 149–150

Grootendorst J, de Kloet ER, Dalm S and Oitzl MS (2001) Reversal of cognitive deficit of apolipoprotein E knockout mice after repeated exposure to a common environmental experience. Neuroscience 108: 237–247

Huang Y, Weisgraber KH, Mucke L and Mahley RW (2004) Apolipoprotein E: diversity of cellular origins, structural and biophysical properties, and effects in Alzheimer's disease. J Mol Neurosci 23: 189–204

Hyman BT, Gomez-Isla T, Briggs M, Chung H, Nichols S and Kohout F et al. (1996) Apolipoprotein E and cognitive change in an elderly population. Ann Neurol 40: 55–66

Joseph JA, Shukitt-Hale B, Denisova NA, Prior RL, Cao G and Martin A et al. (1998) Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. J Neurosci 18: 8047–8055

Lass A, Forster MJ and Sohal RS (1999) Effects of coenzyme Q10 and alpha-tocopherol administration on their tissue levels in the mouse: elevation of mitochondrial alpha-tocopherol by coenzyme Q10. Free Radic Biol Med 26: 1375–1382

Lee Y, Aono M, Laskowitz D, Warner DS and Pearlstein RD (2004) Apolipoprotein E protects against oxidative stress in mixed neuronal–glial cell cultures by reducing glutamate toxicity. Neurochem Int 44: 107–118

Lomnitski L, Chapman S, Hochman A, Kohen R, Shohami E and Chen Y et al. (1999) Antioxidant mechanisms in apolipoprotein E deficient mice prior to and following closed head injury. Biochim Biophys Acta 1453: 359–368

Mahley RW and Rall SC Jr (2000) Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 1: 507–537

Martin A, Janigian D, Shukitt-Hale B, Prior RL and Joseph JA (1999) Effect of vitamin E intake on levels of vitamins E and C in the central nervous system and peripheral tissues: implications for health recommendations. Brain Res 845: 50–59

Masliah E, Mallory M, Ge N, Alford M, Veinbergs I and Roses AD (1995) Neurodegeneration in the central nervous system of apoE-deficient mice. Exp Neurol 136: 107–122

Masliah E, Samuel W, Veinbergs I, Mallory M, Mante M and Saitoh T (1997) Neurodegeneration and cognitive impairment in apoE-deficient mice is ameliorated by infusion of recombinant apoE. Brain Res 751: 307–314

Matthews RT and Beal MF (1996) Increased 3-nitrotyrosine in brains of Apo E-deficient mice. Brain Res 718: 181–184

McDonald SR, Lal H and Forster MJ (1997) Accelerated cognitive decline in apolipoprotein E deficient aging mice is independent of sensorimotor decline. Soc Neurosci Abstr 23: 2005

McDonald SR, Sohal RS and Forster MJ (2005) Concurrent administration of coenzyme Q_{10} and α-tocopherol improves learning in aged mice. Free Radic Biol Med 38: 729–736

Miyata M and Smith JD (1996) Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat Genet 14: 55–61

Montine TJ, Montine KS, Olsen SJ, Graham DG, Roberts LJ and Morrow JD et al. (1999) Increased cerebral cortical lipid peroxidation and abnormal phospholipids in aged homozygous apoE-deficient C57BL/6 mice. Exp Neurol 158: 234–241

Nicolle MM, Gonzalez J, Sugaya K, Baskerville KA, Bryan D and Lund K et al. (2001) Signatures of hippocampal oxidative stress in aged spatial learning-imparied rodents. Neuroscience 107: 415–431

Oitzl MS, Mulder M, Lucassen PJ, Havekes LM, Grootendorst J and de Kloet ER (1997) Severe learning deficits in apolipoprotein E-knockout mice in a water maze task. Brain Res 752: 189–196

Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM and Maeda N (1992) Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci USA 89: 4471–4475

Pratico D, Tangirala RK, Rader DJ, Rokach J and Fitzgerald GA (1998) Vitamin E suppresses isoprostane generation *in vivo* and reduces atherosclerosis in apoE-deficient mice. Nat Med 4: 1189–1192

Raber J, Wong D, Yu GQ, Buttini M, Mahley RW and Pitas RE et al. (2000) Apolipoprotein E and cognitive performance. Nature 404: 352–354

Ramassamy C, Krzywkowski P, Averill D, Lussier-Cacan S, Theroux L and Christen Y et al. (2001) Impact of apoE deficiency on oxidative insults and antioxidant levels in the brain. Brain Res Mol Brain Res 86: 76–83

Reed T, Carmelli D, Swan GE, Breitner JC, Welsh KA and Jarvik GP et al. (1994) Lower cognitive performance in normal older adult male twins carrying the apolipoprotein E epsilon 4 allele. Arch Neurol 51: 1189–1192

Reich EE, Montine KS, Gross MD, Roberts LJ II, Swift LL and Morrow JD et al. (2001) Interactions between apolipoprotein E gene and dietary alpha-tocopherol influence cerebral oxidative damage in aged mice. J Neurosci 21: 5993–5999

Roses AD (1996) Apolipoprotein E alleles as risk factors in Alzheimer's disease. Annu Rev Med 47: 387–400

Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA and Joo SH et al. (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 43: 1467–1472

Shea TB, Rogers E, Ashline D, Ortiz D and Sheu MS (2002) Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue. Free Radic Biol Med 33: 1115–1120

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J and Salvesen GS et al. (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 90: 1977–1981

Sumien N, Forster MJ and Sohal RS (2003) Supplementation with vitamin E fails to attenuate oxidative damage in aged mice. Exp Gerontol 38: 699–704

Sumien N, Heinrich KR, Sohal RS and Forster MJ (2004) Short-term vitamin E intake fails to improve cognitive or psychomotor performance of aged mice. Free Radic Biol Med 36: 1424–1433

Veinbergs I, Mallory M, Mante M, Rockenstein E, Gilbert JR and Masliah E (1999) Differential neurotrophic effects of apolipoprotein E in aged transgenic mice. Neurosci Lett 265: 218–222

Veinbergs I, Mallory M, Sagara Y and Masliah E (2000) Vitamin E supplementation prevents spatial learning deficits and dendritic alterations in aged apolipoprotein E-deficient mice. Eur J Neurosci 12: 4541–4546

Veurink G, Liu D, Taddei K, Perry G, Smith MA and Robertson TA et al. (2003) Reduction of inclusion body pathology in ApoE-deficient mice fed a combination of antioxidants. Free Radic Biol Med 34: 1070–1077

Yaffe K, Haan M, Byers A, Tangen C and Kuller L (2000) Estrogen use, APOE, and cognitive decline: evidence of gene–environment interaction. Neurology 54: 1949–1954