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Editorial

Homocysteine and Folate Status in an Era of Folic Acid Fortification: Balancing Benefits, Risks, and B-vitamins

Mandatory folic acid (vitamin B_9) fortification of flour products to a level of 140–150 μg/100g was implemented in the US and Canada in 1998. The rationale was to improve folate status in women of childbearing age and thereby decrease the incidence of neural tube defects (NTDs). Studies with complete ascertainment of prenatally diagnosed NTD cases indicate that fortification has prevented up to 50% of NTDs *(1)*. Fortification has therefore been highly successful in achieving its primary objective.

Folate concentration in serum and erythrocytes is a marker of folate status, which is inversely associated with the plasma concentration of total homocysteine (tHcy). Cobalamin (vitamin B_{12}) status and renal function are also strong determinants of plasma tHcy *(2)*, and moderate hyperhomocysteinemia is associated with an increased risk of chronic diseases, including cardiovascular disease, dementia, and impaired cognition *(3)*.

In this issue of *Clinical Chemistry*, Pfeiffer and colleagues *(4)* report on concentrations of tHcy in approximately 26 000 participants in the National Health and Nutrition Examination Survey (NHANES) from 1991 through 1994, and from 3 postfortification surveys (1999–2004).

When comparing the postfortification to the prefortification period, Pfeiffer and colleagues found a tHcy decrease of approximately 10%, and a pronounced decrease in the prevalence of individuals with elevated tHcy (>13 μmol/L). These observations complement recent data published by the same authors, which demonstrate an increase in serum and erythrocyte folates, and no change in serum cobalamin in NHANES samples during the same time period *(5)*. Results from these recent studies by Pfeiffer et al. confirm previous studies on folate and homocysteine in NHANES *(6, 7)* and the Framingham cohort *(8)*.

A slight decrease in blood folate between the first (1999–2000) and third (2003–2004) postfortification periods *(5)*, attributed to a decreased fortification level and promotion of low-carbohydrate weight-loss diets *(6, 9)*, was not associated with a significant increase in tHcy *(4)*. This finding may reflect the fact that tHcy is a sensitive indicator of low or low-normal folate status *(3)*, whereas the postfortification decrease in blood folate between 1999–2000 and 2003–2004 involved the portion of the population with the highest folate status *(5)*. Quinlivan and Gregory addressed the consequences of lower folate intake in women of childbearing age, and predicted the resulting increase in NTD incidence to be moderate (approximately 5%), and less than if the reduction had been uniform across the whole folate distribution *(9)*.

The decrease in tHcy after folic acid fortification has been related to the acceleration in the decline in stroke mortality that was observed in the US and Canada after 1998. Notably, no such change in the decline in stroke mortality was observed in England and Wales, where fortification with folic acid is not mandatory *(10)*. These observations, from what could be considered a quasi-experimental intervention, were essentially in agreement with results from reanalyses of data from 2 recent secondary intervention trials with folate, vitamin B_6, and cobalamin in cardiovascular patients (Hope-2 and VISP), demonstrating reduced risk of stroke following high-dose vitamin therapy in patients with normal kidney function and without cobalamin deficiency *(11)*.

Coronary heart disease may respond differently to homocysteine-lowering therapy with B-vitamins, as indicated by 4 recent secondary intervention trials (VISP, HOPE 2, NORVIT and CHAOS-2) that demonstrated no decreased risk *(12)*. Treatment effects may have been blunted in the background of low tHcy in areas of folic acid fortification (the VISP and HOPE 2 trials). But negative results were also obtained in 2 trials (NORVIT and CHAOS-2) carried out in nonfortified populations. There is therefore no evidence of beneficial effects from short-term (<5 years) folic acid supplementation in patients with established coronary heart disease *(12)*.

A small (6%) but significant decline in the prevalence of orofacial clefts in the US occurred following the implementation of folic acid fortification *(13)*. Moreover, folic acid supplementation may protect against adverse pregnancy outcomes, including placental abruption *(14)*, and reduce the rate of cognitive decline *(15)* and risk of cancer, in particular colorectal cancer *(16)*. However, the view that folate protects against cancer has recently been challenged by results from 3 large intervention trials with folic acid. In the Aspirin/Folate Polyp Prevention Study, folic acid supplementation for 6 years did not protect against adenoma recurrence, but increased the number of adeno-

mas and the incidence of advanced lesions. In the NORVIT and HOPE-2 trials, there was a trend towards increased risk of total cancer or colon cancer in the treatment groups *(16)*. Finally, Mason et al. *(17)* reported on a sudden reversal in the decline in colorectal cancer in US and Canada at the time of implementation of fortification. These observations in humans should be considered in the light of results from experimental studies indicating that folic acid supplementation and high folate status may promote the progression of already existing preneoplastic or neoplastic lesions (the so-called cancer acceleration phenomenon) *(16)*. Different mechanisms may prevail in normal tissues, however, in which carcinogenesis may be promoted by folate deficiency and suppressed by excess folate *(16)*.

An upper tolerable intake for folic acid from supplements and fortified foods was somewhat arbitrarily set at 1000 μg/day for adults by the Institute of Medicine, primarily to avoid the alleged masking of cobalamin deficiency *(18)*. There have been concerns that a significant portion of the US population, in particular older supplement users, is exposed to amounts of folic acid exceeding the upper tolerable intake *(19)*. Unmetabolized folic acid in blood has been detected in individuals taking vitamin supplements *(20–22)*. There may be direct adverse effects from unmetabolized folic acid in blood, which was found to be inversely related to natural killer cell cytotoxicity, a component of the nonspecific immune response *(20)*. Moreover, folic acid is a fully oxidized folate species, which enters the folate pool by a route involving reduction to dihydrofolate and further reduction to tetrahydrofolate. It has been hypothesized that folic acid in this way bypasses the so-called methylfolate trap, which may occur in cobalamin deficiency. The resulting imbalanced folate metabolism may explain the lack of macrocytosis under conditions of clinically significant cobalamin deficiency *(23)*.

In a folic acid–fortified population, cobalamin status rather than folate status is the predominant cause of increased tHcy *(24)*, a situation that may enhance the utility of tHcy measurements in the assessment of cobalamin status. This finding is important because the proportion of individuals with the combination of low cobalamin and no macrocytosis has increased after implementation of folic acid fortification, and the lack of macrocytosis may delay the diagnosis of cobalamin deficiency *(25)*.

There even have been concerns that folic acid intake in cobalamin-deficient individuals may aggravate clinical manifestations, and a recent study demonstrated that high folate increased the risk of cognitive impairment and anemia in seniors with low cobalamin status *(23)*. In cobalamin-deficient seniors, both tHcy and methylmalonic acid increased with serum folate above 20 nmol/L *(26)*. This metabolic response indicates that high doses of folic acid from fortified food or supplements causes a disruption of intracellular cobalamin metabolism in cobalamin-deficient individuals.

The ability to normalize the metabolic indices of mild cobalamin deficiency by oral supplementation with high doses of cobalamin has been established *(27)*, and a recent study demonstrated that low oral doses of crystalline cobalamin, in the range of 5–10 μg/day, increased serum cobalamin in elderly individuals with low to normal serum concentrations due to food-bound cobalamin malabsorption, which is an important cause of low cobalamin in the general population *(28)*. Cobalamin fortification of food should be considered as a public health strategy to improve cobalamin status in older adults. To restore cobalamin stores in depleted individuals, the daily cobalamin doses required would probably exceed the recommended daily allowance of 2.4 μg/day, but there is still no consensus on the appropriate level of cobalamin fortification *(22)*.

In conclusion, the marked reduction in the incidence of NTDs is an indisputable benefit of the folic acid fortification policy in the US and Canada. In addition, fortification may reduce the occurrence of diseases related to folate deficiency and hyperhomocysteinemia. These benefits should be weighted against possible adverse effects in the elderly, in whom preneoplastic lesions and cobalamin deficiency are common. However, the masking of cobalamin deficiency may be overcome by cobalamin supplementation. The folate-cobalamin interaction exemplifies the complexity of the targeted metabolic network, which also involves nutrients such as riboflavin, vitamin B_6 *(29)*, and betaine *(30)*. Future fortification regimens to prevent chronic disease related to one-carbon metabolism should be optimized to provide balanced and physiological doses of other B-vitamins in addition to folic acid.

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