



● PERSPECTIVE

## Impact of dietary supplementation of one-carbon metabolism on neural recovery

In the cell, one-carbon metabolism modulates nucleotide synthesis, DNA repair, as well as methylation through the reduction of homocysteine (Figure 1). High levels of plasma homocysteine have been associated with negative health outcomes in humans (Murray et al., 2017). Folates, B-vitamins, are a major component of one-carbon metabolism and play an important role in brain function. Specifically, they are involved in nucleotide synthesis, DNA repair, methylation, second messenger systems, ion channels, protein, and neurotransmitter synthesis, as well as the metabolism of homocysteine (Murray et al., 2017). Folate is the natural form found in foods, whereas folic acid is the chemically synthesized and often found in supplements. In 1998, the importance of folates was noted in the prevention of neural tube defects by both the Canadian and US governments and mandatory folic acid fortification laws were put into place. The neuroprotective properties of folic acid during development suggest that it may modulate growth and differentiation in the brain. Another component of one-carbon metabolism is the nutrient choline. In the brain, choline's primary role is in the synthesis of the neurotransmitter, acetylcholine, and lipid metabolism. In the rest of the body, choline also generates a methyl group to remethylate homocysteine to methionine. This reaction is done to a lesser extent in the brain.

Dietary supplementation using components of one-carbon metabolism in patients diagnosed with different neurological diseases might be effective in managing symptoms or reducing disease progression. A study published in 2010 reported that B-vitamin supplementation for 2 years reduced brain atrophy in patients with mild cognitive impairment within the UK (Smith et al., 2010). More recently, a clinical study in China showed that hypertensive patients treated with Enalapril and folic acid for 4.5 years had a reduced risk of developing a stroke compared to those treated with enalapril alone (Huo et al., 2015). Both these studies were conducted in countries that do not have mandatory folic acid fortification laws in place. It is important to note that not all individuals benefit from mandatory folic acid fortification. For example, individuals with a polymorphism in methylenetetrahydrofolate reductase (MTHFR) cannot reduce folic acid as effectively as someone without the polymorphism and therefore have increased levels of homocysteine. Interestingly, there are alternative ways to reduce homocysteine levels. For example, cytidine 5'-diphosphocholine (CDP-choline), a metabolite of choline, has been reported to aid in neural repair in the central and peripheral nervous systems as well as increase levels of acetylcholine (Arenth et al., 2011). The mechanisms through which one-carbon supplementation may change the brain to reduce disease severity are not well understood.

We recently reported that supplementation with components of one-carbon metabolism after ischemic damage to the sensorimotor cortex increased neuroplasticity and anti-oxidant activity at the damage site as well as reduced sensorimotor impairment in a mouse model (Jadavji et al., 2017). The mice in our study were maintained on a folic acid deficient diet prior to damage to increase levels of plasma homocysteine. After ischemic damage via photothrombosis we supplemented the diet of these mice with folic acid, vitamins B<sub>12</sub>, B<sub>6</sub>, and choline for 4 weeks after which motor function of animals was assessed and tissue and blood was collected. In the supplemented mice, we observed reduced impairment on the accelerating rotarod and ladder beam task, as well as increased use of impaired forelimb on cylinder task after ischemic damage. These behavioral changes were mirrored with increases in neuronal brain derived neurotrophic factor (BDNF) and immediate early gene, FosB, levels, as well as increased phospho-AKT (pAKT) expression within the damage cortex. We also reported reduced p53 levels in

supplemented mice. Additionally, both stroke and increased levels of homocysteine result in oxidative stress, so we evaluated the impact of one-carbon metabolism on antioxidant activity. We observe increased levels of nuclear factor erythroid 2-related factor 2 (Nrf2) and superoxide dismutase 2 (SOD2) at the damage site. The results from this study show that supplementation with one-carbon metabolism may be beneficial for recovery.

Damage to the spinal cord allows for detailed analysis of neuronal regeneration and dissection of potential mechanisms. The impact of folic acid on regeneration was shown in a study by Iskander et al. (2004). Adult rats underwent damage to the spinal cord using the spinal cord regeneration model. Three days prior to and after damage animals were administered folic acid. The study reported a daily dose of 80 µg/kg resulted in 54 labeled neurons per ganglion which was similar to regenerating axons on the ipsilateral side to peripheral nerve injury in untreated mice. Using the Basso, Beattie, and Bresnahan (BBB) scoring system, neurological recovery was assessed in the animals with spinal cord injury. Folic acid treatment produced significant improvement in the BBB starting at 7 days after injury and was maintained throughout the study period (42 days after injury). Additionally, folic acid supplementation after optic nerve injury increased the number of retinal ganglion cells (RGCs) per retina to 1,373 ± 73.42 compared to controls 913.4 ± 11.83. Another study from the same group reports that increased expression of the folate receptor 1 (Folr1) enables increased folic acid to enter the cell which then leads to positive benefits. Additionally, folic acid regulates Folr1 activation in a dose-dependent fashion (Iskandar et al., 2010). The interaction of folic acid and Folr1 is governed by dehydrate folate reductase (DHFR) and *de novo* methylation (Figure 1).

Recent research demonstrates a strong rationale to investigate one-carbon supplementation as an epigenetic therapeutic to counteract neurodegenerative disease including Alzheimer's disease (AD). In primary neuronal cells cultured with amyloid beta (Aβ) oligomers, cell viability and methylation status increased when folic acid was present in the media (Li et al., 2015). Methylation status in the 40 µM folic acid treatment group showed activity similar with controls including an increase in S-adenosylmethionine (SAM): S-adenosylhomocysteine (SAH) ratio, as well as reduced mRNA and protein expression of amyloid precursor protein (APP) and Presenilin-1 (PS-1) to levels observed at baseline. *In vivo*, 7-month-old APP/PS1 (APP<sup>swe</sup>/PS1<sup>dE9</sup>) mice supplemented with 600 µg/kg folic acid showed consistently lower expression of APP, PS1, and Aβ (Li et al., 2015). SAM only treatment did not alter PS-1 nor Aβ expression of mRNA or proteins in brain tissue of mice. Whereas, a combined treatment of folic acid and SAM had a similar effect as only the folic acid treatment, highlighting the key role of folic acid. Increased methylation status for both the APP and PS1 genes in the

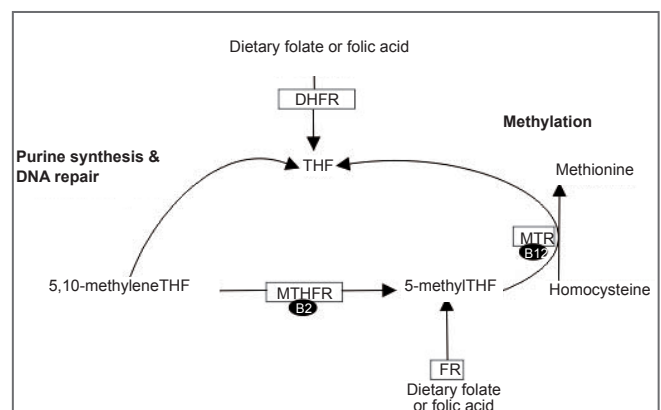


Figure 1 Simplified folate metabolism in the cell.

Dietary folate or folic acid enters the cycle and is involved in the synthesis of purines, DNA repair or methylation through reduction of homocysteine levels. DHFR: Dihydrofolate reductase; FR: folic receptor; MTHFR: methylenetetrahydrofolate reductase; MTR: methionine synthase; THF: tetrahydrofolate.



supplemented diet compared to folate deficient diet was reported both *in vitro* and *in vivo*. Overall, these data suggest that supplementation with folic acid may regulate gene expression thereby reducing the magnitude of AD pathology.

The mechanisms through which one-carbon metabolism affects AD progression have been investigated. Using a mouse model, animals were subjected to hypobaric hypoxia (5,500 meters for 4 weeks) using a decompression chamber or normoxic conditions after supplementation with one-carbon metabolism components (Yu et al., 2016). Supplementation consisted of 5-fold higher dose of B-vitamins (folic acid, vitamins B<sub>6</sub> and B<sub>12</sub>), choline, or combination of both prior to hypoxic treatment. Hypoxia exposure induced significant impairments in working and spatial memory, similarly characterized in AD patients, these impairments were reduced when animals were supplemented with B-vitamins. Tau hyperphosphorylation is one hallmark of AD and was reported both in cortical and in hippocampal tissue of hypoxic mice. Between the supplemented groups, the choline treatment provided a greater benefit than B-vitamins in maintaining normal tau phosphorylation after hypoxia, which is beneficial for AD pathology. Although B-vitamin treatment alone was beneficial, choline treatment as well as a combined treatment significantly improved tau phosphorylation after hypoxia in both cortical and hippocampal tissue. Homocysteine concentration was significantly elevated after hypoxia compared to controls and was reduced in all treatment groups, showing synergistic effects. These findings suggest that supplementation with components of one-carbon metabolism after hypoxia may be effective at reducing AD related pathology in mouse models.

The effects of folate supplementation have also been examined in animal models of Parkinson's disease (PD). In a study by Haghdoost-Yazdi et al. (2012), the effect of supplementation with several B vitamin doses and combinations was examined both behaviourally and on levels of homocysteine. A model for PD-associated dopaminergic degeneration using the neurotoxin 6-hydroxydopamine (6-OHDA) was stereotaxically administered into the striatum of mice. The group that received 10× the amount of folic acid normally found in the diet performed better on rotational behaviour testing, with 60% fewer rotations post-surgery at both test points compared to control group. The same pattern was observed for rotarod, with this group as well as the 5× folic acid and the B complex groups performing at close to control levels. Contrary to what was expected, levels of homocysteine were not reduced in these or any group, and were in fact elevated compared to control levels. This finding suggests that the mechanism by which folic acid supplementation limits the effect of 6-OHDA is not through decreasing amounts of homocysteine, as was theorized. Unfortunately, authors did not quantify dopaminergic cells in either the striatum or substantia nigra. Srivastav et al. (2015) studied the effect of supplemental folate in a *Drosophila* model of early-onset familial PD. A novel recessive allele for the *Parkin* gene was used, producing reduced mRNA and null amounts of the Parkin protein, which is involved in the degradation of unfolded proteins and is also linked to proper mitochondrial function. This resulted in several deficiencies in homozygous flies, including increased lethality at the pupal stage, decreased transition rates to later life stages, and impaired motor function. It also increased oxidative stress, reduced antioxidant activity, and affected mitochondrial functionality, as evidenced by lower levels of ATP. In flies given a 10 to 250 μM effective dose of folic acid, these deficiencies were at least partially reversed. Lethality was reduced, more flies transitioned to later life stages, and motor function improved. In addition, levels of oxidative stress decreased and the amount of ATP present increased, suggesting improved mitochondrial function. Based on these results, folic acid supplementation may be useful for attenuating some of the effects of dopaminergic degeneration associated with PD.

Animal models have been used to describe mechanisms through which dietary supplementation with one-carbon metabolism components has a beneficial role in regeneration within the central nervous system. Functional benefits from supplementation has been reported in both animal models and humans. However, the regenerative properties of folate may not be apparent in populations with mandatory folic acid fortification laws in place. It is important

to note that the aging process reduces the ability to absorb many needed nutrients and vitamins from our diet. Therefore, dietary supplementation with one-carbon metabolism components could be considered for this population even in countries with mandatory folic acid fortification.

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