

SUPPLEMENTAL MATERIAL

Potential for Elimination of Folate and Vitamin-B₁₂ Deficiency in India Using Vitamin-Fortified Tea—A Preliminary Study

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SUPPLEMENTAL METHODS

Blood Tests. Women between 18- to 25-years from the Kulloli Institute of Nursing Sciences, Sangli, with no medical history of illness in the past 6-months, who were not consuming iron or multivitamins and were not pregnant, were invited to help us assess their baseline folate and vitamin-B₁₂ status ($n=60$). Venous blood was drawn from these women at the Ghodawat Diagnostic Center, Sangli, to assess basal serum folate and vitamin-B₁₂ levels. Of 5-mL drawn from each woman, 1-mL was sent for a complete blood count at the Ghodawat Diagnostic Center and the remainder processed for measurement of serum folate and vitamin-B₁₂. Following separation of serum within 1-hour of blood collection, samples were kept chilled in ice-packs during courier transport to the Thyrocare Laboratory, Navi Mumbai, where analysis of serum folate and vitamin-B₁₂ levels was performed within 1-day of receipt using a chemiluminescence immunoassay. Normal values for serum folate from this laboratory was over 5.4-ng/ml [coefficient of variation (% CV) <10%]; the reference range for normal vitamin-B₁₂ was 211- to 911-pg/ml [intra-assay (% CV) 4%, inter-assay (% CV) 4.4%].

SUPPLEMENTAL RESULTS

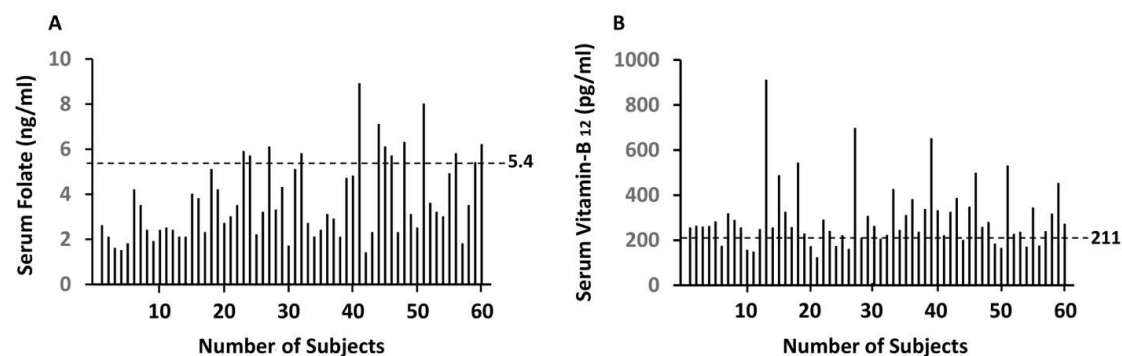
Preliminary data on preferences of Indian women of childbearing age. While conducting several educational lectures on the importance of optimizing nutrition among scores of young Indian women attending undergraduate colleges in several states in the North (Punjab), West (Maharashtra), East (Uttar Pradesh and Bihar), and South India (Kerala and Tamil Nadu), we also carried out informal focus group assessments on their propensity to regularly take iron-vitamin supplements. Most complained of having experienced side effects of abdominal discomfort, nausea, bloating, constipation, diarrhea, which are in large part the side effects of oral iron. As a result, the vast majority of these women uniformly expressed a strong aversion and resistance to taking daily supplements of vitamins in tablet form, despite being educated on the potential adverse risks of low folate and vitamin-B₁₂ deficiency to their future progeny. However, they unanimously and enthusiastically voiced preference for a food-based approach from within their extant diet to supplement their low dietary intake of folate and vitamin-B₁₂ (Unpublished Observations).

Dissolution of the vitamins, camouflage and organoleptic assessment of vitamins dissolved in brewed tea. Both pure USP-grade folic acid (yellow color) and vitamin-B₁₂ (brilliant red color) are stable for several months at room temperature when protected from light, and are water soluble. Organoleptic testing of folate and vitamin-B₁₂ dissolved in water was informally assessed in the USA among women of Indian origin; when diluted to final concentrations of 1- to 5-mg per cup of water, both pure folate and vitamin-B₁₂ imparted a faint yellow and pink color, respectively, but were both tasteless in water, and did not impart any altered smell or taste to tea (with or without added milk and sugar). Following the addition of either 1-mg of folate or 1-mg vitamin-B₁₂ (from

a stock solution dissolved in water) to a teabag, and drying, there was an immediate release of each of these colored vitamins from the teabag into a cup of room temperature water. This predicted that both vitamins would likewise be readily released from teabags during the brewing of hot tea. Moreover, addition of 1-mg of folate and vitamin-B₁₂ to a teabag steeped in hot water for 2-5 minutes was easily camouflaged in the amber-reddish colored brewed tea. Similar studies using golden-yellow and super-red food coloring agents [for mock-fortified tea] led to similar results as observed with the vitamins. Thus, fortification of tea with either vitamins or food coloring agents led to no adverse organoleptic effects to brewed tea. These findings predicted that upon daily consumption of folate- and vitamin-B₁₂-fortified tea, most women with low folate and vitamin-B₁₂ status would respond with a measurable rise in their serum vitamin levels; indeed, the results observed among women in the experimental arm (*Main Document*), supported these assumptions.

Distribution of low folate and vitamin-B₁₂ status among women of childbearing-age in Sangli:

As shown in **Supplemental Figure S1A**, 48 of 60 (80%) nursing students of the Kulloli Institute of Nursing Sciences (Sangli) had serum folate levels consistent with frank folate deficiency (less than 5.4 ng/ml, when measured at Thyrocare Lab, Navi Mumbai). There were an additional 9 of 60 women with serum folate levels in the low-normal range (between 5.4- to 7-ng/ml). Thus, a full 57 of 60 (95%) of these women had low-folate status (less than 7-ng/ml). In addition, **Supplemental Figure 1B** revealed that 14 of 60 (23%) women had serum levels of vitamin-B₁₂ consistent with frank vitamin-B₁₂ deficiency (less than 211-pg/ml, when also measured at Thyrocare Lab). An additional 24 of 60 women had vitamin-B₁₂ levels in the low-normal range (between 211- to 300-pg/ml). These data indicated that 38 of the 60 women studied (63%) had low-vitamin-B₁₂ status.



Supplemental Figure S1. Distribution of the baseline concentration of serum folate (Panel A, left) and serum vitamin-B₁₂ (Panel B, right) among 60 women of childbearing-age in Sangli (average age = 22 ±3 SD). Blood samples were assayed for serum folate and vitamin-B₁₂ at Thyrocare Lab (Navi Mumbai). The serum folate and vitamin-B₁₂ levels for each subject along the horizontal axes are coincident when viewed from left to right. The horizontal dashed line across the *left* panel (----) depicts the cut-off level of 5.4-ng/mL, below which the serum folate level in any subject is consistent with frank folate deficiency. The horizontal dashed line across the *right* panel (----) depicts the cut-off level of 211-pg/mL, below which the serum vitamin-B₁₂ level in any subject is consistent with frank vitamin-B₁₂ deficiency.