

Apple juice concentrate prevents oxidative damage and impaired maze performance in aged mice

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Abstract. Oxidative stress contributes to age-related cognitive decline. In some instances, consumption of fruits and vegetables rich in antioxidant can provide superior protection than supplementation with purified antioxidants. Our prior studies have shown that supplementation with apple juice concentrate (AJC) alleviates oxidative damage and cognitive decline in a transgenic murine model compromised in endogenous antioxidant potential when challenged with a vitamin-deficient, oxidative stress-promoting diet. Herein, we demonstrate that AJC, administered in drinking water, is neuroprotective in normal, aged mice. Normal mice aged either 9–10 months or 2–2.5 years were maintained for 1 month on a complete diet or a diet lacking folate and vitamin E and containing iron as a pro-oxidant, after which oxidative damage was assayed by thiobarbituric acid-reactive substances and cognitive decline as assayed by performance in a standard Y-maze. Mice 9–12 months of age were unaffected by the deficient diet, while older mice demonstrated statistically-increased oxidative damage and poorer performance in a Y-maze test. Supplementation with AJC prevented these neurodegenerative effects. These data are consistent with normal aged individuals being susceptible to neurodegeneration following dietary compromise such as folate deficiency, and a hastened onset of neurodegeneration in those individuals harboring a genetic risk factor such as ApoE deficiency. These findings also support the efficacy of antioxidant supplementation, including consumption of antioxidant-rich foods such as apples, in preventing the decline in cognitive performance that accompanies normal aging.

Keywords: Aging, neurodegeneration, antioxidants, cognitive decline, nutrition, apple juice

1. Introduction

One factor contributing to the age-related decline in cognitive performance is increased oxidative stress [1–5]. In addition to pharmacological approaches, increased oxidative stress can also be counteracted by dietary supplementation with fruits and vegetables that are high in antioxidant potential [6–12]; these have been shown to be effective against cognitive impairment and progression of Alzheimer's disease [13–19].

Experimental studies substantiate these clinical findings. For example, supplementation with blueberries, spinach or strawberries has been shown to reverse age-related declines in signal transduction as well as cognitive and behavioral deficits in rats [17,18,20,21]. So-called “natural” antioxidants obtained by consumption of fresh fruit can in some cases provide superior antioxidant activity than dietary supplements of purified antioxidants [6], which may result from simultaneous consumption of multiple antioxidants and compounds with antioxidant potential.

Our prior studies have demonstrated that supplementation with apple juice concentrate (AJC) prevented the oxidative damage and decline in performance in maze trials that normally accompanies maintenance of mice lacking ApoE (“ApoE^{-/-} mice”), which are a model for

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Table 1

Supplementation with AJC prevents oxidative damage to central nervous system and cognitive impairment in aged mice due to vitamin deficiency and oxidative challenge

	Adult mice		Aged Mice	
	TBARs	% change*	TBARs	% change
Complete diet	1.5 ± 0.2	—	1.6 ± 0.1	—
Deficient diet	1.6 ± 0.1	6.7 ± 8.2	1.9 ± 0.1	22.9 ± 8.7**
Deficient diet + APC	1.4 ± 0.2	-6.8 ± 1**	1.7 ± 0.1	7.7 ± 5.1
	Y Maze	% change	Y maze	% change
Complete diet	62.2 ± 1	—	63.8 ± 2.7	—
Deficient diet	61.3 ± 5	-1.4 ± 4.0	56.9 ± 4.1	-10.8 ± 4.3**
Deficient diet + APC	66.1 ± 4	6.7 ± 6.0	64.2 ± 8.9	8.2 ± 7.3

Normal mice approximately 9 months of age ("Adult mice") and 2–2.5 years of age ("Aged mice") were maintained on the complete diet, the deficient diet, and the deficient diet supplemented with AJC for 1 month, subjected to the Y maze test, then sacrificed the following day and TBARs were determined in homogenates of total central nervous tissue as described in Materials and Methods. Values for the Y maze are presented as the mean % alternations (± standard error of the mean) and those for TBARs are presented as $\times \text{mol} \times 10^{-1}$ TBARs/mg total protein.

*% change compared to values obtained for mice of the same age on the complete diet

**statistically different from mice of the same age on the complete diet ($p < 0.05$).

increased oxidative stress [22–27], on a folate- and vitamin E-deprived diet and subjected to oxidative challenge via dietary iron [28]. Normal mice of the same age (9–10 months of age) did not demonstrate similar trauma when maintained on this challenge diet [25]. Since oxidative stress can impart age-related neurodegeneration even in the absence of genetic compromise [1,29,30], we examined herein whether or not supplementation with AJC would diminish oxidative damage and maintain performance in maze trials in aged, normal mice.

2. Materials and methods

Mice and diet Normal C57/BalbC mice either approximately 9–10 months of age ("adult") or 2–2.5 years of age ("aged") 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. [25,26]). 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 [25,26,28]. 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." Additional groups received apple juice concentrate (AJC) from a stock of 70 brix (generous gift of Veryfine Inc. Littleton MA), AJC (stored frozen until used) was administered *ad libitum* at a final concentration of 0.5% (vol/vol) as the sole

source of drinking water for this month; drinking water was changed daily. This concentration of AJC was previously demonstrated to provide maximal neuroprotection to genetically-compromised (ApoE^{-/-}) mice under these dietary conditions and did not alter food or water consumption nor induce differences in weight among animals [28]. Since a portion of the antioxidant activity of apples is recovered in apple juice [31–35], we elected in the present study, as in our prior studies [28,36], to administer AJC as a source of apple-derived antioxidants, which avoided potential differential quenching of antioxidants in apple supplements due to presence of iron added to some diets. Mice were therefore maintained on 4 total diets: complete ± ACJ and deficient ± AJC.

Y-maze tests Mice maintained on the above diets for 1 month were subjected to standard Y maze tests as described previously [37]. The pattern of exploration of the Y 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 during any 3-arm visitation sequence (37 and refs. therein). Mazes were cleaned and dried between tests to avoid influence of the prior mouse on subsequent exploration. Mice were sacrificed following maze trials for analyses of oxidative species (below).

TBAR analyses: Thiobarbituric acid-reactive substances (TBARs) were quantified in total brain homogenates as an index of endpoint oxidative damage as utilized previously for ApoE^{-/-} mouse central nervous system as well as in AD [23–26,38,39]. Briefly, brain tissue homogenates (50 μg 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, 10 min). The resulting supernatants were aspirated and quantified in a fluorescent spectrophotometer (excitation 520 nm, emission 553 nm) by comparison with a standard curve of tetramethoxypropane in HCl.

Statistical analyses All analyses are derived from 2–4 independent experiments, with 3–4 mice of each age under each dietary condition for each experiment, for a total n of 6–16 mice under each condition. Statistical analyses were carried out with Student's t test and ANOVA.

3. Results and discussion

As shown previously, normal mice approximately 9 months of age displayed identical levels of TBARs in central nervous tissue, and performed equally well in Y maze analyses, when maintained on the complete or deficient diets (Table 1). Mice aged 2 to 2.5 years displayed statistically-identical TBARs and cognitive performance as did 9-month old mice when maintained on the complete diet. Unlike these relatively-younger mice, however, aged mice displayed statistically-increased TBARs and statistically-decreased performance in the Y maze when maintained on the deficient diet (Table 1); these values are similar to the extent of neurodegeneration observed in adult ApoE^{-/-} mice maintained on the deficient diet [28]. Supplementation with AJC prevented the increase in TBARs and the decline in cognitive performance that otherwise accompanied maintenance of aged mice on the deficient diet (Table 1). As previously demonstrated [28], supplementation of the deficient diet with AJC statistically improved TBARs in adult mice on the normal diet (Table 1). These data are consistent with normal aged individuals being susceptible to neurodegeneration following dietary compromise such as folate deficiency, and a hastened onset of neurodegeneration in those individuals harboring a genetic risk factor such as ApoE deficiency.

The findings of the present study demonstrate that dietary supplementation with an antioxidant-rich fruit such as apples can prevent oxidative damage to the central nervous system that can otherwise arise from vitamin deficiency during aging. The neuroprotective efficacy of AJC was reflected by reduction in oxida-

tive species in brain tissue as well as by prevention of cognitive impairment that otherwise accompanies these deficiencies. On a total weight basis, the amount of AJC consumed by mice in these and prior studies [28], corresponds to 2–3 8oz glasses of diluted AJC per day by humans.

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Apples and products derived from them are a source of significant antioxidant activity [6,31–38,40–42]. We have not determined which components present in AJC were responsible for the neuroprotective effects demonstrated herein nor in our prior study [28]. Our prior studies eliminated the possibility that extra energy derived from the high sugar content of AJC provided neuroprotection [28], which prompted the conclusion that the antioxidant potential of apple juice was responsible for neuroprotection; this conclusion is supported by the recent demonstration that antioxidants in apple products provided neuroprotection against oxidative stress in culture neuronal cells [42,43], and by the demonstration that AJC alleviated the compensatory increase in endogenous antioxidants in ApoE^{-/-} mice maintained on the deficient diet [36]. However, recent studies suggest that the sugar content of apples is also beneficial for plasma antioxidant levels [44,45].

The findings presented herein support the notion that consumption of foods rich in antioxidant potential can provide neuroprotection; they extend our prior demonstration of neuroprotection in genetically-compromised mice [28,36], and demonstrate that antioxidant-rich foods are also effective against oxidative damage and decline in memory that accompanies normal aging. Our studies were confined to examining the efficacy of supplementation with AJC under short-term dietary challenge. Of interest would be to examine whether or not supplementation with AJC would remain effective during longer feeding trials, and whether delayed supplementation could reverse, or attenuate continued progression of, the detrimental effects of maintaining ApoE^{-/-} mice on the deficient diet as shown herein. Also of interest would be to examine how rapidly withdrawal of AJC supplementation would result in a loss of neuroprotection. Further such studies, as well as to determine the neuroprotective efficacy of AJC supplementation during other dietary deficiencies, are warranted.

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