JOURNAL CLUB

Does Supplementation With Vitamin B_{12} and/or Folic Acid Improve Growth?

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Summary

In this randomized, placebo-controlled, double-blind trial in 1000 North Indian children, the investigators measured the effect of giving daily (for 6 mo) vitamin B_{12}, folic acid, or the combination of both on linear and ponderal growth. They also identified predictors for growth in multiple linear regression models and effect modifiers for the effect of folic acid or vitamin B_{12} supplementation on growth. The overall effect of either of the vitamins was significant only for weight; children who received vitamin B_{12} increased their mean weight-for-age z scores (WAZ) by 0.07 (95% CI 0.01, 0.13). WAZ and height-for-age z scores (HAZ) increased significantly after vitamin B_{12} supplementation in wasted, underweight, and stunted children. These subgrouping variables significantly modified the effect of vitamin B_{12} on growth. Vitamin B_{12} status at baseline predicted linear and ponderal growth in children not receiving vitamin B_{12} supplements but not in those who did.

Commentaries

Evidence-based Medicine Viewpoint

Relevance: Macro- and micronutrient deficiencies are known to adversely impact child health in the short- as well as long-term. This study [1] was designed to evaluate the effect of dietary supplementation with vitamin B_{12}, or folic acid, or both (I=Intervention), on growth parameters (O=outcome), in a cohort of infants and young Indian children (P=population), over a period of six months (T=time-frame), compared to placebo (C=comparison), through a randomized controlled trial (S=study design) in a community-based setting. The trial is a part of a study [2] evaluating the efficacy of supplementing these vitamins, on the frequency of respiratory infections and diarrhea. The trial [1] details are summarized in ***Table* I**.

Critical appraisal: This randomized controlled trial (RCT) included all the methodological refinements [3], qualifying for a high-quality (low risk of bias trial) (***Table* II**). The RCT had several additional noteworthy methodological refinements. Field workers measuring outcomes (weight and height) received rigorous training to confirm accuracy and precision of measurements. Further, the instruments for measuring weight and height were calibrated daily, thus ensuring accuracy. It is remarkable to note that interventions were administered by field workers themselves on a daily basis (except holidays). A different team of field workers assessed outcomes. Since the main study [2] was designed to identify children developing respiratory infections or diarrhea, the field workers visited each enrolled child twice a week. Perhaps for these reasons, there was remarkably high compliance to the protocol and very low attrition of enrolled children.

The investigators also made efforts to objectively confirm supplementation by measuring blood (serum) levels of vitamin B_{12} and folic acid before and after supplementation. They also measured levels of total homocysteine (an indirect metabolic marker for vitamin B_{12} and/or folic acid function). The laboratory methodology followed standard practices. Data entry was done in duplicate by two operators and discrepancies resolved within 72 hours. For these reasons, the data generated in this study can be taken as robust and reliable. However there are certain caveats. Of 1377 children identified for recruitment to the trial, 299 (21.7%) had to be excluded because of illness requiring hospitalization. This reflects a very high frequency of potentially serious problems affecting over one-fifth of the children. This is unusually high even considering the overall poor baseline nutritional and hemoglobin status of the enrolled children. Although baseline vitamin B_{12}, folic acid and homocysteine levels were comparable in the four arms of the trial, it appears that the levels changed significantly at the end of the trial even in the placebo group. In these children, the median (IQR) for vitamin B_{12} increased from 266 (165, 381) to 318 (191, 404), folic acid

TABLE I SUMMARY OF THE TRIAL DETAILS

Population (P)	Infants and pre-school children (6-30 mo-old) were identified through house-to-house visits by trained field workers, in a community with 60,000 households (total population 300,000). Eligible children were not included in the trial if they had any of the following characteristics: severe acute malnutrition (defined as weight-for-age z score <–3), severe illness necessitating admission to hospital (definition/criteria not mentioned), severe anemia (Hb <7g/dL (measurement timing and details not specified), or pre-supplementation with vitamin B12 or folic acid or both.
Intervention (I)	Intervention groups comprised participants receiving vitamin B_{12} or folic acid or both.Vitamin B_{12} supplementation was intended at twice the recommended daily allowance (RDA). Infants (< 1y) received 0.9 µg and those older than 1 y received 1.8 µg; daily for 6 mo. Folic acid supplementation also was planned to be double the RDA; infants received 75 µg and those older than 1 y received 150 µg;daily for 6 mo. The supplements were prepared mixed in a paste, and administered daily by field workers (except on holidays when family members were expected to administer).
Comparison (C)	Infants and children in the placebo group received only the paste, in the same dose and frequency as those in the intervention groups.
Outcomes (O)	Changes in weight, length/height, weight-for-age z score, length/height-for-age z score and weight-for-length z score
Time-frame (T)	6 months
Study design	Randomized controlled trial (RCT) with factorial design. Thus four allocation groups were created *viz.* vitamin B_{12} supplementation, folic acid supplementation, vitamin B_{12} + folic acid supplementation, and placebo.
Sample size	The investigators calculated sample size based on anticipated differences (between supplemented and un-supplemented participant groups) in pre- *vs* post-supplementation change in z scores for weight-for-age and length/height-for-age, using alpha error of 0.05 and beta error of 0.10. Standard deviation of 0.6 was assumed. The trial recruited more participants than required based on the primary outcomes in the main study [2] viz frequency of respiratory infections and diarrhea.

increased from 11.4 (6.8, 19.5) to 15.3 (9.8, 21.5), and homocysteine decreased from 11.9 (9.1, 16.9) to 10.7 (8.5, 13.9). Unfortunately, the authors have not presented statistical tests to confirm whether these differences were significant. Similarly, it is interesting that in the arm that was supplemented only with vitamin B_{12}, a similar increase in folic acid level was evident. In the arm that received only folic acid supplementation, the magnitude of increase in vitamin B_{12} level was similar to the placebo group. However, no inter-group statistical comparisons have been presented.

It appears that 6 months supplementation with vitamin B_{12} at twice the RDA could increase the geometric mean vitamin B_{12} level only by 28%, although the corresponding increase in folate was over three-fold. It is unclear why the two vitamins behaved so differently. It is even more interesting that despite this dramatic difference, the reduction in homocysteine levels were comparable between those who received vitamin B_{12} (22%) and folic acid (17%). The authors have not explored these issues further.

The investigators showed that vitamin B_{12} supplementation resulted in 0.07 WAZ change in comparison to non-supplementation; however there was no significant difference in change in length/height or weight-for-length, between the supplemented and non-supplemented groups. Folic acid supplementation did not show any differences compared to no supplementation for any of the anthropometric parameters. The investigators did not directly compare the changes in anthropometric indices between placebo group *versus* dual supplementation group, although they mentioned that mean weight and length were significantly higher in the former.

Subgroup analysis also showed some interesting patterns. It appears that vitamin B_{12} supplementation had marginal benefits on weight gain in children with pre-existing stunting, wasting and underweight. It also seems to be efficacious in girls compared to boys. Children with baseline low(er) vitamin B_{12} levels also showed better weight gain (compared to placebo). In contrast, none of these parameters seemed to influence weight in children supplemented with folic acid. In terms of change in length/height, those with pre-existing stunting, easting, underweight and low(er) baseline folic acid levels seemed to have beneficial effect of vitamin B_{12} supplementation as well as folic acid supplementation also. These findings suggest that among the recruited children, there are specific subgroups that may show some benefit with supplementation.

TABLE II EVALUATION OF METHODOLOGICAL QUALITY USING THE COCHRANE RISK OF BIAS TOOL

Criteria	*Assessment*
Sequence generation	Adequate. The randomization sequence was generated by personnel not involved in the execution of the trial, using a computer program. Block randomization was done with fixed block sizes of 16 each.
Allocation concealment	Adequate. The investigators and field workers were provided identical bottles (containing intervention or placebo) pre-labelled with participant serial numbers. Thus they could not predict which group a particular participant would be randomized to.
Blinding of participants, personnel and outcome assessors	Adequate. The intervention and placebo were delivered in identical medium (lipid-rich paste) having identical physical appearance and taste. Thus the investigators (conducting the RCT), field workers (administering the intervention), families (also administering the intervention) and infants/ children (participants) were blinded to the contents of the paste. However, there is no description of whether any of the above people were independently interviewed to assess whether they could guess/ perceive the allocations.
Incomplete outcome data reporting	Adequate. There was very low attrition rate (0-1.2% in the four groups). The data were analyzed using intention-to-treat analysis.
Selective outcome reporting	Adequate. All relevant outcomes related to efficacy were reported. However, it is unclear whether any adverse effects of intervention and/or the vehicle used to deliver the intervention (paste) were evaluated. This is important as B group vitamin supplementation is often associated with gastro-intestinal side effects. Further the lipid-rich paste may also cause intolerance. These issues are important especially because the authors themselves previously reported that folic acid supplementation increased the risk of diarrhea [2].
Other sources of bias	No obvious bias.
Overall assessment	Low risk of bias

Based on these data, it is difficult to accept the authors' main conclusion that the trial demonstrates that low vitamin B_{12} levels are associated with poor growth. Another important question is whether the relative benefit of vitamin B_{12} and/or folic acid supplementation seen in the subgroup of children with pre-existing undernutrition, can be interpreted as effects of the vitamins alone. Although these changes were recorded comparative to placebo (thereby eliminating the Hawthorne effect), it is still possible that supplementation may be beneficial only in the presence of poor overall health status. In such settings, any regular contact with the health-care system can induce positive influences (that may have nothing to do with the intervention *per se*).

Extendibility: The RCT was conducted in a very familiar setting with overcrowding, overall low socioeconomic status, poor health and growth parameters, and possibly low(er) access to health-care (based on frequent identification of children requiring hospitalization). It would be expected that such a setting would amplify the beneficial effect (if any) of vitamin supplementation. The absence of significant increase in weight and length/height parameters, suggests that there is no urgency for large-scale supplementation with these vitamins in Indian children.

Conclusions: This elegantly designed RCT does not suggest that routine vitamin B_{12} and/or folic acid supplementation for 6 months has significant, long-term beneficial effects on growth of infants and young children. Viewed in conjunction with the previously reported trial data [2] showing the absence of benefit on frequency of respiratory infections and diarrhea, routine supplementation is not warranted.

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Pediatrician's Viewpoint

Vitamin B_{12} and folate are closely linked to DNA synthesis, and hence affect the cell growth and differentiation. Several studies have demonstrated role of vitamin B_{12} in maturation of brain and peripheral nerves with its deficiency leading to developmental delay and host of other neurological abnormalities [1]. How much vitamin B_{12} deficiency affects the somatic growth? – is a lesser explored subject. In recent years, researchers have demonstrated maternal vitamin B_{12} deficiency (particularly during pregnancy) having a role in intrauterine growth retardation [2-4].

In this study, Strand, *et al*. have shown improvement in weight-for-age z sores (WAZ) and weight-for-height z scores (WHZ) with vitamin B_{12} and folate supplementation, particularly with vitamin B_{12} supplementation in subgroup of children who were wasted, stunted or underweight. The dose used in the study is only the double the RDA dose over a six month period. End study vitamin B_{12} and homocysteine levels were still abnormal which would mean that with supplementation at this dose, the deficiency might not be completely taken care off. As the authors say, for more meaningful effect on growth, higher doses over longer period may be required. In addition, children with undernutrition have deficiency of several micronutrients which may hamper response to supplementation of one micronutrient. However, the results of the study should lead to further exploration of the topic.

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Pediatric Endocrinologist's Viewpoint

In this well-planned and well-conducted randomized controlled trial (RCT), the authors have studied a change in growth parameters over a 6 month period in children aged 6-35 months, randomized to receive twice daily allowance of vitamin B_{12}, folic acid or both in comparison to placebo. There was no significant difference in mean weight/length gain or in Z scores for weight-for-age (WAZ), height-for-age (HAZ) or weight-for-height in the group receiving folic acid as against placebo. In the group receiving B_{12} supplementation, there was a statistically significant, though clinically modest, increase in WAZ of 0.07 (95% CI 0.01,0.31) over that observed in the placebo group. There was no difference in other anthropometric parameters between the two groups. On sub-group analysis, only those children who were stunted, wasted, undernourished or had low plasma B_{12} levels at baseline, showed a significant increase in WAZ and HAZ as compared to the placebo group. A favorable effect of folic acid supplementation on HAZ, though not on WAZ, was observed only in the subgroup of children who were deficient in folic acid at baseline.

Multiple environmental factors influence growth as reflected by changes in anthropometric parameters, during infancy and early childhood. Since many of these factors, like low socioeconomic status, poor sanitation, nutritional deficiencies, may co-exist, it is difficult to tease out the influence of a single factor upon growth. This becomes even more difficult, if the growth parameters are followed over a short period of 6 months, and the effect observed is a modest difference in a single parameter as was observed in this study. Further, to attribute this observed increase in WAZ to B_{12} supplementation, which had anyway resulted in only 28% increase in geometric mean plasma B_{12} concentration in the supplemented *versus* the placebo group, is perhaps over-interpretation of study findings. No robust evidence exists in literature that supplementation with single/multiple micronutrients has a definite impact on anthropometric parameters of young children in communities [1-3]. Certainly, it is good to see beneficial effect of B_{12} and folic acid supplementation on anthropometric parameters of children with malnutrition, and these children should be supplemented with B_{12} and folic acid along with other vitamins and micronutrients.

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