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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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Abstract

Background: Vitamin B_{12} (B_{12}) deficiency is common in Indians and a major contributor to hyperhomocysteinemia, which may influence fetal growth, risk of type 2 diabetes and cardiovascular disease.

Objective: To study the effect of physiological doses of B_{12} and folic acid on plasma total homocysteine (tHcy).

Design: A cluster randomized, placebo-controlled, double-blind, 2x3 factorial trial, using the family as the randomization unit. Vitamin 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}, $B_{10}F_{200}$). Plasma tHcy was measured before and after 4 and 12 mo of supplementation.

Results: Three hundred individuals from 119 families in the Pune Maternal Nutrition Study were randomised. There was no interaction between B_{12} and folic acid (P=0.14) in relation to tHcy change and their effects were analyzed separately: **B_0** vs. **B_2** vs. **B_{10}**; and **F_0** vs. **F_{200}**. At 12 mo, tHcy fell 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 non-significant rise of 1.2 (95% CI: −0.5, 2.9) μmol/L in B_0. B_2 and

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B_{10} did not differ significantly. In F_{200}, tHcy fell 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/d) showed no additional benefit, neither had any unfavourable effects.

Keywords

Cyanocobalamin; folic acid; homocysteine; randomised 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 and 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 (IR) in the offspring (Yajnik *et al.,* 2008). Based on these results we propose that B_{12} supplementation in women of child bearing age may be a simple and effective mass measure to lower the incidence of LBW, adiposity and insulin resistance and thus of T2D and CVD, and also improve neurocognitive function of the children.

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

METHODS

Participants

The participants were families from an ‘extended’ cohort of the PMNS. The PMNS methodology has been reported in detail by Rao in 2001 (Rao *et al.,* 2001). In brief, 2675 married women of childbearing age, living in 6 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, in order 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 the current study.

The study was approved by the KEM Hospital Ethics Committee. Exclusion criteria were: unwillingness to participate, pregnancy, anemia (hemoglobin (Hb) <9 g/dL), 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} (e.g. phenytoin,

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antacids). We obtained informed written consent from the parents and informed written assent from the children (mean age 9 y).

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 6 groups (A = B_0F_0, B = B_2F_0, C = $B_{10}F_0$, D = B_0F_{200}, E = B_2F_{200}, 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} concentrations; those below and above the median value were equally distributed in six groups. Within each group, the statistician randomly allocated codes (A to 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 through 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 were 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 mo, 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 mo after supplementation and were measured in separate batches. The samples were collected in EDTA tubes, kept on ice and spun within one h (2500 *g* × 30 min) and plasma aliquots were stored (−70°C) until further analysis. Hemoglobin was measured within one hour of blood collection on a Beckman Coulter analyser (A^C.T diff ™, Miami, Florida). Plasma creatinine was measured on an Alcyon 300 automated analyser (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 *L. leichmanii* (Kelleher *et al.,* 1987, 1991) and a chloramphenicol-resistant strain of *L. casei* (Horne and Patterson, 1988; Tamura *et al.,* 1990), with inter-batch CV <8% and <7% respectively. Plasma tHcy was measured by fluorescence polarization immunoassay (Abbott, IL, USA; 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 mo.

Definitions

Compliance was defined as taking ≥ 80% of the dispensed capsules, hyperhomocysteinemia as plasma tHcy concentrations >15 μmol/L (in adults), >10 μmol/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 (SD). 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 ANOVA to test the differences between supplementation groups, adjusting for the cluster design. There was no difference between clustered and un-clustered 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 μg 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 (NNT) was calculated as the reciprocal of the absolute risk reduction (ARR) for hyperhomocysteinemia at the end of 12 mo. All analyses were done using STATA, version 7.0 (STATA Inc. College Station, Texas, USA).

RESULTS

Recruitment and Participant flow (Figure 1)

Of the 119 families (349 individuals) in the extended PMNS cohort, 307 individuals were willing to participate (88% response), seven (1 pregnant, 6 anemic) were excluded and 300 (106 children, 93 fathers and 101 mothers) were randomized. During the intervention, 1 woman became pregnant and 5 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 mo sample; they were analysed using the Last Observation Carried Forward (LOCF) 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% 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 mo 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, fall in plasma tHcy concentration and prevalence of hyperhomocysteinemia at 4 and 12 mo were similar in the compliers (n=210) and non-compliers (n=84) (Table 1 in supplementary information). Overall compliance rates were similar at 4 and 12 mo.

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

Plasma vitamin B_{12} and folate concentrations (Table 2)

At baseline 48% participants were B_{12} deficient. Plasma B_{12} concentrations increased significantly in those who received B_{12} supplements. At 12 mo 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 mo. Plasma B_{12} concentrations were higher in the B_{10} compared to the B_2 group. After 12 mo 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 mo.

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 mo this reduced to 0% in the supplemented and to 6% in the non-supplemented group.

Plasma total homocysteine (tHcy) concentration

B_{12} supplementation **(Figure 2, Table 2)—**Plasma tHcy concentrations fell in the B_2 and B_{10} groups, and showed little change in the B_0 group. The fall 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 fall 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 non-significant rise of 1.2 (95% CI: −0.5, 2.8) μmol/L. Eighty-two percent of the fall was achieved by 4 mo. After 12 mo, in the B_2 group the proportion of hyperhomocysteinemic participants fell 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 **(Figure 3, Table 2)—**The F_0 and F_{200} groups showed similar fall 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. In the F_0 group the proportion of hyperhomocysteinemic participants fell 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 mo. 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 (NNT) was 4 for B_2, 2 for B_{10} and 10 for F_{200} group.

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 wellbeing; $n=40$) 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/d 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 mo. 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 vitamin 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 mo period. Using the cut-point of 15 μmol/L (adults) and 10 μmol/L (children), we found there was a 13% fall in the hyperhomocysteinemia with 2 μg/d and 35% fall with 10 μg/d of B_{12} from the baseline. The large effect of supplementation was also evident in the small numbers needed to treat: only 4 hyperhomocysteinemic individuals needed to be treated with 2 μg/d of B_{12} for 12 mo for 1 to become normohomocysteinemic, and only 2 with 10 μg/d B_{12}. If the relationship of maternal B_{12} deficiency and hyperhomocysteinemia with fetal outcomes is causal vitamin B_{12} intervention could translate into a substantial reduction in the incidence of low birth weight, 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 mo, the proposal for folic acid fortification in India for prevention of first occurrence neural tube defects (The Flour Fortification Initiative, 2007) 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 mo 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 mo. 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 five 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 demonstrated that 3 doses of 2 μg of B_{12} at 6 hr

intervals not only raised plasma B_{12} concentrations but also caused a significant (though small) fall in tHcy concentrations within 24 hr 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 which saturates after a 1.5 to 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 wk) (Yajnik *et al.*, 2007). In 2 wk (total dose 3 mg B_{12}) plasma tHcy concentrations fell from 18.0 to 13.0 μmol/L, which remained static over the next 4 wk (total dose 9 mg B_{12}). In the present study 0.72 mg of B_{12} (2 μg/d × 12 mo) achieved a similar effect, 82% of which was achieved by 4 mo with 0.24 mg B_{12}.

The majority of published studies of B vitamin supplementation have been in predominantly non-vegetarian 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 towards B_{12} deficient groups like 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 Vliet *et al.*, 2007; Winkles *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 demonstrated 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 J, 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 G, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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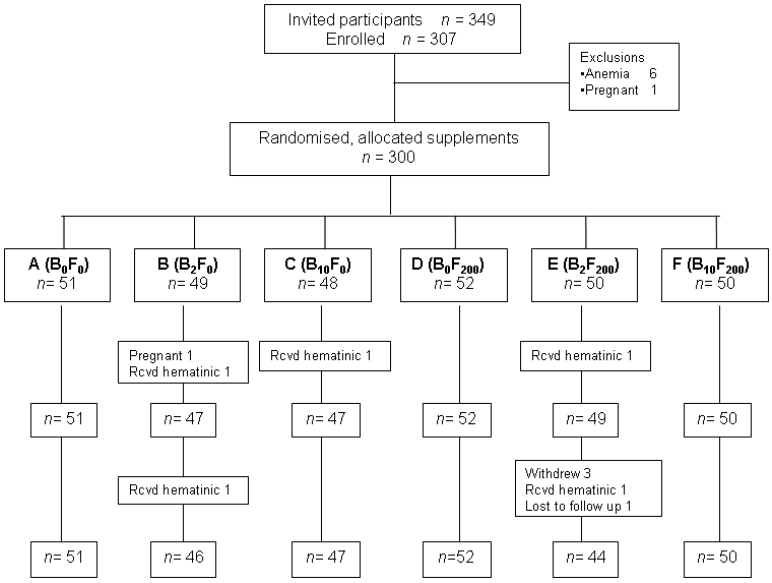

FIGURE 1.
Participant flow and follow up

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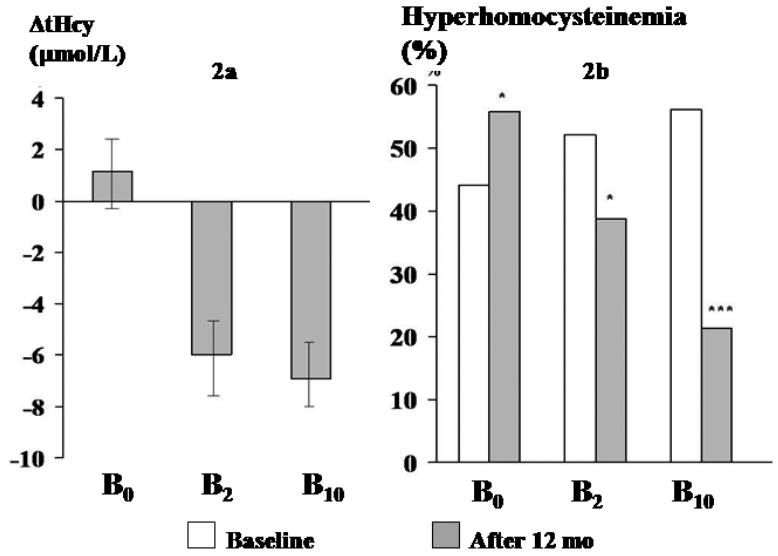

FIGURE 2.

Effect of 12 mo of supplementation with vitamin B_{12} (B_0, B_2, B_{10}) on plasma tHcy

2a Change in **t**Hcy (mean and 95% CI) over 12 mo in 3 groups (0 line indicates baseline tHcy concentration); **2b** Proportion with hyperhomocysteinemia at baseline and at 12 mo in 3 groups

*P < 0.05, **P < 0.01, ***P < 0.001

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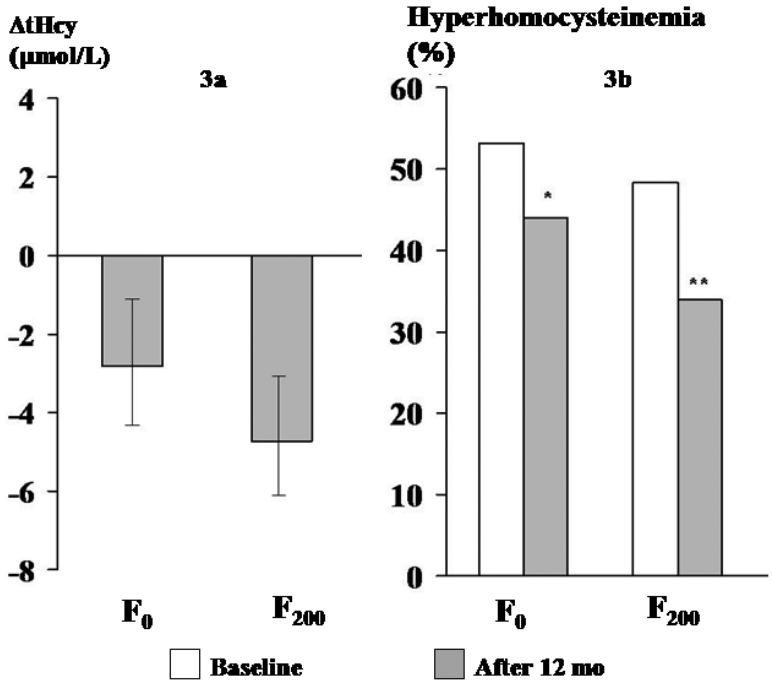

FIGURE 3.

Effect of 12 mo of supplementation with folic acid (F_0, F_{200}) on plasma tHcy

3a Change in **t**Hcy (mean and 95% CI) over 12 mo in 2 groups (0 line indicates baseline tHcy concentration), **3b** Proportion with hyperhomocysteinemia at baseline and at 12 mo in 2 groups

*P < 0.05, **P < 0.01, ***P < 0.001

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TABLE 1

Baseline parameters in children and parents

Physical and biochemical parameters	Children *n*= 106	Fathers *n*= 93	Mothers *n*= 101
Age (y)	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[1]	21.5	37.6
> 25 kg/m^2 (%)	Girls 55.8[1]	17.2	13.9
Hemoglobin (g/dL)	12.5 (0.9)	14.3 (1.2)	12.2 (1.4)
Plasma creatinine (mg/dL)	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

All values are mean (SD) unless specified.

[1]% of children < −2 SD of age and gender specific BMI (WHO Reference population).

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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 mo

	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 mo	181 (141)	267 (158)***	326 (158)***	267 (191)***	248 (131)***
• 12 mo	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 mo	24.9 (15.6)***	24.6 (15.7)***	24.2 (14.3)***	15.5 (6.4)***	33.1 (16.1)***
• 12 mo	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 mo	18.5 (17.1)	14.2 (10.6)***	12.9 (9.4)***	17.2 (14.5)*	13.4 (11.3)***
• 12 mo	19.3 (16.8)	12.9 (7.9)***	11.6 (7.4)***	16.3 (13.7)**	13.1 (10.3)***

Values in mean (SD);

* $P < 0.05$,

** $P < 0.01$,

*** $P < 0.001$ different from baseline concentration

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TABLE 3

Relative benefit and number needed to treat (NNT) for hyperhomocysteinemia in different supplementation groups after 12 mo

Groups	Responded *(n)*	Not responded *(n)*	Relative benefit (95% CI)	Number needed to treat (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

(Responded = number of hyperhomocysteinemic participants who became normohomocysteinemic, Not responded = number of hyperhomocysteinemic participants who remained hyperhomocysteinemic at the end of trial).