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ORIGINAL ARTICLE

Effect of physiological doses of oral vitamin B_{12} on plasma homocysteine: a randomized, placebo-controlled, double-blind trial in India

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Background/Objectives: Vitamin B_{12} (B_{12}) deficiency is common in Indians and a major contributor to hyperhomocysteinemia, which may influence fetal growth, risk of type II diabetes and cardiovascular disease. The purpose of this paper was to study the effect of physiological doses of B_{12} and folic acid on plasma total homocysteine (tHcy) concentration.
Subjects/Methods: A cluster randomized, placebo-controlled, double-blind, 2×3 factorial trial, using the family as the randomization unit. B_{12} was given as 2 or 10 μg capsules, with or without 200 μg folic acid, forming six groups (B_0F_0, B_2F_0, $B_{10}F_0$, B_0F_{200}, B_2F_{200} and $B_{10}F_{200}$). Plasma tHcy concentration was measured before and after 4 and 12 months of supplementation.
Results: From 119 families in the Pune Maternal Nutrition Study, 300 individuals were randomized. There was no interaction between B_{12} and folic acid ($P = 0.14$) in relation to tHcy concentration change and their effects were analyzed separately: B_0 vs. B_2 vs. B_{10}; and F_0 vs. F_{200}. At 12 months, tHcy concentration reduced by a mean 5.9 (95% CI: −7.8, −4.1) μmol/l in B_2, and by 7.1 (95% CI: −8.9, −5.4) μmol/l in B_{10}, compared to nonsignificant rise of 1.2 (95% CI: −0.5, 2.9) μmol/l in B_0. B_2 and B_{10} did not differ significantly. In F_{200}, tHcy concentration decreased by 4.8 (95% CI: −6.3, −3.3) μmol/l compared to 2.8 (95% CI: −4.3, −1.2) μmol/l in F_0.
Conclusion: Daily oral supplementation with physiological doses of B_{12} is an effective community intervention to reduce tHcy. Folic acid (200 μg per day) showed no additional benefit, neither had any unfavorable effects.
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Keywords: cyanocobalamin; folic acid; homocysteine; randomized controlled trial; South Asian Indians; vitamin B_{12}

Introduction

Hyperhomocysteinemia is a risk factor for cardiovascular disease (CVD) (Wald *et al*., 2002), psychiatric disorders (dementia and Alzheimer's disease) (Smith, 2008) and in pregnancy for adverse outcomes including early pregnancy loss, birth defects and low birth weight (LBW) (Vollset *et al*., 2000; Selhub, 2008). Low vitamin B_{12} (B_{12}) status and hyperhomocysteinemia are common among Indians living in India (Refsum *et al*., 2001; Yajnik *et al*., 2006), and those migrated abroad (Chambers *et al*., 2000; Chandalia *et al*., 2003). This is largely due to B_{12} deficiency, even with normal folate status, reflecting vegetarian food habits. In recent years, this has been particularly well documented from Pune, India (Refsum *et al*., 2001; Yajnik *et al*., 2006, 2008). In the Pune Maternal Nutrition Study (PMNS), maternal hyperhomocysteinemia predicted LBW (Yajnik *et al*., 2005), and neurocognitive impairment in children (Bhate *et al*., 2008), and low maternal B_{12} with high erythrocyte folate predicted higher adiposity and higher insulin resistance in the

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offspring (Yajnik *et al*., 2008). On the basis of these results, we propose that B_{12} supplementation in women of childbearing age may be a simple and effective mass measure to lower the incidence of LBW, adiposity and insulin resistance and thus of type II diabetes and CVD, and also improve neurocognitive function of the children.

In an earlier 'proof of concept' trial (Yajnik *et al*., 2007), we showed that high-dose oral B_{12} supplementation (500 μg alternate day, for 6 weeks) reduced circulating total homocysteine (tHcy) concentrations. We report results of a randomized, placebo-controlled trial of B_{12} supplementation on plasma homocysteine, using physiological doses over 12 months.

Methods

Participants

The participants were families from an 'extended' cohort of the PMNS. The PMNS methodology has been reported in detail by Rao *et al*. (2001). In brief, 2675 married women of childbearing age, living in six rural villages near Pune city were recruited, and those who became pregnant were followed up. After the main study, we enrolled an additional 153 pregnant women from the same recruited sample to study the early fetal growth. They did not contribute to the main study, and nutritional and blood measurements were not available during pregnancy. Of these, 119 families remain in follow-up, and the child and parents (349 individuals) were invited to take part in this study.

The study was approved by the KEM Hospital Ethics Committee. Exclusion criteria were the following: unwillingness to participate, pregnancy, anemia (hemoglobin <9 g per 100 ml), already taking supplements containing iron, folic acid and/or B_{12} for 10 or more days, or on treatment with drugs known to impair the absorption or utilization of folic acid or B_{12} (for example, phenytoin, antacids). We obtained informed written consent from the parents and informed written assent from the children (mean age 9 years).

For blood collection (June to November 2006), the families were brought to the Research Centre, the evening before the study. A standard vegetarian dinner was provided, after which they rested. A fasting blood sample was collected in the morning.

Study design and intervention

The trial was double blinded. We planned to test three levels of B_{12} supplementation (none, 2 and 10 μg) and each of these at two levels of folic acid supplementation (none and 200 μg), forming six groups ($A = B_0F_0$, $B = B_2F_0$, $C = B_{10}F_0$, $D = B_0F_{200}$, $E = B_2F_{200}$ and $F = B_{10}F_{200}$) (Figure 1). Randomization was computer-based. The unit of randomization was the family, making it a cluster-randomized trial. We stratified the families by the children's baseline plasma B_{12}

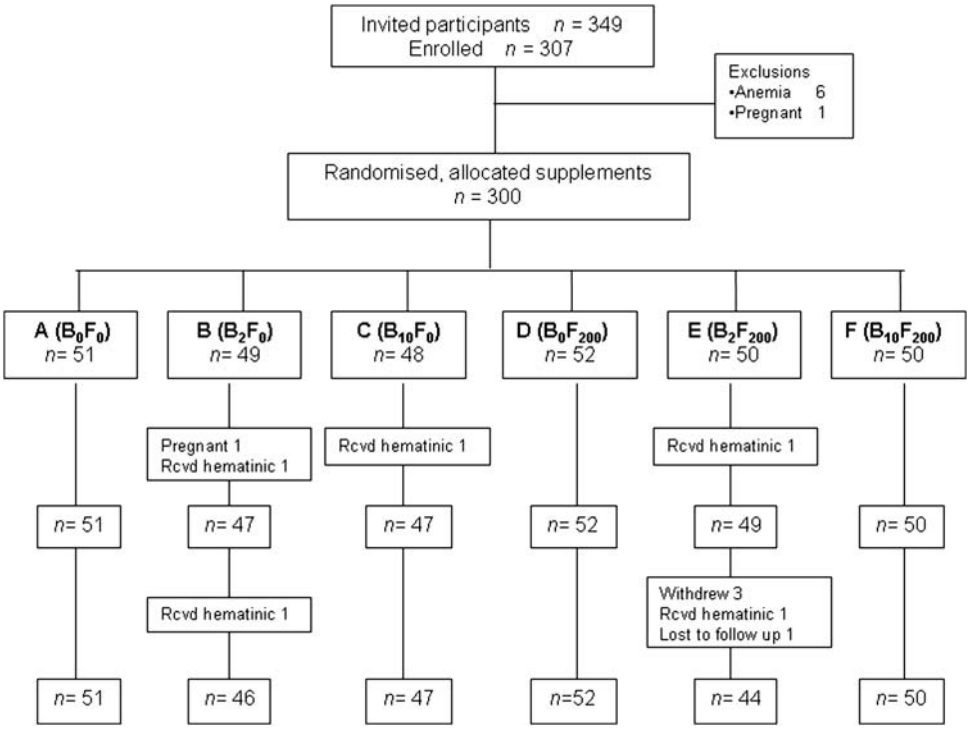

Figure 1 Participant flow and follow-up.

concentrations; those below and above the median value were equally distributed in six groups. Within each group, the statistician randomly allocated codes (A–F) to the participant families. The contents of the capsules were known only to the pharmacist until the end of the trial. The codes were revealed only after data analysis. The study capsules were manufactured in six different colors. The supplements were dispensed monthly in containers labeled with the participant's name and capsule group (A–F). All members of the family received the same colored capsules. The participants were advised to take one capsule orally, daily before breakfast. The number of dispensed capsules and those returned at each monthly home visit was counted to calculate the compliance. At each monthly home visit, we recorded adverse events and treatment of intercurrent illnesses, if any. Participants who took medicine containing folic acid and/or B_{12} for more than 10 days were omitted from data analysis. The duration of supplementation was 12 months, and took place between April 2007 and March 2008. Laboratory analysis of the study medication at the beginning and end of the study period revealed similar potency of the capsules.

Measurements

Blood samples were collected at baseline and 4 and 12 months after supplementation and were measured in separate batches. The samples were collected in EDTA tubes, kept on ice and spun within 1 h ($2500\,g \times 15$ min) and plasma aliquots were stored ($-70\,^{\circ}C$) until further analysis. Hemoglobin was measured within 1 h of blood collection on a Beckman Coulter Analyzer (A^C.T diff; Miami, Florida). Plasma creatinine was measured on an Alcyon 300 automated analyzer (Abbott Laboratories, Abbott Park, IL, USA) using Jaffe's method. Plasma B_{12} and folate were measured by microbiological assay using a colistin sulfate-resistant strain of *Lactobacillus leichmannii* (Kelleher *et al*., 1987; Kelleher and Broin, 1991) and a chloramphenicol-resistant strain of *Lactobacillus casei* (Horne and Patterson, 1988; Tamura *et al*., 1990), with inter-batch CV <8 and $<7\%$, respectively. Plasma tHcy concentration was measured by fluorescence polarization immunoassay (Abbott Laboratories; CV $<8\%$) (Shipchandler and Moore, 1995).

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (CMS Instruments, London, UK) and body weight to the nearest 0.005 kg (Conveigh, Electronic Instruments Ltd, Mumbai, India). Dietary intake of B_{12}- and folate-rich foods was recorded by food frequency questionnaire in children at the beginning and end of 12 months.

Definitions

Compliance was defined as taking $\geqslant 80\%$ of the dispensed capsules, hyperhomocysteinemia as plasma tHcy concentrations $>15\,\mu$mol/l (in adults), $>10\,\mu$mol/l (in children) (Refsum *et al*., 2004), and B_{12} and folate deficiency as concentrations <150 pmol/l (Refsum *et al*., 2001) and <7 nmol/l (Clarke *et al*., 2004), respectively.

Statistical methods

The data are presented as mean and standard deviation (s.d.). Though B_{12} and tHcy concentrations were not normally distributed, we used parametric tests for differences between group means, which are normally distributed as per the central limit theorem. We used analysis of variance to test the differences between supplementation groups, adjusting for the cluster design. There was no difference between clustered and unclustered analysis (statistically insignificant intraclass correlation). The change in the prevalence of hyperhomocysteinemia from baseline was tested by McNemar's test.

The change in plasma tHcy concentrations was adjusted for the baseline tHcy concentrations, age and gender. There was no significant interaction between the effects of B_{12} and folic acid supplementation on change in plasma tHcy concentration ($P=0.14$; Figure 1 in Supplementary Information). Therefore, the effects of 2 and 10 μg B_{12} supplementation were tested against no B_{12}, combining the folic acid supplementation groups ($B_0 = B_0F_0$, B_0F_{200}; $B_2 = B_2F_0$, B_2F_{200}; $B_{10} = B_{10}F_0$, $B_{10}F_{200}$) and the effect of 200 μg folic acid was tested against no folic acid by combining the B_{12} supplementation groups ($F_0 = B_0F_0$, B_2F_0, $B_{10}F_0$; $F_{200} = B_0F_{200}$, B_2F_{200}, $B_{10}F_{200}$). The relative benefit for hyperhomocysteinemia was calculated by taking ratios of absolute benefit in different supplementation groups against non-supplemented group. The number needed to treat was calculated as the reciprocal of the absolute risk reduction for hyperhomocysteinemia at the end of 12 months. All analyses were conducted using Stata, version 7.0 (Stata Inc. College Station, TX, USA).

Results

Recruitment and participant flow

Of the 119 families (349 individuals) in the extended PMNS cohort, 307 individuals were willing to participate (88% response), 7 (1 pregnant, 6 anemic) were excluded and 300 (106 children, 93 fathers and 101 mothers) were randomized (Figure 1). During the intervention, one woman became pregnant and five participants received B_{12}-containing medication (from family physician) and were excluded from the analysis. Three participants withdrew and one was lost to follow-up after collection of the 4-month sample; they were analyzed using the Last Observation Carried Forward method. Thus, the final analysis includes 294 participants (106 children, 92 fathers and 96 mothers).

Baseline characteristics

Table 1 shows the basic characteristics of the 300 participants. Seventy-two percent fathers, 48% mothers and 27%

Table 1 Baseline parameters in children and parents

Physical and biochemical parameters	*Children (n = 106)*	*Fathers (n = 93)*	*Mothers (n = 101)*
Age (years)	9.0 (0.2)	36.8 (3.7)	30.4 (3.1)
Weight (kg)	21.9 (2.9)	59.2 (10.0)	47.9 (8.3)
Height (cm)	126.4 (5.4)	165.6 (7.0)	155.3 (5.4)
BMI (kg/m^2)	13.7 (1.4)	21.6 (3.3)	20.4 (3.5)
$<18.5\,kg/m^2$ (%)	Boys 42.6[a]	21.5	37.6
$>25\,kg/m^2$ (%)	Girls 55.8[a]	17.2	13.9
Hemoglobin (g per 100 ml)	12.5 (0.9)	14.3 (1.2)	12.2 (1.4)
Plasma creatinine (mg per 100 ml)	0.6 (0.1)	0.9 (0.1)	0.8 (0.1)
Plasma B_{12} concentration (pmol/l)	203 (83)	130 (65)	161 (77)
Plasma vitamin B_{12} <150 pmol/l (%)	26.7	72.2	48.4
Plasma folate concentration (nmol/l)	18.9 (6.3)	16.3 (5.5)	16.8 (6.8)
Plasma folate <7 nmol/l (%)	1.9	14.4	8.3
Plasma tHcy concentration (μmol/l)	10.7 (3.8)	31.4 (22.6)	14.6 (7.8)
Plasma tHcy >15 μmol/l (adults) and >10 μmol/l (children) (%)	47	75.3	34.7

Abbreviations: BMI, body mass index; tHcy, total homocysteine.
All values are mean (s.d.) unless specified.
[a]Percentage of children <−2 s.d. of age- and gender-specific BMI (WHO Reference population).

Table 2 Mean concentrations of plasma vitamin B_{12}, folate and tHcy in the B_0, B_2, B_{10}, F_0 and F_{200} groups at baseline, 4 and 12 months

	B_0 (n = 102)	*B_2 (n = 94)*	*B_{10} (n = 98)*	*F_0 (n = 143)*	*F_{200} (n = 151)*
Mean compliance at 12 months (%)	82	84	87	86	82
Plasma B_{12} (pmol/l)					
Baseline	171 (76)	168 (85)	159 (83)	163 (84)	169 (79)
4 months	181 (141)	267 (158)***	326 (158)***	267 (191)***	248 (131)***
12 months	201 (69)***	242 (73)***	307 (119)***	252 (114)***	247 (84)***
Plasma folate (nmol/l)					
Baseline	13.9 (5.7)	12.6 (4.3)	13.5 (3.8)	13.2 (5.6)	13.5 (5.6)
4 months	24.9 (15.6)***	24.6 (15.7)***	24.2 (14.3)***	15.5 (6.4)***	33.1 (16.1)***
12 months	23.7 (15.2)***	20.2 (11.4)**	19.7 (11.5)	14.6 (6.3)***	27.8 (14.4)***
Plasma tHcy (μmol/l)					
Baseline	17.6 (15.3)	19.7 (19.0)	18.5 (14.3)	19.8 (17.3)	17.5 (15.1)
4 months	18.5 (17.1)	14.2 (10.6)***	12.9 (9.4)***	17.2 (14.5)*	13.4 (11.3)***
12 months	19.3 (16.8)	12.9 (7.9)***	11.6 (7.4)***	16.3 (13.7)**	13.1 (10.3)***

All values are mean (s.d.).
*$P<0.05$, **$P<0.01$, ***$P<0.001$ different from baseline concentration.

children were B_{12} deficient, and 75% fathers, 35% mothers and 47% children were hyperhomocysteinemic. In contrast, only 14% fathers, 8% mothers and 2% children had folate deficiency. Baseline B_{12}, folate and tHcy concentrations were similar in the different supplementation groups.

Compliance

Seventy-one percent ($n = 210$) participants returned <20% of the dispensed capsules over 12 months and were defined as 'compliers'. Fourteen percent participants consumed 70–80%, 6% consumed 60–70%, another 6% consumed 50–60% and remaining 3% consumed <50% of the dispensed dose. The mean plasma tHcy concentration, decrease in plasma tHcy concentration and prevalence of hyperhomocysteinemia at 4 and 12 months were similar in the compliers ($n = 210$) and noncompliers ($n = 84$) (Table 1 in Supplementary Information). Overall compliance rates were similar at 4 and 12 months.

The frequency of consumption of folate- and B_{12}-rich foods in children was similar at baseline and after 12 months.

Plasma B_{12} and folate concentrations

At baseline 48% participants were B_{12} deficient. Plasma B_{12} concentrations increased significantly in those who received B_{12} supplements (Table 2). At 12 months the rise was 64% in those who received 2 μg (B_2) and 119% in those who received 10 μg (B_{10}). In both groups this was similar to the rise

achieved by 4 months. Plasma B_{12} concentrations were higher in the B_{10} compared to the B_2 group. After 12 months of supplementation, 6% of the B_2 and 2% of the B_{10} group remained B_{12} deficient. Participants who did not receive B_{12} (B_0) also showed a rise in plasma B_{12} concentration (33% above baseline) after 12 months.

Plasma folate concentrations increased by 112% in those who received folic acid (F_{200}) and by 18.8% in the group who did not (F_0). At baseline 8% participants were folate deficient; after 12 months this reduced to 0% in the supplemented and to 6% in the non-supplemented group.

Plasma total homocysteine concentration

B_{12} supplementation. Plasma tHcy concentrations decreased in the B_2 and B_{10} groups, and showed little change in the B_0 group (Figure 2, Table 2). The decrease was greater in those with higher baseline concentrations ($r=-0.6$, $P=0.000$). We therefore adjusted the change in plasma tHcy concentrations for baseline concentrations. The change in plasma tHcy concentrations was not related to the baseline plasma B_{12} and folate concentrations. The baseline-adjusted decrease was 5.9 (95% CI: −7.8, −4.1) μmol/l in the B_2 group and 7.1 (95% CI: −8.9, −5.4) μmol/l in the B_{10} group (not significantly different). The B_0 group showed a nonsignificant rise of 1.2 (95% CI: −0.5, 2.8) μmol/l. Eighty-two percent of the decrease was achieved by 4 months. After 12 months, in the B_2 group the proportion of hyperhomocysteinemic participants decreased from 52 to 39% ($P=0.02$), in the B_{10} group from 56 to 21% ($P<0.000$) and in the B_0 group it increased from 44 to 56% ($P=0.02$).

Folic acid supplementation. The F_0 and F_{200} groups showed similar decrease in plasma tHcy concentration: F_0 2.8 (95% CI: −4.3, −1.2) μmol/l and F_{200} 4.8 (95% CI: −6.3, −3.3) μmol/l (Figure 3, Table 2). In the F_0 group, the proportion of hyperhomocysteinemic participants decreased from 53 to 44%, $P=0.07$ and in the F_{200} group from 48 to 34%, $P=0.003$.

Table 3 shows the number of hyperhomocysteinemic individuals in different supplementation groups who became normohomocysteinemic ('responded') or remained hyperhomocysteinemic ('not responded') after 12 months. The relative benefit of supplementation was similar in the two B_{12} supplemented groups (B_2 and B_{10}), but was higher in the B_{10} compared to the F_{200} group. The number needed to treat was 4 for B_2, 2 for B_{10} and 10 for F_{200} group.

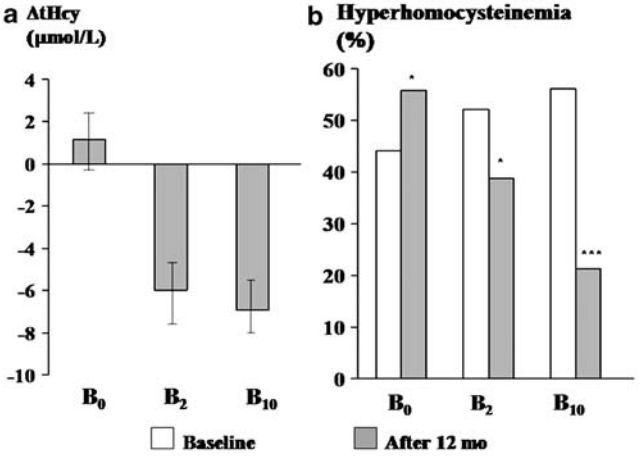

Figure 2 Effect of 12 months of supplementation with vitamin B_{12} (B_0, B_2, B_{10}) on plasma total homocysteine (tHcy) concentration. (**a**) Change in tHcy (mean and 95% CI) over 12 months in three groups (0 line indicates baseline tHcy concentration). (**b**) Proportion with hyperhomocysteinemia at baseline and at 12 months in three groups. *$P<0.05$, ***$P<0.01$.

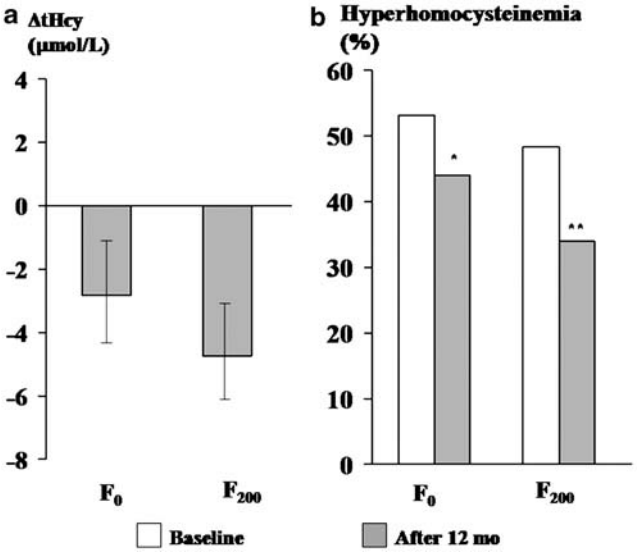

Figure 3 Effect of 12 months of supplementation with folic acid (F_0, F_{200}) on plasma total homocysteine (tHcy) concentration. (**a**) Change in tHcy (mean and 95% CI) over 12 months in two groups (0 line indicates baseline tHcy concentration). (**b**) Proportion with hyperhomocysteinemia at baseline and at 12 months in two groups. *$P<0.05$, **$P<0.01$.

Table 3 Relative benefit and NNT for hyperhomocysteinemia in different supplementation groups after 12 months

Groups	*Responded (n)*	*Not responded (n)*	*Relative benefit (95% CI)*	*NNT*
B_0	6	39		
B_2	18	29	2.87 (1.25, 6.58)	4
B_{10}	39	16	5.32 (2.48, 11.4)	2
F_0	29	47		
F_{200}	34	37	1.25 (0.86, 1.82)	10

Abbreviation: NNT, number needed to treat.
Responded, number of hyperhomocysteinemic participants who became normohomocysteinemic; not responded, number of hyperhomocysteinemic participants who remained hyperhomocysteinemic at the end of trial.

Side effects

There were 62 responses from 46 participants during the study period. One woman reported an accidental injury requiring hospital admission, which was not attributable to supplementation. Other responses were classified into positive (increased appetite, weight gain, sense of well-being; $n=40$) and negative (abdominal pain and acidity, feeling unwell; $n=22$). There was no obvious clustering of side effects in any particular intervention group.

Discussion

This is the first community-based randomized trial of B_{12} supplementation in an Indian population with substantial B_{12} deficiency due to low dietary intake. We found that both 2 and 10 μg per day of oral B_{12} (cyanocobalamin) significantly reduced plasma tHcy concentrations in otherwise healthy, free-living, rural participants. Eighty-two percent of the effect was achieved by 4 months. Overall, the two doses of B_{12} were similarly effective in reducing plasma tHcy concentrations. Folic acid by itself had no additional effect on plasma tHcy reduction, over placebo or in combination with B_{12} (Table 2 in Supplementary Information).

The relatively large effect of such small doses of B_{12} is probably related to the high prevalence of B_{12} deficiency and hyperhomocysteinemia in this population (Refsum *et al*., 2001; Yajnik *et al*., 2006, 2008). Without B_{12} supplementation, hyperhomocysteinemia increased by 12% over the 12-month period. Using the cut point of 15 μmol/l (adults) and 10 μmol/l (children), we found there was a 13% decrease in the hyperhomocysteinemia with 2 μg per day and 35% decrease with 10 μg per day of B_{12} from the baseline. The large effect of supplementation was also evident in the small numbers needed to treat: only four hyperhomocysteinemic individuals needed to be treated with 2 μg per day of B_{12} for 12 months, for one to become normohomocysteinemic and only two with 10 μg per day B_{12}. If the relationship of maternal B_{12} deficiency and hyperhomocysteinemia with fetal outcomes is causal B_{12} intervention could translate into a substantial reduction in the incidence of LBW, diabetes and CVD in this community as well as improvement in cognitive function based on our previous findings (Yajnik *et al*., 2005, 2008; Bhate *et al*., 2008).

Although we knew that B_{12} deficiency was common in this population (Refsum *et al*., 2001; Yajnik *et al*., 2006, 2008), we used a placebo to maintain the scientific rigor and included an arm with only folic acid to test comparative effects, especially in view of proposed food fortification in India. Despite doubling of plasma folate concentrations, folic acid by itself had no effect on circulating tHcy concentrations; neither did it enhance the effect of B_{12} (Figure 1, Supplementary Information). This supports our contention that folate deficiency is not common in this population (Refsum *et al*., 2001; Yajnik *et al*., 2006, 2008). Although this dose of folic acid was not associated with any adverse effects over 12 months, the proposal for folic acid fortification in India for prevention of first occurrence neural tube defects (The Flour Fortification Initiative website, 2009) needs to be formally investigated, including co-fortification with B_{12}.

The effect of supplementation on plasma tHcy was unrelated to the compliance (Table 1 in Supplementary Information), perhaps because the overall compliance was good (72%). Another explanation is that the dose of B_{12} over 4 and 12 months was more than necessary to achieve the effect. This observation is reassuring for future public health interventions.

Major strengths of our study are that it was community-based and included apparently healthy children and adults, rather than being targeted at high-risk groups or patients. The participation rate was high and compliance was maintained at high levels throughout the 12 months. The factorial design allowed us to look at independent effects of B_{12} and folic acid in comparison to their combination and the placebo. We used physiological rather than pharmacological doses of vitamins, with a view to translate our findings into future public health programs. The difficulty in obtaining specially manufactured capsules led to a 5 months gap between the baseline data collection and commencement of the intervention. However, this was distributed similarly in different groups and therefore should not affect our results.

The striking reduction in plasma tHcy concentrations with small doses of 2 and 10 μg of B_{12} merits discussion. In a recent study, we have shown that three doses of 2 μg of B_{12} at 6 h intervals not only raised plasma B_{12} concentrations but also caused a significant (though small) decrease in tHcy concentrations within 24 h of the first dose (Bhat *et al*., 2009). This is perhaps a reflection of a B_{12}-deficient state and high baseline plasma tHcy concentrations. The almost similar effect of 2 and 10 μg doses is perhaps related to characteristics of intestinal B_{12} absorption, which is predominantly by an active (intrinsic factor-mediated) mechanism that saturates after a 1.5–2 μg dose (Carmel, 2008). Only about 1% of absorption is by passive absorption (by diffusion).

In addition to these considerations, the duration of supplementation is also an important determinant of the effect. Small doses over a long time might be equally effective as a large dose over a short time (Carmel, 2008). Our previous study (in vegetarian women) used a large dose of oral B_{12} (500 μg every alternate day for 6 weeks) (Yajnik *et al*., 2007). In 2 weeks (total dose 3 mg B_{12}) plasma tHcy concentrations decreased from 18.0 to 13.0 μmol/l, which remained static over the next 4 weeks (total dose 9 mg B_{12}). In this study, 0.72 mg of B_{12} (2 μg per day × 12 months) achieved a similar effect, 82% of which was achieved by 4 months with 0.24 mg B_{12}.

The majority of published studies of B vitamin supplementation have been in predominantly nonvegetarian western populations, in whom folate deficiency is the main determinant of hyperhomocysteinemia (Selhub, 2008). After folic acid fortification of foods in these populations, the

attention has now shifted toward B_{12}-deficient groups such as the elderly, in whom B_{12} deficiency is thought to be due to 'atrophic gastritis', rather than dietary deficiency. This causes food cobalamin malabsorption, which could require large doses of B_{12} to be effective (Eussen *et al*., 2005), although recent studies have shown efficacy with smaller doses (Bor *et al*., 2006; Blacher *et al*., 2007) as well as foods fortified with folic acid, B_{12} and/or B_6 in the elderly (Tucker *et al*., 2004; Dhonukshe-Rutten *et al*., 2005; van *et al*., 2007; Winkels *et al*., 2008).

Our trial can be considered a public health scale 'proof of principle' study, following on from a high-dose, short-term intervention we reported in a small group of volunteers (Yajnik *et al*., 2007). The two studies have shown an unequivocal role for B_{12} deficiency as contributing to hyperhomocysteinemia in our population.

It is of interest that our interventions have not reduced the plasma tHcy concentrations to those in age-matched Europids, suggesting that other factors also contribute to hyperhomocysteinemia in this population. Such factors may be protein malnutrition (Ingenbleek *et al*., 2002), low methionine intake (Elshorbagy *et al*., 2009) or deficiency of riboflavin (Hustad *et al*., 2000) or pyridoxine (Selhub, 1999). However, it is rewarding that we were able to shift the distribution of plasma homocysteine to more favorable concentrations and this might contribute to a better risk reduction in the population than concentrating on the relatively smaller number with hyperhomocysteinemia (Rose, 1985). There is scope for further investigation to find the etiology of the residual hyperhomocysteinemia, including the role of 'tropical sprue-like' conditions.

In the meanwhile, public health specialists may build on our results and plan large-scale community-based strategies to improve B_{12} nutrition of Indians at different stages of the life cycle. Of particular relevance will be to include B_{12} along with folic acid in the National Nutritional Anemia Control Program or in the proposed food-fortification.

Conflict of interest

The authors declare no conflict of interest.

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