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SUPPLEMENTATION WITH APPLE JUICE CAN COMPENSATE FOR FOLATE DEFICIENCY IN A MOUSE MODEL DEFICIENT IN METHYLENE TETRA HYDROFOATE REDUCTASE ACTIVITY

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Abstract: Folate insufficiency promotes developmental as well as age-related disorders of the nervous system. The C677T variant of 5',10' methylene tetrahydrofolate reductase (MTHFR; which utilizes folate to regenerate methionine from homocysteine) displays reduced activity, and therefore promotes functional folate deficiency. Mice heterozygously lacking this gene (MTHFR+/- mice) represent a useful model for analysis of the impact of MTHFR deficiency and potential compensatory approaches. Since consumption of apple products has benefited mouse models subjected to dietary and/or genetically-induced folate deficiency, we compared the impact of supplementation with apple juice on cognitive and neuromuscular performance of mice MTHFR+/+ and +/- mice with and without dietary folate deficiency. Mice were maintained for 1 month on a standard, complete diet, or a challenge diet lacking folate, and vitamin E and containing a 50g iron/500g total diet as a pro-oxidant. Additional groups received apple juice concentrate (AJC) diluted to 0.5% (vol/vol) in their sole source of drinking water. MTHFR+/- mice demonstrated significantly impaired cognitive performance in standard reward-based T maze and the non-reward-based Y maze tests as compared to MTHFR+/+ when maintained on the complete diet; supplementation with AJC improved the performance of MTHFR+/- to the level observed for MTHFR+/+ mice. Maintenance for 1 month on the deficient diet reduced the performance of both genotypes in both tests, but supplementation with AJC prevented these reductions. MTHFR+/+ and +/- displayed virtually identical neuromuscular performance in the standard paw grip endurance test when maintained on the complete diet, and displayed similar, non-significant declines in performance when maintained on the deficient diet. Supplementation of either diet with AJC dramatically improved the performance of both genotypes. The findings presented herein indicate that supplementation with AJCs can compensate for genetic as well as dietary insufficiency in folate in a murine model of genetic folate compromise, and support the notion that dietary supplementation may be more critical under conditions of latent genetic compromise.

Key words: Apple products, nutrition, cognition, genetics, Alzheimer's disease.

Introduction

Folate insufficiency promotes developmental as well as age-related disorders of the nervous system (1). In addition to the deleterious impact of dietary compromise (2), functional folate deficiency resulting from polymorphisms in the enzymes that perturb the so-called "one-carbon" or methionine cycle can also foster nervous system disorders and can potentiate the impact of dietary folate deficiency (3). One such polymorphism, the C677T variant of 5',10' methylene tetrahydrofolate reductase (MTHFR; which utilizes folate to regenerate methionine from homocysteine), which displays reduced activity, is associated with multiple nervous system disorders (4-8). Individuals homozygous for this polymorphism exhibit mild hyperhomocysteinemia, which is further augmented by diminished dietary folate (9-11).

Increased awareness of the impact of folate deficiency on development has led to more frequent supplementation during pregnancy. In this regard, a 36% increase in MTHFR polymorphisms is present among young people, indicating that increased maternal dietary folate allowed an increase in fetal viability despite latent MTHFR deficiency (12). This indicates the growing presence within the population of individuals with genetic deficiencies in folate metabolism; such individual may therefore require increased dietary folate to prevent nervous system compromise, although this possibility has been debated (13, 14). Such individuals may be particularly susceptible to age-related decline in neuronal function pending only following with age-related nutritional decline.

Mice heterozygously lacking this gene (MTHFR+/- mice) represent a useful model for analysis of the impact of MTHFR deficiency and potential compensatory approaches. These mice display increased sensitivity to dietary folate deprivation. Supplementation with folate and/or S-adenosylmethionine, an intermediate of the methionine cycle, can compensate for the increased homocysteine, oxidative damage and cognitive impairment characteristic of these mice (15).

Several studies have demonstrated that consumption of apple products can reduce oxidative species and resultant oxidative damage, as well as maintain or even improve cognitive performance in mouse models subjected to dietary and/or genetically-induced oxidative challenge (16-18). Herein, we compared the impact of supplementation with apple juice on cognitive and neuromuscular performance of mice MTHFR+/+

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and +/- mice with and without dietary folate deficiency.

Materials and methods

Adult BALB/cAnNCrlBR mice heterozygously lacking MTHFR (MTHFR+/-) along with normal littermates (MTHFR+/+; genotypes of which were determined by PCR as described previously; 19) received a diet ("AIN-76"; Purina/Mother Hubbard, Inc.) either containing folic acid (4 mg/kg total diet wet weight) and vitamin E (50IU/kg total diet wet weight; defined as the "complete diet") or the same basal diet lacking folate and vitamin E and containing iron (50g/500g total diet) as a pro-oxidant. Mice were maintained in groups of 3 or 4 on these diets for 1 month (16). Additional groups of mice maintained on each diet received apple juice concentrate (AJC) diluted to 0.5% (vol/vol) in their sole source of drinking water for this month; AJC was utilized for ease of dispensing while avoiding potential oxidation of apple constituents that could result from mixing with chow (16).

Cognitive impairment was monitored after maintenance on the above diets for 1 month using standard reward-based "T maze" and non-reward-based "Y maze" systems as described (16). Mice were placed at the bottom of a T-shaped maze, with one arm of the maze blocked, and therefore could explore only one arm. Each arm of the maze contained a depression containing a small amount of sweetened milk. Mice were allowed to locate and consume the milk in the available arm, then were returned to the bottom of the maze and the block was removed from the other arm. If the mouse entered the opposite (newly-unblocked arm), it was scored as passing; if it instead re-explored the previously-visited arm, it was scored as failing. Mice were tested 3 times in succession, allowing only sufficient time for cleaning of the maze between tests (approx 1 min), and the percentage of passing trials calculated for each mouse. For Y maze analyses, the pattern of exploration 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. Notably, these same mice were subjected to both maze trials on successive days (Y maze prior to T maze to avoid any influence of reward expectations on the behavior in the Y maze), Mazes were cleaned and dried between tests to avoid influence of the prior mouse on subsequent exploration.

Neuromuscular performance was also assayed using the standard "paw grip endurance" test which quantifies the length of time each mouse can cling to a wire suspended above their cage (20-23).

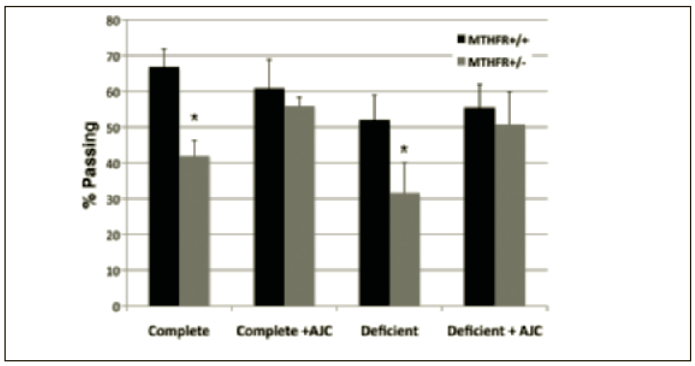
Data were derived from 2 independent experiments with 4-6 mice from each strain on each diet per experiment. Statistical comparisons were carried out with Student's t test (or individual comparisons between two diets or genotypes.

Results

MTHFR+/- mice demonstrated significantly impaired performance ($p<0.05$) in the T maze test as compared to MTHFR+/+ when maintained on both the complete diet or deficient diet. Supplementation of the complete diet with AJC for 1 month did not alter the performance of MTHFR+/+ mice, but improved the performance of MTHFR+/- mice to a level indistinguishable from MTHFR+/+ mice. Maintenance for 1 month on the deficient diet reduced the performance of MTHFR+/+ and MTHFR+/- mice in the Tmaze test by approximately 10% each compared to their respective performance when maintained on the complete diet; this did not differ significantly from their performance on the complete diet. By contrast, supplementation of the deficient diet with AJC maintained levels of performance identical to that of supplementation of the complete diet (Fig. 1).

Figure 1

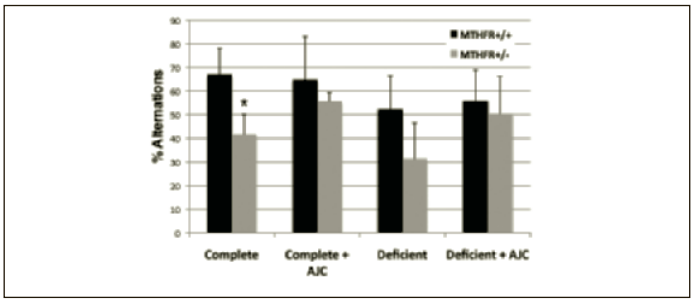
MTHFR+/- mice demonstrated impaired cognitive performance in the T maze: exacerbation by dietary folate deficiency and improvement following AJC supplementation



MTHFR+/+ and MTHFR+/- were maintained on the complete or deficient diet for 1 month then subjected to T maze analyses. MTHFR+/- mice demonstrated significantly ($p0<05$) impaired performance vs. MTHFR+/+ when maintained on the complete or deficient diet (asterisks); supplementation with AJC improved their performance to a level indistinguishable from MTHFR+/+ mice. Maintenance on the deficient diet reduced the performance of both genotypes by approximately 10% each, which did not differ significantly from their respective performance on the complete diet; supplementation of the deficient diet with AJC maintained performance levels of performance identical to that of supplementation of the complete diet.

MTHFR+/- mice also demonstrated significantly ($p<0.05$) reduced performance in the Y maze when maintained on the complete diet; supplementation with AJC improved the performance of MTHFR+/- to a level statistically identical to that of MTHFR+/+ mice (Fig. 2). Maintenance on the deficient diet reduced performance of both genotypes to a similar extent (21% and 24% for +/+ and +/- mice, respectively); these reductions did not differ significantly from performance of either genotype on the complete diet. Supplementation of the deficient diet with AJC attenuated the reduction in performance of MTHFR+/+ mice, and completely prevented the reduction in performance of MTHFR+/- mice (Fig. 2).

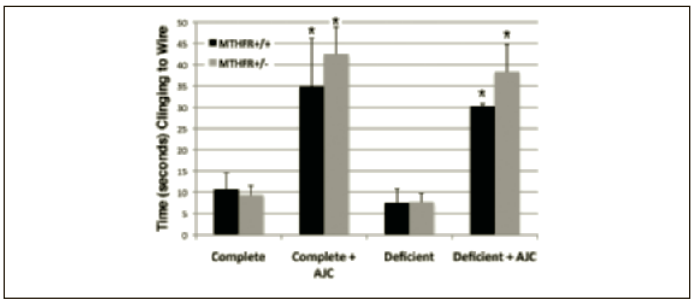
Figure 2
MTHFR+/- mice demonstrated impaired cognitive performance in the Y maze: exacerbation by dietary folate deficiency and improvement following AJC supplementation



MTHFR+/+ and MTHFR+/- were maintained on the complete or deficient diet for 1 month then subjected to Y maze analyses. MTHFR+/- mice demonstrated significantly ($p<0.05$) impaired performance vs. MTHFR+/+ when maintained on the complete diet (asterisk); supplementation with AJC improved their performance to a level statistically indistinguishable from MTHFR+/+ mice. No other values differed significantly. Maintenance on the deficient diet reduced performance of both genotypes to a similar extent, but these reductions did not differ significantly from their performance on the complete diet. Supplementation of the deficient diet with AJC attenuated the reduction in performance of MTHFR+/+ mice, and completely prevented the reduction in performance of MTHFR+/- mice.

MTHFR+/+ and +/- displayed virtually identical performance in the paw grip endurance test when maintained on the complete diet, and displayed similar, non-significant declines in performance when maintained on the deficient diet. Supplementation of either diet with AJC significantly (3-4-fold; $p<0.01$ for both genotypes on both diets) improved the performance of both genotypes (Fig. 3).

Figure 3
Supplementation with AJC improved neuromuscular performance in both MTHFR+/+ and MTHFR+/- mice



MTHFR+/+ and MTHFR+/- were maintained on the complete or deficient diet for 1 month then subjected to the paw grip endurance test. Both genotypes displayed similar performance on the complete diet, and displayed similar, non-significant declines in performance maintained on the deficient diet. However, AJC supplementation statistically ($p<0.01$) improved the performance of both genotypes for both genotypes on both diets; asterisks indicate values differing significantly from the complete diet without AJC.

Discussion

The findings presented herein indicate that supplementation with AJCs can compensate for genetic as well as dietary insufficiency in folate in a murine model of genetic folate compromise. AJC alleviated the compromise in cognitive performance displayed by MTHFR+/- versus +/+ mice, as well as prevented the additional compromise that accompanied maintenance on the deficient diet. Prior studies have demonstrated that the decline in performance following maintenance on the deficient diet was reversible following return of mice to the complete diet, indicating that impaired performance was unlikely to be due to permanent neuronal depletion (16). Rather, impaired cognitive performance was likely due to the reduction in acetylcholine that accompanied maintenance of these mice on the deficient diet (15). Notably, supplementation with AJC at levels utilized herein has been demonstrated to prevent the reduction in acetylcholine and decline in cognitive performance that otherwise accompanies maintenance of mice on the deficient diet (16, 24). In support of this possibility, cholinergic depletion itself is sufficient to induce impairments in memory in rodents (25-29).

No neuromuscular impairment was detected in MTHFR+/- versus MTHFR+/+ mice as indicated by the paw grip endurance test, and both genotypes displayed similar, non-significant declines when maintained on the deficient diet. This neuromuscular impairment could also be due to reduction in acetylcholine, but this has not been investigated. Folate is present within the neuromuscular junctions (30), and can display anti-esterase activity in neuromuscular preparations (31), and has in some cases alleviated neuromuscular compromise (32, 33). However, AJC statistically improved neuromuscular performance in both genotypes, indicating that the beneficial effects of AJC extend beyond that of cognition.

We have not attempted to determine which AJC component(s) are responsible for the beneficial effects observed herein nor in our prior studies. Whether or not constituents of apples cross the blood-brain barrier (34), the antioxidant potential of apples (35-37) reduces oxidative species within central nervous system tissue (16-18). AJC apparently provides neuroprotection beyond that of simple antioxidant potential, since, in addition to maintaining acetylcholine levels (15), supplementation with AJC compensated for multiple deleterious consequences of folate deprivation associated with Alzheimer's disease, including suppression of presenilin-1 (PS-1) overexpression (38), and increased generation as well as toxicity of Abeta (38, 39).

A critical consequence of folate deficiency is reduction of S-adenosyl methionine, the major methyl donor. Individuals harboring polymorphisms of MTHRF with diminished function can display reduced levels of SAM, which can be exacerbated by dietary insufficiency (40). The analogous situation is observed in MTHFR+/- (15). Notably, regulation of PS-1 expression, maintenance of acetylcholine levels, and efficacy of glutathione are all critically dependent upon SAM-mediated methylation events (15, 41-43). Alleviation of disregulation in all of these events following supplementation with AJC suggests that AJC may promote or maintain SAM-dependent methylation (15, 18, 38).

The increased incidence of both the C677T and A1298C MTHFR variants in young populations as a result of maternal

folate supplementation (12) underscores the possibility of a growing, latent predisposition to age-related neurodegenerative conditions such as Alzheimer's disease (44-46) that could manifest with the onset of deficiencies in nutrition/metabolism that often accompany aging (47).

Our studies in MTHFR+/- mice presented herein and in a prior report (15) support the likelihood of this possibility, since dietary folate deficiency and genetic deficiency MTHFR exert synergistic deleterious impact on cognitive performance and oxidative damage to brain tissue. These studies underscore that MTHFR polymorphisms may place individuals at increased risk for developing age-related neurodegenerative disorders, even in the presence of what is normally considered sufficient dietary folate. For example, folate supplementation several-fold above normal levels in culture medium prevented Abeta neurotoxicity in cortico-hippocampal cultures (48). Dietary folate supplementation may represent important/essential intervention for such individuals, although this has been disputed (13, 14, 49).

Supplementation with folate or SAM also provided neuroprotection in a murine model of motor neuron disease (50, 51), and has been hypothesized as potentially beneficial in humans affected with motor neuron disease (52). While no relationship of MTHFR deficiency has been established with motor neuron disease, a report of peripheral neuropathy for an individual harboring MTHFR deficiency 6 warrants further investigation.

The findings of the present study provide further support for the benefits of supplementation with AJC. While consumption of fruit and/or fruit juice, including apple, can be beneficial throughout life, it may have enhanced benefit during aging (34, 52, 53). In this regard, a recent clinical study demonstrated a positive impact of AJC on mood and behavior in individuals with advanced Alzheimer's disease (54, 55). AJC also stimulated organized synaptic activity in cultured neurons (56). The findings presented herein support the notion that supplementation with fruit or fruit juice may be more critical under conditions of latent genetic compromise.

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