Folate and Vitamin B\textsubscript{12}: Function and Importance in Cognitive Development

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Abstract

The importance of the B vitamins folate and vitamin B\textsubscript{12} for healthy neurological development and function is unquestioned. Folate and vitamin B\textsubscript{12} are required for biological methylation and DNA synthesis. Vitamin B\textsubscript{12} also participates in the mitochondrial catabolism of odd-chain fatty acids and some amino acids. Inborn errors of their metabolism and severe nutritional deficiencies cause serious neurological and hematological pathology. Poor folate and vitamin B\textsubscript{12} status short of clinical deficiency is associated with increased risk of cognitive impairment, depression, Alzheimer’s disease and stroke among older adults and increased risk of neural tube defects among children born to mothers with low folate status. Folate supplementation and food fortification are known to reduce incident neural tube defects, and B vitamin supplementation may have cognitive benefit in older adults. Less is known about folate and vitamin B\textsubscript{12} requirements for optimal brain development and long-term cognitive health in newborns, children and adolescents. While increasing suboptimal nutritional status has observed benefits, the long-term effects of high folate intake are uncertain. Several observations of unfavorable health indicators in children and adults exposed to high folic acid intake make it imperative to achieve a more precise definition of folate and B\textsubscript{12} requirements for brain development and function.
are critical for central nervous function, such as the epigenetic regulation of
gene expression by DNA and histone methylation, the methylation of myelin
basic protein and membrane phosphatidylcholine, the synthesis of hormones
and neurotransmitters such melatonin and epinephrine, and the inactivation of
dopamine and catecholamines. Folate (in the form of 5-methyltetrahydrofolate)
and vitamin B\textsubscript{12} are required in all tissues to maintain adequate cellular methionine, which in turn is converted to S-adenosylmethionine the universal methyl
donor for these reactions. Methylation produces the sulfur amino acid homocysteine, which serves as precursor for the cyclical regeneration of methionine.
Choline is another important dietary methyl donor, providing an alternative
source of labile methyl groups for regeneration of methionine by an alternative
pathway which is only expressed in the liver and kidney.

Disrupting these pathways can have wide-ranging direct and indirect con-
sequences for neural tissue. Folate and B\textsubscript{12} deficiency inhibit methylation and
cause homocysteine to accumulate in circulation. Homocysteine may be cyto-
toxic, and mildly elevated plasma homocysteine is associated with increased
risk of neurodegenerative and cardiovascular disease. By increasing choline
utilization or by depleting membrane phosphatidylcholine, folate deficiency
might also indirectly limit the synthesis of the key neurotransmitter ace-
tylcholine. Folate and B\textsubscript{12} are also required for activity that is independent
of each other. Folate is needed to synthesize thymidylate and purine nucle-
otides. Thus, folate deficiency can compromise DNA replication and integ-
rity. Vitamin B\textsubscript{12} serves as a coenzyme for methylmalonyl-CoA mutase which
catalyzes the final step in the mitochondrial degradation of odd chain fatty
acids and branched amino acids. Thus, in addition to impinging upon folate
and methylation, B\textsubscript{12} deficiency leads to abnormal membrane fatty acid com-
position and elevation of circulating methylmalonic acid. These biochemical
effects of deficiency can be theoretically linked to the observed neuropa-
thological, cognitive and developmental abnormalities that are associated with
deficiency of these vitamins [1].

Neurological Consequences of Acquired and Congenital Defects in One-
Carbon Metabolism

The importance of the B vitamins folate and vitamin B\textsubscript{12} for healthy brain develop-
ment and function is evident in the serious neurological consequences of
acquired nutritional deficiencies and inborn errors of their metabolism. Severe
acquired deficiencies of vitamin B\textsubscript{12} and folate manifest with either hematologi-
cal or neurological abnormalities or a combination of both [2–6]. A large pro-
portion of cases develop neurological symptoms without exhibiting any major
hematological abnormality, and this tendency is thought to be more prevalent
among the elderly [7]. The primary neurological feature is a progressive
neuropathy in which sensation and motor control are gradually lost, typically beginning with the lower limbs [6]. In addition, these conditions are often accompanied by behavioral changes ranging from mild irritability to severe depression, hallucinations, confusion and memory loss [8]. If the condition is left untreated, it may eventually lead to sub-acute combined degeneration of the spinal cord. The dominant histopathological finding in this condition is a patchy loss of myelin in the white matter of the spinal cord where myelin-laden macrophages surround the empty spaces, although brain and peripheral myelin are also affected [7]. Prognosis depends on intervention. Although vitamin supplementation is an effective treatment in many cases, cumulative neurodegenerative damage may become irreversible if it is allowed to progress too far. It is not clear whether the behavioral changes are due to vitamin-related neurochemical imbalances or to structural changes in the brain caused by demyelination [8].

Genetic impairment of one-carbon metabolism can produce similar pathology. For example, mutations that severely limit the synthesis of 5-methyltetrahydrofolate by methylene tetrahydrofolate reductase (MTHFR) typically result in abnormal neurological development, progressive demyelinating neuropathy and cognitive impairment [9–11]. Although congenital defects in MTHFR activity usually become evident soon after birth, they have been known occasionally to remain silent until the onset of neurological symptoms and psychotic or schizophrenic behavior in adolescence or adulthood [12–15]. Gene–gene and gene–nutrient interactions appear to influence the severity and expression of phenotype in these cases. This is implied by cases where some family members carrying the same mutation are symptomatic while others are not [13, 14]. In many cases, MTHFR deficiency is partially responsive to supplementation with folate or betaine [12, 13]. Similar findings are observed in mice with a targeted mutation in the MTHFR gene. In homozygotes for the mutation, CNS development is compromised, but this can be partially mitigated by dietary supplementation with betaine [16]. Cerebral folate deficiency is another example of folate-dependent impairment of CNS development and function. In this rare pediatric autoimmune condition, auto-antibodies to the choroid plexus high-affinity folate transporter are associated with low cerebrospinal folate despite normal serum folate, and with progressive impairment of neurological development, myelination and cognition [17]. In some cases, the symptoms can be partially mitigated, at least temporarily, by folinic acid (5-formyl tetrahydrofolate), which can enter the CNS through an alternative reduced folate transporter. Vitamin B12 deficiency in newborns and infants who have smaller liver and tissue stores [18] is often due to maternal deficiency. Symptoms of clinical deficiency can be treated with intramuscular injections and high-dose oral supplementation, leading to hematologic and neurologic recovery; however in the long-term, resolving the deficiency and acute pathology does not guarantee full restoration of cognitive development [19].
Subclinical Deficiencies in CNS Development and Aging

The neuropathology common to both congenital and severe acquired vitamin \( B_{12} \) and folate deficiencies demonstrates the importance of methylation for brain development and function, yet these deficiencies are relatively rare. More often, individuals who fall in the lower range of population folate and vitamin \( B_{12} \) intake and plasma concentrations, but who are not clinically deficient, have significantly higher risk of poor neurological and cognitive outcomes. These include increased risk of neural tube defects (NTDs) among children born to mothers with low folate status, and increased risk of cognitive impairment, depression, Alzheimer’s disease and stroke among older adults.

With respect to aging, the association between low B vitamin status and increased risk of neurodegenerative disease has been extensively reviewed elsewhere [20, 21]. Data from over 100 observational cross-sectional and prospective studies, encompassing over 50,000 subjects in total, provide compelling evidence for the association, with approximately 90% of the studies reporting significant associations and the remainder not. Taken together, they provide the basis for the tenable hypothesis that ‘low-normal intake or blood concentrations of B vitamins (folate, \( B_{12} \) and \( B_6 \)) and/or moderately elevated plasma total homocysteine increase the risk of brain atrophy and developing cognitive impairment in the elderly’ [1, 21, 22]. As demyelination is not a dominant feature of these diseases, a variety of alternative mechanisms has been proposed to account for these associations, relating both vitamin deficiencies and elevated homocysteine to different aspects of cognitive dysfunction and neuropathology [23]. The efficacy of B vitamin supplementation for neurocognitive protection has yet to be determined, with several trials reporting null findings [24–26] and others suggesting benefit [27, 28].

With respect to early life, the prevention of NTDs by folic acid provides strong evidence of folate’s critical importance to CNS development, although the precise mechanism for the phenomenon is still unknown. Early observations that poor folate status is associated with increased risk of spina bifida and anencephaly led to highly successful clinical trials in which use of periconceptual folic acid supplements reduced the risk of incident NTDs by as much as 50% or more [29]. Further evidence for folate’s importance for neural development comes from genetic association studies. A meta-analysis of more than 17 different studies including several thousand subjects calculated a pooled odds ratio giving an approximately twofold increase in risk of an NTD for mothers and children who are TT homozygotes compared with CC homozygotes for the C677->T single nucleotide polymorphism in the MTHFR gene, where the TT genotype encodes a slightly less active enzyme. Risk for NTDs among the TT homozygotes decreased with increased folate status [30]. Folate supplementation before conception is critical for preventing NTDs given that neural tube closure occurs early in embryonic development when the mother may not be
Folate and Vitamin B12: Function and Importance in Cognitive Development

aware of the pregnancy. Despite this, most women of childbearing age do not take supplements, particularly those at risk for nutritional deficiency. For this reason, mandatory food fortification of all cereal grains with folic acid has been enacted in over 50 countries. These public health programs have been highly successful with respect to reduction of incident NTDs. They have also significantly increased folic acid intake across populations and lowered the prevalence of hyperhomocysteinemia. In the Framingham study in the USA, the prevalence of folate deficiency decreased from 4.9 to 1.9% after fortification [31], but at the same time, the proportion of individuals exceeding the recommended upper intake limit of 1 mg folic acid/day grew from 1.3 to 11.3% [32]. Interestingly, following fortification, vitamin B_{12} and choline status have been found to determine NTD risk [33, 34].

Folate and B_{12} Supplementation and Cognitive Development

All of these examples relate to pathological consequences of vitamin deficiency. But what effect does supplementation beyond replacement have on brain development and attainment of full intellectual potential? Here, the data are surprisingly scarce. Answering this question is important in light of the possibility that exposure to high levels of folic acid may have unintended effects, for better or for worse. The upper limit of folate intake for adults was conservatively set at 1 mg per day out of concern for the possibility that in individuals with frank B_{12} deficiency, high folate might either exacerbate the symptoms or ‘mask’ the associated anemia, delaying early detection and treatment and thus allowing it to progress [35]. This concern relates to a relatively small though not insignificant proportion of the population. There is deeper controversy over whether fortification has increased cancer risk and decreased risk of cardiovascular disease and stroke [36–38]. Finally, despite scant data, concerns have also been raised over theoretical mechanisms through which high folate intake might exert subtle but substantial life-long effects on metabolism and neurological development through altered epigenetic programming [39, 40].

A small number of recent observations show unexpected evidence for metabolic interaction of high folate status with low-marginal B_{12} status. Cross-sectional data from the NHANES and SALSA cohorts show that the metabolic effects of vitamin B_{12} deficiency are more pronounced in adults with high folic acid intake. MMA and Hcy levels are elevated in individuals with low B_{12} and high folate status compared to those with normal folate and normal or low B_{12} [41, 42]. These metabolic findings are mirrored by poorer neuropsychological test scores on the symbol digit test and by lower hemoglobin values [43]. Similar associations between worse metabolic and functional impairments with B_{12} deficiency were found when unmetabolized folic acid in plasma was used as a putative proxy for high folic acid intake [44]. Irrespective of B_{12} status, high folic acid intake has also
been observed to associate with more rapid cognitive decline in older adults [45] and with decreased immune function in older postmenopausal women [46].

The same pattern of a potentially undesirable interaction between high folate and low $B_{12}$ has been seen in children. A large study of 2,812 Columbian school children aged 5–12 years found that hemoglobin concentrations decreased with increasing erythrocyte folate [47]. There was a significant interaction between folate and $B_{12}$ status such that differences in hemoglobin concentrations between the highest and lowest quartile of folate were greatest in children with low $B_{12}$ status ($<148$ pmol/l) followed by marginal $B_{12}$ status (between 188–221 pmol/l) and least in $B_{12}$-sufficient children. Notably, the prevalence of anemia in this cohort was only 3.7%, and data were adjusted for relevant biological and socioeconomic factors. The same interaction pattern has been observed in an entirely different setting, the Maternal Nutrition Study in Pune, India, where higher maternal folate during pregnancy predicted greater insulin resistance in offspring 6 years later, particularly in mothers with low $B_{12}$ [48].

Cognitive development was not reported in these studies; however, a handful of other studies yield inconsistent associations between maternal folate and $B_{12}$ status with cognitive development in children. In a cohort of 536 children from Mysore, India, higher maternal folate was associated with better performance on a battery of neuropsychological tests after appropriate adjustment for confounding, irrespective of vitamin $B_{12}$ status, and despite the fact that 42.5% of mothers had low vitamin $B_{12}$ during pregnancy. In this cohort, maternal $B_{12}$ and homocysteine concentrations did not predict children's cognitive performance [49]. In contrast to these findings, maternal $B_{12}$ status predicted cognitive performance in 9-year-old offspring in a small subset of the Pune cohort. Children born to mothers in the lowest $B_{12}$ decile ($<77$ pmol/l) performed significantly worse than those whose mothers were in the highest decile of $B_{12}$ ($>224$ pmol/l) on two out of 7 neuropsychological tests used for the examination (digit span backward and color trail test A). Plasma folate, homocysteine and methylenemalonic acid were not associated with cognition [50]. A cross-sectional study of 598 Indian children aged 6–10 years revealed an unexpected inverse association between plasma $B_{12}$ status and factor scores for cognitive domains of short-term memory, retrieval ability and an overall composite score for mental processing. In this cohort, increasing hemoglobin concentration was positively associated with better cognitive performance, but iron, iodine, fatty acids and folate showed no association [51]. A comparison of cognitive performance among adolescents (age 10–16) who were either vegan-macrobiotic or omniverous until the age of 6, found poorer performance on cognitive tests among the previously macrobiotic group, which also had a higher prevalence of low but not deficient vitamin $B_{12}$ status. Finally, an early study found severe mental retardation among children born to mothers with folate deficiency [3], but a more recent study found no relation of neuropsychological development in 5-year-old children to maternal folate status during the second half of pregnancy [52].
A Role of Folate and Vitamin B$_{12}$ in Epigenetic Programming of Neurocognitive Development?

The prevention of NTDs by relatively modest doses of folic acid demonstrates the profound effect that folate-dependent metabolism has during CNS development. However, there is no reason to assume that the mechanism for this effect is specific only to neural tube closure. A leading hypothesis for the mechanism involves epigenetic mechanisms – the stable and heritable regulation of gene without altering gene sequence that is regulated by DNA and histone methylation. Epigenetic patterns that are established early in life program life-long effects on development, function and aging. Aberrant DNA methylation is critically involved in the neurodevelopmental disorders Praeder Willi and Angelman syndromes, and is also hypothesized to be involved in Rett syndrome, schizophrenia and autism [53, 54]. Moreover, recent animal studies have shown that experience-dependent, reversible changes in DNA and histone methylation are critically involved in the regulation of synaptic plasticity, learning and memory in nondividing neurons of the adult brain. Thus, it is reasonable to hypothesize that even mild alterations of methylation during brain development as a function of intake of folic acid, vitamin B$_{12}$, choline or other nutrients would have long-lasting impact on brain development and function [55, 56].

Evidence that methyl donor availability can determine stable epigenetic changes in brain development and cognitive function comes largely from animal studies. In rodents, providing dams with choline at critical windows during embryonic development changes DNA methylation and gene expression in the brain of the offspring, induces structural changes involving neuronal stem cell proliferation and angiogenesis in hippocampus, and enhances memory. Moreover maternal choline supplementation prevents normal age-related cognitive decline in elderly rats [57]. However, the beneficial effects of supplementation may have upper limits. Folic acid has been shown to significantly improve nerve regeneration following spinal cord injury in adult rats. The effect of folate followed a nonlinear dose-dependent relationship with regeneration, where the enhancement of regeneration peaked at 80 μg folic acid per kg body weight and declined significantly thereafter. This inverse U-shaped relationship was paralleled by increasing expression of the DNA methyltransferase enzyme 3 (DNMT3) up to 80 μg/kg and decreased expression at higher doses [58].

Although data from human studies are limited, the available data show that even modest supplementation within the normal range of dietary intake can have significant epigenetic effects. A recent study documented the effect of using periconceptual folic acid (400 μg/day) on epigenetic regulation of the insulin-like growth factor 2 gene (IGF2) in offspring. Lymphocyte DNA methylation in the promoter region of the IGF2 gene was significantly (4.5%) higher in children born to mothers who took folic acid supplements (n = 86), compared to those not exposed to folic acid (n = 34). Although birthweight was similar in the
two groups, a 1.7% higher methylation was associated with one standard deviation decrease in birthweight (584 g) independent of periconceptual exposure to folic acid or gestational age at delivery [59]. Another human study found a nonlinear U-shaped association of plasma folate with DNA telomere length in a non-fortified population of older Italian adults. Telomere length (a marker for biological aging) declined with increasing folate until the median folate level, and then increased again as folate increased above the median [60]. The authors speculated that epigenetic mechanisms were involved. If such changes to peripheral DNA are also reflected in the brain, this would suggest that brain development, cognitive function and aging can be modulated by folate and B<sub>12</sub> availability across the current range of vitamin intake from diet, fortification and supplement use. Given the clear importance of folate and vitamin B<sub>12</sub>-dependent metabolism in brain development and function, it would not be surprising if these processes also influenced the development and realization of full cognitive and intellectual potential.

Conclusions

Taken together, the demonstrable importance of folate supplementation for NTD prevention, the paucity of data on the impact of folic acid and B<sub>12</sub> supplementation on neurocognitive development in human populations, the observation of both favorable and potentially unfavorable outcomes in association with folate intake alone or in association with low B<sub>12</sub> status, and the uncertainty regarding the mechanisms that underlie such observations all suggest it is important to improve folate and vitamin B<sub>12</sub> status in deficient populations, possibly in conjunction with choline, and to avoid excess. In order to improve on current guidelines and recommendations by revisiting the upper limits, and to delineate their risks and benefits, we require a much more precise understanding than is currently available of the impact of these nutrients on brain development and cognitive function, and the mechanisms through which they act on the brain throughout the lifespan.

References


