

Differential susceptibility of transgenic mice lacking one or both apolipoprotein alleles to folate and vitamin E deprivation

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Abstract. The E4 allele of apolipoprotein E (ApoE) is associated with neurodegeneration in part due to increased oxidative stress. Transgenic mice lacking ApoE (–/–) represent a model for the consequences of deficiencies in ApoE function. Dietary deficiency in folate and vitamin E has previously been shown to potentiate the impact of ApoE deficiency; ApoE–/– mice deprived of folate and vitamin E for 1 month demonstrated increased oxidative damage in brain tissue and impaired cognitive performance as compared to ApoE+/+ mice. Since individuals homozygous for E4 can demonstrate more increased risk for neurodegeneration and an earlier age of onset than individuals heterozygous for E4, we tested the impact of folate and vitamin E deprivation on ApoE+/- mice. Thiobarbituric acid-reactive substances in brain tissue of ApoE+/- were significantly increased compared to ApoE+/+ mice, but this increase was less than that observed in ApoE–/– mice. By contrast, livers of ApoE+/- and –/– mice displayed an identical increase over that of +/+ mice. ApoE–/– mice, but not +/- or +/+ mice, exhibited impaired cognitive performance in maze trials when deprived of folate and vitamin E. These findings support the notion that homozygous deficiency of ApoE function can be more severe than heterozygous deficiency. They further suggest that the impact of partial deficiency in ApoE function may present a latent risk that may manifest only when compounded by other factors such as dietary deficiency.

Keywords: Apolipoprotein E, folate, vitamin E, neurodegeneration, oxidative stress

1. Introduction

Folate deficiency contributes to age-related neurological and psychological disorders including dementia, impaired cognition, depression, psychosis, Alzheimer's disease (AD) and Parkinson's disease (PD) [1], for reviews, see [2–4]. These deleterious effects arise at least in part by the increase in oxidative stress that accompanies folate deficiency. Folate deficiency increases neuronal oxidative stress by increasing levels of the neurotoxin homocysteine – levels of which

are related to the progression and severity of AD [5], by decreasing endogenous antioxidants, by depleting overall cellular methylation reactions, and by inducing DNA damage and depleting energy reserves [6–10]. Folate deprivation has been shown to impair multiple aspects of learning and memory in humans [11] as well as in experimental animals [12–14]. Folate deprivation also potentiates the deleterious impact of certain other risk factors for AD including amyloid beta, glutamate and metal neurotoxicity [6,7,9,15] and deficiency in apolipoprotein E (ApoE) [10,14,16,17].

The epsilon 4 allele (E4) of ApoE is linked with an increase in, and an earlier age of onset, of sporadic and familial AD [18–20]. It remains unclear whether this risk is derived from the diminished function(s) of ApoE4, or instead derives from the absence of protective effects provided by ApoE3 and/or ApoE2, or yet

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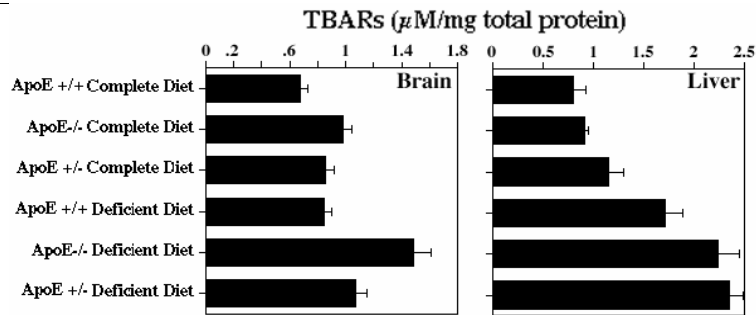


Fig. 1. Individual and combinatorial influence of dietary and genetic deficiencies on oxidative damage in brain and liver. Mice received dietary regimens as indicated for 1 month, after which total brain tissue was harvested and analyzed for TBARS as described in Materials and Methods. Panels present μmol TBARS/mg total protein (mean \pm standard deviation for brain and liver as indicated) compiled from 2 independent experiments, with 4–8 mice of each genotype maintained with both diets for each experiment, yielding a total n of 8–16 mice of each strain under each condition. TBARS in brain tissue of both ApoE +/- and -/- mice differed from +/+ mice for both diets. Brain tissue from all mice maintained on the deficient diet displayed increased TBARS relative to those on the complete diet. For liver analyses, all mice on the complete diet displayed identical TBARS. All mice on the deficient diet displayed statistically-increased TBARS over those on the complete diet. In addition mice lacking one or both ApoE genes displayed increased TBARS versus those bearing both genes.

instead derives from the actual presence of ApoE4 [20, 21]. The E4 allele can exhibit gene dosage effects such that individuals homozygous for E4 demonstrated the most severe impairments in cognition and/or earliest age of onset of dementia, while those heterozygous for E4 in various studies demonstrated either no statistical difference from otherwise normal individuals, or symptoms intermediate between individuals homozygous for E4 and lacking E4 [22–27].

Pre-clinical memory decline can occur earlier in E4 homozygotes than in E4 heterozygotes [28]. The dosage of E4 therefore remains an important risk factor [29], and leaves open the possibility that individuals heterozygous for E4 may harbor a latent risk for neurodegeneration that will reach clinical proportions only if augmented by one or more additional risk factors.

Transgenic mice lacking ApoE (“ApoE-/-”) exhibit increased oxidative stress and, under certain conditions, impaired cognition, and therefore represent a useful model for the impact of deficiencies in ApoE function on neurodegeneration [10,16,17,30–34]. Dietary deficiencies in folate and vitamin E deficiency have been shown to potentiate oxidative damage to brain tissue and impair cognitive ability of ApoE-/- mice [10,14,16,17,35,36]. We tested herein whether or not deficiencies in dietary folate and vitamin E adversely affected mice heterozygous for ApoE.

2. Materials and methods

2.1. Mouse strains and diet

Normal C57BL/6J mice and ApoE -/- mice on the same genetic background, originally obtained from

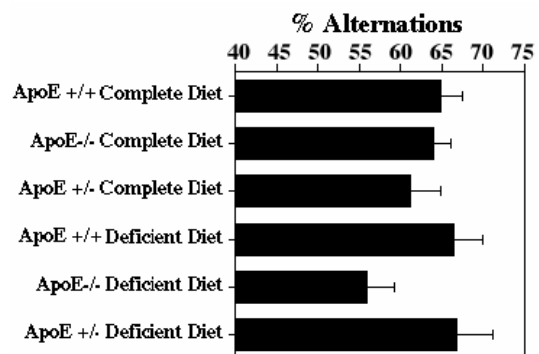


Fig. 2. Folate and vitamin E deficiency impairs cognitive performance in ApoE-/- but not ApoE +/+ or +/- mice. Mice were maintained on the indicated diets, then subjected to the Y maze test and the % alternations determined as described in Materials and Methods. Values present the mean \pm standard deviation for % alternations derived from 2 independent experiments, with 4–8 mice of each genotype maintained with both diets for each experiment, yielding a total n of 8–16 mice of each strain under each condition. Note that only ApoE-/- mice demonstrate impaired performance ($p < 0.05$ versus all other conditions) and only when maintained on the deficient diet; all other conditions were statistically identical (ANOVA).

Jackson Laboratories, were crossed to yield ApoE +/- mice. Mice of all three genotypes between the ages of 9–12 months of age were maintained on a basal, folate and vitamin E-free chow and drinking water ad libitum for 1 month (“AIN-76”; Purina/Mother Hubbard, Inc.) [10,16]. For some groups, this basal diet was supplemented with folic acid (2 mg/kg total diet wet weight), vitamin E (1 g/kg total diet wet weight), and iron (50 g/500 g total diet;) as a pro-oxidant [10,16, 17]. Supplementation with folic acid and vitamin E

without iron was defined as the “complete diet;” supplementation with iron without folic acid or vitamin E was defined as the “deficient diet.”

2.1.1. TBAR analyses

Thiobarbituric acid-reactive substances (TBARs) were quantified in homogenates of frontal cortex as an index of endpoint oxidative damage as utilized previously for ApoE $-/-$ mouse central nervous system as well as in AD [10,16,32,37,38]. Briefly, brain tissue homogenates (50 μ g total protein) were mixed with 1 μ M copper sulfate in 5 mM HEPES (total volume 400 μ l). Samples then received 1 ml of a 0.375% TBA/15% trichloroacetic acid in 0.25 N HCl, incubated for 30 min at 90°C, and were clarified by centrifugation (1,500 rpm for 10 min). The resulting supernatants were aspirated and fluorescence quantified in a fluorescent spectrophotometer (excitation 520 nm, emission 553 nm) by comparison with a standard curve of tetramethoxypropane in HCl.

2.2. Y-maze tests

One day prior to sacrifice for the above TBAR analyses, mice were subjected to standard Y maze tests as described previously [14]. The pattern of exploration of the maze was recorded over 5 min intervals and the % alternations determined, which was defined as the frequency in which mice visited each of the 3 arms sequentially during any 3-arm visitation sequence [14] and refs. therein.

2.3. Statistical analyses

All analyses are derived from 2 independent experiments, with 4–8 mice of each genotype maintained with both diets for each experiment, yielding a total n of 8–16 mice of each strain under each condition. Statistical analyses were carried out with Student’s t test and ANOVA; values were considered statistically different if $p \leq 0.05$.

3. Results and discussion

ApoE $-/-$ exhibited statistically increased TBARs in brain tissue versus ApoE $+/+$ and ApoE $+/-$ mice when maintained on the complete diet. A further statistical increase in TBARs was observed for both ApoE $-/-$ and $+/-$ mice when maintained on the deficient diet; the increase for ApoE $-/-$ mice was sta-

tistically greater than that observed for $+/-$ mice. By contrast, liver TBARs were statistically identical for all genotypes when maintained under the complete diet, and all genotypes demonstrated statistically-increased TBARs when maintained on the deficient diet. ApoE $-/-$ and $+/-$ mice on the deficient diet displayed a further identical increase over that observed for $+/+$ mice. These data highlight differential sensitivity of liver and brain to the impact of dietary- and genetically-induced oxidative damage [10].

Since maintenance on the deficient diet for 1 month has been shown to affect selectively the cognitive performance of ApoE $-/-$ mice in the Y-maze test [14], we examined whether or not the deficient diet would similarly affect ApoE $+/-$ mice. As shown previously [14], ApoE $-/-$ mice displayed a significant reduction in % alternations when maintained on the deficient diet (Fig. 2; $p \leq 0.005$ vs. both other genotypes). By contrast, ApoE $+/-$ mice exhibited statistically identical % alternations as those of ApoE $+/+$ mice (Fig. 2).

These studies demonstrate that dietary deficiencies in folate and vitamin E foster increased brain oxidative damage in ApoE $+/-$ and $-/-$ mice, and that the degree of potentiation is dependent upon the number of copies of the murine ApoE gene. As in our prior investigations of ApoE $-/-$ mice (Shea and Rogers, 2002b), liver demonstrated more pronounced oxidative damage than did brain tissue. One interpretation of these data is that oxidative damage to brain tissue can remain more latent than that of liver; this interpretation is consistent the lack of compromise in cognitive ability in ApoE $+/-$ mice under dietary conditions that do impair cognitive ability in ApoE $-/-$ mice [see also [14]]. Of interest would be to examine the effect of maintaining ApoE $+/-$ mice on the deficient diet for longer periods, which may induce cognitive deficits. In this regard, while short-term (1 month) vitamin E deprivation alone was not problematic for ApoE $-/-$ mice [10], vitamin E deprivation for 9 months induced cognitive deficits in ApoE $-/-$ mice [34]. This would reveal whether mice with one functional ApoE allele can compensate for long-term dietary deficiencies in folate and vitamin E, or whether the increased oxidative damage to brain tissue in ApoE $+/-$ mice, which was less than that observed in ApoE $-/-$ mice, merely takes longer to be reflected in cognitive function.

This study addressed only relative decreases in ApoE function, obtained by absence of one or both copies of the murine ApoE gene. Of interest would be to examine the consequences of mice expressing one or

more copies of E4 versus other ApoE alleles [20,21]. Increased oxidative stress, which can arise from dietary, environmental and/or genetic sources, is a major risk factor contributing to the age-related decline in cognitive performance [39–43]. Oxidative damage in the brain is elevated in AD patients, and the extent of this damage correlates with the presence of the E4 allele [31]. Since the timing and severity of cognitive decline associated with ApoE4 can be a function of the number of copies of E4 [28,29], the findings of the present study leave open the possibility that individuals heterozygous for E4 may harbor a latent risk for neurodegeneration that may reach clinical proportions under conditions that induce additional oxidative stress such as dietary deficiency.

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References

- [1] E.H. Mizrahi, D.W. Jacobsen, S.M. Debanne, F. Traore, A.J. Lerner, R.P. Friedland and G.J. Petot, Plasma Total Homocysteine Levels, Dietary Vitamin B6 and Folate Intake In AD and Healthy Aging, *J Nutr Health Aging* **7** (2003), 160–165.
- [2] M.M. Mattson and T.B. Shea, Folate and Homocysteine in Neural Plasticity and Neurodegenerative Disorders, *Trends Neurosci* **26** (2002), 137–146.
- [3] T.B. Shea and E. Rogers, Homocysteine as a risk factor for Alzheimer's disease, *New Eng J Med* **346** (2002), 2007.
- [4] T.B. Shea, J. Lyons-Wieler and E. Rogers, Homocysteine, folate deprivation and Alzheimer's disease, *J Alz Dis* **4** (2002), 261–268.
- [5] A. Postiglione, G. Milan, A. Ruocco, G. Gallotta, G. Guiotto and G. Di Minno, Plasma folate, vitamin B(12), and total homocysteine and homozygosity for the C677T mutation of the 5,10-methylene tetrahydrofolate reductase gene in patients with Alzheimer's dementia. A case-control study, *Gerontology* **47** (2001), 324–329.
- [6] H. Kruman, C. Culmsee, S.L. Chan, Y. Kruman, Z. Guo, L. Penix and M.P. Mattson, Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity, *J Neurosci* **20** (2000), 6920–6926.
- [7] H. Kruman, T.S. Kumaravel, A. Lohani, W.A. Pedersen, G. Roy, R.G. Cutler, Yuri Kruman, N. Norman Haughey, J. Lee, M. Michele Evans and M.P. Mattson, Folic Acid Deficiency and Homocysteine Impair DNA Repair in Hippocampal Neurons and Sensitize Them to Amyloid Toxicity in Experimental Models of Alzheimer's Disease, *J. Neurosci.* **22** (2002), 1752–1762.
- [8] P.I. Ho, D. Ortiz, E. Rogers and T.B. Shea, Multiple aspects of homocysteine neurotoxicity: glutamate excitotoxicity, kinase hyperactivation and DNA damage, *J Neurosci Res* **70** (2002), 694–702.
- [9] P. Ho, D. Ashline, S. Dhitavat, S. Collins, E. Rogers and T.B. Shea, Folate Deprivation Induces Neurodegeneration: Roles Of Oxidative Stress And Increased Homocysteine, *Neurobiol Dis* **14** (2003), 32–42.
- [10] T.B. Shea and E. Rogers, Folate quenches oxidative damage in brains of apolipoprotein E-deficient mice: augmentation by vitamin E, *Mol Brain Res* **108** (2002), 1–6.
- [11] L. Hassing, A. Wahlin, B. Winblad and L. Backman, Further evidence on the effects of vitamin B12 and folate levels on episodic memory functioning: a population-based study of healthy very old adults, *Biol Psychiatry* **45** (1999), 1472–1480.
- [12] S.F. Crowe and C.K. Ross, Effect of folate deficiency and folate and B12 excess on memory functioning in young chicks, *Pharmacol Biochem Behav* **56** (1997), 189–197.
- [13] R. Lalonde, The neurobiological basis of spontaneous alternation, *Neurosci Biobehav Rev* **26** (2002), 91–104.
- [14] S.M. Mihalick, D. Ortiz, R. Kumar, E. Rogers and T.B. Shea, Folate and vitamin E deficiency impair cognitive performance in mice subjected to oxidative stress: differential impact on normal mice and mice lacking apolipoprotein E, *Neuromol Med*, in press.
- [15] A.R. White, X. Huang, M.F. Jobling, C.J. Barrow, K. Beyreuther, C.L. Masters, A.I. Bush and R. Cappai, Homocysteine potentiates copper- and amyloid beta peptide-mediated toxicity in primary cultures: possible risk factors in the Alzheimer's-type neurodegenerative pathways, *J Neurochem* **76** (2001), 1509–1520.
- [16] T.B. Shea, E. Rogers, D. Ortiz and M-S. Sheu, Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue, *Free Rad Biol Med* **33** (2002), 1115–1120.
- [17] T.B. Shea, E. Rogers, D. Ashline, D. Ortiz, N. Duarte, T.A. Wilson, R.J. Nicolosi and M-H. Sheu, Vitamin E deficiency does not induce compensatory antioxidant increases in central nervous system tissue of apolipoprotein E-deficient mice, *J Alz Dis* **4** (2002), 1–6.
- [18] J. Growdon, Incorporating biomarkers into clinical drug trials in Alzheimer's disease, *J Alz Dis* **3** (2001), 287–292.
- [19] R. Parasuraman, P.M. Greenwood and T. Sunderland, The apolipoprotein E gene, attention, and brain function, *Neuropsychology* **16** (2002), 254–274.
- [20] G.W. Rebeck, M. Kindy and M.J. LaDu, Apolipoprotein E and Alzheimer's disease: the protective effects of ApoE2 and E3, *J Alz Dis* **4** (2002), 145–154.
- [21] B. Teter, J. Raber, B. Nathan and K.A. Crutcher, The presence of apoE4, not the absence of apoE3, contributes to AD pathology, *J Alz Dis* **4** (2002), 155–163.
- [22] J.C. Breitner, G.P. Jarvik, B.L. Plassman, A.M. Saunders and K.A. Welsh, Risk of Alzheimer disease with the epsilon4 allele for apolipoprotein E in a population-based study of men aged 62–73 years, *Alzheimer Dis Assoc Disord* **12** (1998), 40–44.
- [23] Z. Hawi, K. Sheehan, A. Lynch, I. Evans, N. Lowe, B. Lawlor and M. Gill, Late onset Alzheimer's disease and apolipoprotein association in the Irish population: relative risk and attributable fraction, *Ir J Med Sci* **172** (2003), 74–76.
- [24] M.J. Finton, J.A. Lucas, J.D. Rippeth, D.L. Bohac, G.E. Smith, R.J. Ivnik, R.C. Petersen and N.R. Graff-Radford, Cognitive asymmetries associated with apolipoprotein E genotype in patients with Alzheimer's disease, *J Int Neuropsychol Soc* **9** (2003), 751–759.
- [25] C. Geroldi, M. Pihlajamaki, M.P. Laakso, C. DeCarli, A. Beltramello, A. Bianchetti, H. Soininen, M. Trabucchi and G.B. Frisoni, APOE-epsilon4 is associated with less frontal

- and more medial temporal lobe atrophy in AD, *Neurology* **53** (1999), 1825–1832.
- [26] W.B. Growdon, B.S. Cheung, B.T. Hyman and G.W. Rebeck, Lack of allelic imbalance in APOE epsilon3/4 brain mRNA expression in Alzheimer's disease, *Neurosci Lett* **272** (1999), 83–86.
- [27] R.H. Myers, E.J. Schaefer, P.W. Wilson, R. D'Agostino, J.M. Ordovas, A. Espino, R. Au, R.F. White, J.E. Knoefel, J.L. Cobb, K.A. McNulty, A. Beiser and P.A. Wolf, Apolipoprotein E epsilon4 association with dementia in a population-based study: The Framingham study, *Neurology* **46** (1996), 673–677.
- [28] R.J. Caselli, N.R. Graff-Radford, E.M. Reiman, A. Weaver, D. Osborne, J. Lucas, A. Uecker and S.N. Thibodeau, Pre-clinical memory decline in cognitively normal apolipoprotein E-epsilon4 homozygotes, *Neurology* **53** (1999), 201–207.
- [29] M.S. Tsai, E.G. Tangalos, R.C. Petersen, G.E. Smith, D.J. Schaid, E. Kokmen, R.J. Ivnik and S.N. Thibodeau, Apolipoprotein E: risk factor for Alzheimer disease, *Am J Hum Genet* **54** (1994), 643–649.
- [30] G.S. Huang, S.M. Yang, M.Y. Hong, P.C. Yang and Y.C. Liu, Differential gene expression of livers from ApoE deficient mice, *Life Sci* **68** (2000), 19–28.
- [31] C. Ramassamy, D. Averill, L. Beffert, L. Throux, S. Lussier-Cacan, J.S. Cohn, Y. Christen, J. Schoofs and J. Davignon, Oxidative damage and protection by antioxidants in the frontal cortex of Alzheimer's disease is related to the apolipoprotein E genotype, *Free Rad Biol Med* **27** (1999), 544–553.
- [32] C. Ramassamy, D. Averill, U. Beffert, L. Theroux, S. Lussier-Cacan, J.S. Cohn, Y. Christen, J. Schoofs, J. Davignon and J. Poirier, Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain, *Neurobiol Dis* **7** (2000), 23–37.
- [33] C. Ramassamy, P. Krzywkowski, D. Averill, S. Lussier-Cacan, L. Throux, Y. Christen, J. Sch Davignon and J. Poirier, Impact of apolipoprotein E deficiency on oxidative insults and antioxidant levels in the brain, *Mol Brain Res* **86** (2002), 76–83.
- [34] I. Veinbergs, M. Mallory, Y. Sagara and E. Masliah, Vitamin E supplementation prevents spatial learning deficits and dendritic alterations in aged apolipoprotein E-deficient mice, *Eur J Neurosci* **12** (2000), 4541–4546.
- [35] E.J. Rogers, S. Mihalick, D. Ortiz and T.B. Shea, Apple juice prevents oxidative stress and impaired cognitive performance caused by genetic and dietary deficiencies in mice, *J Nutr Health Aging* (2003), in press.
- [36] F. Tchanchou, M. Graves, D. Ashline, A. Morin, A. Pimenta, D. Ortiz, E. Rogers and T.B. Shea, Increased transcription and activity of glutathione synthase in response to deficiencies in folate, vitamin E and apolipoprotein E, *J Neurosci Res* (2003), in press.
- [37] D.A. Butterfield and C.M. Lauderback, Lipid peroxidation and protein oxidation in Alzheimer's disease: potential causes and consequences involving amyloid-beta peptide-associated free radical oxidative stress, *Free Rad Biol Med* **32** (2002), 1050–1060.
- [38] M.A. Lovell, W.D. Ehmann, S.M. Butler and W.R. Markesbery, Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease, *Neurol* **45** (1995), 1594–1601.
- [39] C. Berr, Oxidative stress and cognitive impairment in the elderly, *J Nutr Health Aging* **6** (2002), 261–266.
- [40] D.A. Butterfield, A. Castegna, C.M. Lauderback and J. Drake, Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death, *Neurobiol Aging* **23** (2002), 655–664.
- [41] R.A. Floyd and K. Hensley, Oxidative stress in brain aging: Implications for therapeutics of neurodegenerative diseases, *Neurobiol Aging* **23** (2002), 795–807.
- [42] G. Perry, A.D. Cash and M.A. Smith, Alzheimer Disease and Oxidative Stress, *J Biomed Biotechnol* **2** (2002), 120–123.
- [43] G. Perry, A. Nunomura, K. Hirai, X. Zhu, M. Prez, J. Avila, R.J. Castellani, C.S. Atwood, G. Aliev, L.M. Sayre, A. Takeda and M.A. Smith, Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? *Free Radic Biol Med* **33** (2002), 1475–1479.
- [44] P.I. Ho, S.C. Collins, S. Dhritavat, D. Ortiz, D. Ashline, E. Rogers and T.B. Shea, Homocysteine potentiates beta-amyloid neurotoxicity: role of oxidative stress, *J Neurochem* **78** (2001), 249–253.