Vitamin B_{12} supplementation in pregnancy and infant growth and development - a community-based double-blind randomised trial

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Abstract

Background

Vitamin B_{12} is required for normal growth and development, but low and marginal status is endemic in low- and middle-income countries. We aimed to measure the effect of vitamin B_{12} supplementation from early pregnancy until 6 months postpartum on infant growth and neurodevelopment.

Methods

We randomised (1:1) 800 pregnant women within 15 weeks of gestation in a community-based, double-blind, placebo-controlled trial in Nepal to daily supplementation with 50 µg oral vitamin B_{12} or placebo until 6 months postpartum. Independent scientists generated the list linking allocation to participants study ID. The primary outcomes were length-for-age z-scores (LAZ) at 12 months, and the cognitive composite score of the Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III) at 6 and 12 months. All participants with available outcome data were included in the analyses. Trial registration: ClinicalTrials.gov - NCT03071666.

Findings

Women were enrolled between March 28, 2017, and October 15, 2020, with 400 randomized to each arm. Follow up was completed May 18, 2022. At baseline, 71% of the women had plasma cobalamin indicating low or marginal status (<221 pmol/L). Vitamin B_{12} supplementation substantially improved status in mothers and infants. We found no effect of vitamin B_{12} on the primary outcomes. The mean (±SD) LAZ-score at 12 months was -0·6 (1·0) in both study arms (366 and 363 infants) with a mean difference (95% CI) of -0·02 (-0·16, 0·13). The mean difference (95% CI) in cognitive composite scores was 0·5 (-0·6, 1·7) (measured in 364 and 361 infants). Stillbirths or infant deaths occurred in 3 (1%) of 374 women in the vitamin B_{12} arm and 9 (2%) of 379 women in the placebo arm.

Interpretation

Although poor status was prevalent and vitamin B_{12} supplementation from early pregnancy substantially

improved vitamin B_{12} status, supplementation did not improve infant growth or neurodevelopment.

Research in context

Evidence before this study

We searched PubMed and Web of Knowledge for published studies on vitamin B_{12}, growth and neurodevelopment in June 2022 using the search terms "Vitamin B_{12} "[Mesh], “B12”[Text Word], cobalamin*[Text Word] as well as "Clinical Trial"[Publication Type], “trial*”[Text Word], “supplement*”[Text Word], "Dietary Supplements"[Mesh], "Treatment Outcome"[Mesh], and "Vitamins/administration and dosage"[MeSH Terms]. Our searches identified 7 publications from 3 studies on supplementation during pregnancy, 5 publications on supplementation during early childhood and 1 meta-analysis.

The trials during pregnancy were conducted in India (2 studies) and Bangladesh (1 study), with vitamin B_{12} doses of 50, 2 and 250 µg, respectively. The trial in rural Bangalore, India, found an effect on expressive language at 30 months, however no effect on other domains or at other times during follow up. The trial in Pune, India, found higher scores in children at 2 years of age on cognition and language subscales following maternal supplementation pre-pregnancy. Common to these studies were the relatively small sample sizes, ranging from 82 to 366 women and that growth and neurodevelopment were secondary outcomes. Meta-analysis results reported vitamin B_{12} deficiency was associated with low birthweight and preterm birth.

We also searched the registry platform https://clinicaltrials.gov, and identified 2 relevant protocols on the effect of vitamin B_{12} supplementation during pregnancy, none of which has been published yet.

Some very recent publications, particularly targeting the Indian subcontinent, make a strong argument for public action to improve vitamin B_{12} status in women of childbearing age particularly during pregnancy.

Added value of this study

This is the first randomised clinical trial in pregnant women designed to measure the effect of vitamin B_{12} supplementation on infant growth and neurodevelopment. More than two thirds of the study population suffered from low or marginal vitamin B_{12} status at enrolment. Giving vitamin B_{12} during pregnancy and postpartum had a substantial effect on the direct and functional biomarkers of vitamin B_{12} status in the pregnant women and their babies. Supplementation did not, however, improve infant growth and neurodevelopment. The prevalence of spontaneous abortion, low birthweight, preterm delivery, still birth and neonatal deaths reported in our study is comparable with the peri-urban population of Nepal. We found no effect on these outcomes indicating the dose of 50 µg of vitamin B_{12} during pregnancy is generally safe in terms of pregnancy outcomes. A negative effect on early motor performance was observed, giving reasons for caution related to vitamin B_{12} supplementation during pregnancy.

Implications of all the available evidence

Results from the current study showed a high prevalence of low and marginal vitamin B_{12} status among pregnant women. Fifty microgram vitamin B_{12} supplementation given orally for one year starting in early pregnancy substantially improved vitamin B_{12} status in the mother-infant dyads but did not improve infant growth and neurodevelopment during the first year of life. The implications of improved vitamin B_{12} status for other outcomes merit further investigation. Our findings, in conjunction with the scarcity of previous studies, support the current WHO recommendations of no routine vitamin B_{12} supplementation during pregnancy.

Introduction

All too many children from low- and middle-income countries (LMIC) fail to reach their growth and developmental potential.[1] Vitamin B_{12} is required for normal growth and development, but low and marginal vitamin B_{12} status is endemic in many LMIC settings.[2,3] As an enzymatic cofactor for the transmethylation of homocysteine to methionine by methionine synthase, and the rearrangement of methylmalonyl-coenzyme A (CoA) to succinyl-CoA in propionate metabolism by methylmalonyl-CoA mutase, vitamin B_{12} is essential for multiple physiological processes including DNA methylation and histone modification, cell differentiation and growth, energy metabolism, and myelinization of the central nervous system.[3,4] While the significance of vitamin B_{12} throughout the lifecycle is recognized, it is especially important during pregnancy and in early infancy. Failure to thrive, abnormal neurological function, delayed development, and macrocytic anaemia are typical manifestations in infants with severe, clinical vitamin B_{12} deficiency.[5,6] Results from several population-based studies show that even marginal vitamin B_{12} deficiency is associated with adverse pregnancy outcomes,[7] as well as impaired infant growth and poor neurodevelopment in early childhood.[8-10] Findings from three randomised controlled trials (RCT) indicate positive effects of vitamin B_{12} supplementation starting before or during early pregnancy on child health and neurodevelopment in the first 2-3 years of life.[11-14] These recent findings, however, do not suffice to change the current recommendations on routine vitamin B_{12} supplementation in pregnant women from LMICs, which is in line with the current WHO Antenatal Pregnancy Guidelines.[15] Despite the lack of evidence and current public health recommendations, many argue for widespread and high-dose supplementation prior to and during pregnancy.[16,17]

The overall aim of the current study was to measure the effect of daily vitamin B_{12} supplementation from early pregnancy until 6 months postpartum on infant growth and neurodevelopment. The primary

outcomes were linear growth attained at 12 months of age and neurodevelopment measured with the cognitive composite score of the Bayley Scales of Infant and Toddlers Development, 3rd edition (Bayley-III) at 6 and 12 months of age. In the current study, we ensured that the baseline vitamin B_{12} status and the metabolic effect of supplementation were well characterised in both women and infants. Describing the metabolic response to supplementation provides an indication of the compliance as well as the intracellular demand for the vitamin.[18] Moreover, we targeted a population where we previously have described poor vitamin B_{12} status,[19] high rates of stunting,[20] and where maternal and infant vitamin B_{12} status were positively associated with child growth and neurodevelopment.[8,21]

Methods

Study design

This is a community-based, double-blind, randomised, placebo-controlled trial, conducted in Bhaktapur municipality and surrounding areas in Nepal.[22] The ethical review boards in Nepal (NHRC 253/2016) and Norway (2016/1620/REK vest) approved the study. An independent data and safety monitoring board continuously oversaw severe adverse events and undertook an interim analysis when 100 infants had reached 10 weeks.

Participants

Pregnant women aged between 20 and 40 years, no later than 15 weeks' gestation assessed by last menstruation period, were recruited from home visits and gynaecological outpatient departments at three hospitals in the study area and invited for participation. Pregnancy and gestational age were further confirmed by ultrasonography (USG). Additional inclusion criteria were living and planning to reside in the area for the next two years and consenting to participate. Mothers taking or planning to

take dietary or multivitamin supplements containing vitamin B_{12}, with acute or chronic illness, severe anaemia, high-risk pregnancy, or a body mass index (BMI) <18·5 and >30 were excluded. Each pregnant mother provided informed written consent, preferably in the presence of her husband.

Randomisation and masking

Eligible pregnant women were randomised to supplements with or without vitamin B_{12} in a 1:1 ratio in blocks of eight using a computer-generated randomisation list. The supplements, with or without 50 µg vitamin B_{12} were identical in taste and appearance. The rationale for the dose is given in the appendix (p 2). Allocation was concealed, and the participants were linked to the study arms through an identification (ID) number printed on the supplement labels. The list that linked this ID number to the randomisation code was only available to the producers of the supplements and kept with two individuals otherwise not involved in the study. One of these generated the randomization list, and the other labelled the intervention packages according to the instructions given by the producers. These individuals also interacted with the DSMB when the randomization code was needed for the interim analysis. None of the study investigators had access to this list until data collection and data cleaning of the primary outcomes were completed.

Procedures

The supplements were produced specifically for this trial (GCRieber, Norway) and provided in daily rations of solid 7 g biscuits (resembling local sweets) wrapped in foil from early pregnancy to 6 months postpartum. During enrolment procedures, a research assistant provided the first week supply of biscuits to the participants as well as detailed information concerning dose and storage. In weekly home visits, the participants were provided with refills. Participants in both study arms also received daily 400

µg of folic acid during the first trimester, and 60 mg of elemental iron and 0·5-1 g of calcium from the second trimester until 45 days postpartum according to national recommendations.

Details of antenatal supplementation, demographics and socio-economic characteristics were collected within one week of enrolment. Assessment procedures, response categories and source of information are described in the appendix (pp 3-4). During the weekly visits to the participants' home in the follow-up period, we documented day wise consumption of the study intervention, intake of different foods, morbidities, and hospital visits (appendix, pp 5-7). During the COVID-19 pandemic and strict nationwide lockdowns (March-July 2020 and April-June 2021), this information was collected through the phone. Frequency of antenatal visits, laboratory investigations and other treatments were done as per the recommendations of the treating gynaecologist. According to study procedures, planned assessments and blood draw were conducted at the study clinic at 8 months of gestation and when the child was 8-12 weeks, 6, and 12 months.

Outcomes

The primary outcomes of the trial were linear growth expressed as length-for-age z-score (LAZ) at 12 months of age and neurodevelopment as measured by the cognitive composite score of the Bayley-III[23] at 6 and 12 months. Key secondary infant outcomes were LAZ at 6 months, weight-for-age z-score (WAZ), weight-for-length z-score (WLZ), body mass index z-score (BMIZ), infant weight (kg) and length (cm) at 6 and 12 months, Bayley-III language, motor, and socio-emotional composite scores at 6 and 12 months, and early motor performance measured by the Test of Infant Motor Performance (TIMP)[24] at 8-12 weeks of age. Key secondary pregnancy and perinatal outcomes included congenital anomalies, gestational length, birthweight, and haemoglobin concentration.

Length and weight were measured at the study hospital and in the homes. The primary growth outcome was measured at the study hospital. Length was measured to the nearest millimetre using an infantometer (Seca, Germany). An electronic scale was used to measure weight to the nearest 100g (Seca, Germany). During training, the 18 fieldworkers reached an intra-class correlation coefficient (ICC) of 0.93-0.98 compared to an expert. During the implementation of the study, each child was measured twice, independently by 2 fieldworkers. The mean of the two measurements was used for the analyses. The technical error of measurement (TEM) during the study was 0.18 cm for length and 0.03 kg for weight. LAZ, WAZ, and WLZ was calculated using the WHO Child Growth Standards.

Bayley-III assessments were done with the child at the study clinic in well-lit rooms, free from distractions. A caregiver was present with the child during testing, and children were ensured to be well fed and not sick prior to the assessment. Three psychologists experienced in Bayley assessments were standardised in the assessment procedures, reaching an intra-class correlation (ICC) >0·85 when compared with a gold standard ahead of the study and >0·94 for the double-scoring during the study. Raw scores were converted to three composite scores; the cognitive, language and motor composite score (expected standard deviation of 15) based on American norms.[23] The assessments also included the questionnaire-based Bayley-III socio-emotional scale.

The TIMP assessment was done at the study clinic by two trained examiners achieving an ICC>0·94 from the standardisation procedures ahead of the study and an ICC>0·93 from double scoring during the study. The total possible score for the TIMP is 142 with higher scores indicating better motor performance. The TIMP has age specific norms developed in the US, categorising the motor performance into average, low average, below average and far below average range.

Assessment procedures for the pregnancy and perinatal outcomes and for the adverse events including miscarriage, number of medical terminations of pregnancy, still birth, hospitalisation of the mother and infant deaths are described in appendix (pp 3-4).

Laboratory procedures

Blood samples were collected from the mother at enrolment, 8 months of gestation, and 6 months after delivery, and from infants at 6 and 12 months of age. Approximately 3-4 ml of blood were collected into vials containing EDTA. The haemoglobin concentration was estimated immediately with HemoCue (HemoCue 201, Ångelholm, Sweden), calibrated as per guidelines set by the manufacturer. The plasma vials were centrifuged at approx. 2,000 g at room temperature for 10 minutes, stored at --70°C at the field site and always transported on dry ice.

Plasma concentrations of cobalamin and folate were determined in microbiological assays using a colistinsulfate-resistant strain of Lactobacillus leichmannii and chloramphenicol-resistant strain of Lactobacillus casei, respectively. The functional biomarkers plasma total homocysteine (tHcy) and methylmalonic acid (MMA) were analysed by gas chromatography-tandem mass spectrometry (GC-MS/MS) based on methylchloroformate derivatization. The within-day CV was 4% for both folate and cobalamin and ranged from 1% to 5% for tHcy and MMA. The between-day CV was 5% for both folate and cobalamin and ranged from 1% to 3% for tHcy and MMA. These biomarkers were analysed at Bevital Laboratory, Bergen, Norway (www.bevital.no). We also calculated the combined indicator of cobalamin status (3cB12) based on cobalamin, tHcy, and MMA.[25] Higher 3cB12 indicates better status.

Statistical analysis

The analyses were carried out according to a predefined protocol and statistical analysis plan which was uploaded to clinicaltrials.gov before the statistical analyses commenced. The primary analysis was an intention-to-treat analysis. Before unblinding of the trial arms, we completed data cleaning and curation for all the variables included in the analyses. We used generalised linear models (GLM) with the Gaussian distribution family and identity link function for the primary growth outcome and most of the secondary outcomes. Supplementation status was the only independent variable in these GLM models. For the primary outcome cognitive composite scores, where measurements both at 6 and 12 months of age were used, we used generalised estimating equations (GEE) models with an exchangeable covariance structure to take the repeated measurements into account.[26] We compared proportions using GLM with the binomial distribution family and identity link function, yielding differences in proportions (or risks). We log-transformed plasma tHcy and MMA concentrations as these were left-skewed, and present the exponentials of their means, SDs, and mean differences as geometric means (GM), geometric standard deviation factors (GSDF), and geometric mean ratios (GMR). The intervention response in vitamin B_{12} status in mothers and children was visualised using Epanechnikov kernel density plots of the plasma cobalamin and log transferred tHcy and MMA concentrations. We also present the effects of the intervention separately in those with the highest and lowest baseline vitamin B_{12} status (3cB12). As the 3cB12 has not been validated for pregnant women, we categorised the 3cB12 at the 33rd percentile. Adverse events during the supplementation period are presented as the number of days or number of events with episodes of symptoms, hospitalisation, and visits to health facilities by study arm. The sample size was determined using the "power" function in STATA (Stata inc. College Station, TX). For a standardised effect size of 0.25 SD, 676 mothers were required to achieve a statistical power of 90% (alpha=5%). We did not adjust the alpha for multiple comparisons (more than one main outcome). With a potential loss to follow up of 15% (mothers or children) due to dropout or miscarriage[27], the final sample size was set to 800 women. The trial was registered at ClinicalTrials. gov, NCT03071666.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, writing of results, or decision to submit manuscript for publication.

Results

From March 28, 2017, to October 15, 2020, we screened 1,394 pregnant women and enrolled 800 into the study (figure 1). Follow up for the primary outcomes was completed on May 18, 2022. A total of 47 participants (6%) were lost to follow up, mainly due to spontaneous abortion or medical termination based on severe anomaly detected by the USG scan. There were 760 live births, out of which 361 (47%) were caesarean sections. At the 6 and 12 months follow up, 755 (94%) and 753 (94%) children were still in the study, respectively. We were unable to assess 22 children at 6 months and 28 children at 12 months with the Bayley-III within the predefined window period (±45 days) mainly due to the lockdowns during the COVID-19 pandemic or due to illness.

Baseline characteristics are described in table 1. The mean (SD) maternal age was 27·7 (4·0) years, 390 (49%) were primipara and 464 (58%) were enrolled within the first trimester of pregnancy. According to the plasma cobalamin cut-off <221 pmol/L, 569 (71%) of mothers had low or marginal vitamin B_{12} status at enrolment. For all participants, we documented supplement compliance for 276,435 days. Of these days, the supplement was not consumed 5902 out of 137,392 (4%) and 6061 out of 139,043 (4%) days in the in the vitamin B_{12} and placebo arm, respectively. Reasons for not taking supplements were mainly unscheduled travels out of the study area during COVID-19 lockdowns or hospitalisation for childbirth (appendix, p 5). The infant and maternal biomarker concentrations of vitamin B_{12} reported in tables 2

and 3 show a substantial metabolic response to the vitamin B_{12} supplementation (appendix, p 8). The responses over the whole distribution of the biomarkers both in mothers and infants are depicted in figure 2. The frequency of intake of various food items are shown in appendix (p 7).

At the 12-months visit, the mean LAZ was -0·6 (1·0) in both arms with a mean difference between study arms of -0·02 (95% CI -0·2, 0·1). The mean (SD) lengths of infants at 12 months in the vitamin B_{12} and placebo arms were 73·6 (2·7) and 73·8 (2·7) cm, while the mean weights (SD) were 9·0 (1·2) and 9·2 (1·2) kg, respectively (table 2). Other anthropometric indices are also shown in table 2, and there were no differences in any of them.

There was no difference between the arms in any of the Bayley-III scores at any time point. The mean (SD) Bayley-III cognitive composite scores (primary outcome) in the intervention and placebo arm were 97·7 (10·5) and 97·1 (10·2), respectively, with a mean difference of 0·5 (95% CI -0·6, 1·7). Maternal vitamin B_{12} supplementation resulted in 1·8 (95% CI 0·4, 3·3) lower TIMP total score, and a higher risk of having scores below the average range based on American norms (table 2). The negative effect of vitamin B_{12} supplementation on the TIMP score was stronger when restricting the analysis to the 531 (66%) women with the best baseline vitamin B_{12} status (appendix, pp 9-11).

There was no effect of the vitamin B_{12} supplementation on birthweight (mean (SD) of 2990·2 (456·0) g and 3048·1 (446·8) g in the vitamin B_{12} and placebo arm, respectively). Similarly, there was no effect on the proportion of infants born with low birthweight or born preterm (table 3).

Subgroup analyses according to vitamin B_{12} status of the women at enrolment did not reveal any other effects (appendix, pp 9-11). We observed few adverse events which are listed in Table 4; stillbirths or

infant deaths occurred in 3 (1%) of 374 women in the vitamin B_{12} arm and 9 (2%) of 379 women in the placebo arm.

Discussion

The study was conducted in an area with low vitamin B_{12} intake, where low and marginal vitamin B_{12} status is endemic[19], and where we have previously shown associations between maternal vitamin B_{12} status, child growth, and neurodevelopment.[21] Supplementing 50 µg vitamin B_{12} daily from early pregnancy until 6 months postpartum did not improve infant growth and neurodevelopment despite a substantial biomarker response and improved B_{12} status among mothers and infants.

The lack of effect of vitamin B_{12} supplementation on our primary outcomes contrasts with previous studies suggesting that even marginal vitamin B_{12} status contributes to impaired growth[9,21] and neurodevelopment[8,10]. However, our findings are supported by results from a previous RCT conducted in the same area, where a year of daily B_{12} supplementation starting from infancy had no effect.[20] Two recent RCTs, both from India, involving vitamin B_{12} supplementation during pregnancy found positive effects on specific neurodevelopmental domains.[11-13] These studies were small, however, and neurodevelopment was a secondary outcome unlike the present study which was well-powered and had neurodevelopment as the primary outcome.

We did not identify beneficial effects on any of the secondary outcomes or when stratifying the analyses according to maternal vitamin B_{12} status at enrolment. On the contrary, we found a negative effect on motor performance in early infancy. This raises the concern that there might be possible adverse effects of supplementing vitamin B_{12} during pregnancy. It should be noted that there was no effect on motor

scores of the Bayley-III at 6 or 12 months, suggesting that either the negative effect on motor performance did not persist, or that the adverse effect on infant motor performance at 8-12 weeks was a spurious finding. However, the adverse effect on early motor performance give reason for caution when supplementing with B_{12} during pregnancy.

The comprehensive characterisation of biomarkers related to vitamin B_{12} status in both women and their infants at several time points are an important reassurance on both the dose sufficiency and intervention compliance. We observed a high prevalence of low and marginal vitamin B_{12} status in mothers at enrolment. We also observed a favourable metabolic response to vitamin B_{12} supplementation in both the mothers and their infants (figure 2), with a substantial shift in the distribution of different biomarker concentrations between the placebo and intervention group. At the end of supplementation, 6% of the women in the vitamin B_{12} arm were deficient (cobalamin concentration <148 pmol/L), and 46% in the placebo arm. This shift in distribution is interesting beyond documenting compliance to the supplementation. The shift indicates an impact of the supplementation at a biochemical level that may have consequences on clinical outcomes, such as long-term metabolic health, not included in our report.

Our null results could have several explanations. For example, it is possible that most of the women were not deficient enough or the supplementation period did not cover a critical window relevant to our outcomes. Thus, initiating supplementation sooner, such as before pregnancy, could have yielded different results. We could also have seen effects with a higher dose; however, this, in turn, may have increased the risk of negative consequences. Nonetheless, some women and children likely benefitted from supplementation. The effect in these, however, was not measurable as it only contributed to the population average amongst many who had no or even a negative effect. In other words, the positive

effect of the few was diluted by the response in the majority of the study sample. Studies designed to precisely identify those likely to benefit from supplementation are warranted.

While vitamin B_{12} supplementation has been suggested as an intervention among populations where low and marginal vitamin B_{12} status is common[16,17], our study adds to the current literature that evidence is not sufficient to recommend B_{12} supplementation during pregnancy as a routine preventive measure. However, our biomarker results confirm poor vitamin B_{12} status among pregnant women in this region. Notably, using a plasma cobalamin cut-off of 250 pmol/L as suggested by others[28], 82% of the women had subclinical deficiency. It is worthwhile mentioning that the positive predictive value of diagnostic tests can be substantially attenuated when used in non-clinical settings where most of the population is without overt signs and symptoms of deficiency, such as in this study. Caution should therefore be exercised when using biochemical indices to advocate fortification and supplementation. We believe that our findings can be generalised to other populations where poor vitamin B12 status is prevalent which could include populations both in high- and low-income settings.

Strengths of the study include the large sample size, successful randomisation, close follow up and few losses to follow-up, and excellent compliance. Further strengths are the high-quality assessments of outcomes, including the high inter-rater agreement on the neurodevelopmental outcomes, and that we measured neurodevelopment at two time points. The many secondary outcomes included in our study are also an asset, reducing the risk of overlooking critical clinical effects. Furthermore, the tHcy and MMA response to the supplementation confirms excellent compliance and further indicates the poor vitamin B_{12} status in the target population. The results should be interpreted in light of some limitations. Neurodevelopment assessments show low stability in early childhood[29,30] thus, assessments later may give more reliable and detailed information on the effect of the intervention. In addition, due to

lockdowns during the COVID-19 pandemic, we could not assess growth and neurodevelopment within the window period in all children. However, the number of children excluded from the analysis was low.

In summary, there was no beneficial effect of vitamin B_{12} supplementation from early pregnancy until six months postpartum on growth, neurodevelopment, and pregnancy outcomes, but we observed a possible negative effect on motor performance at 8-12 weeks. Findings from the study support the current WHO recommendations of no routine vitamin B_{12} supplementation during pregnancy.[15]

Contributors

TAS was the Principal Investigator for the trial and conceived the study, supervised and performed the statistical analyses, interpretation of results and drafting of the manuscript. RKC and LS were the local Principal Investigators for the trial and contributed to design, data collection, supervision, data analyses and drafted manuscript. IK and MH contributed to study design, supervised the cognitive assessment, contributed to the statistical analysis and drafting of the manuscript. MU, SR, MS, and SB contributed to study design, study implementation, logistic support, supervision and drafting the manuscript. CS contributed with the statistical analyses, literature search and drafting of the manuscript. AM contributed to laboratory analyses, interpretation of results and drafting and review of the manuscript and PMU contributed to laboratory analyses and critical review of manuscript. The corresponding author had full access to all the data in the study and RKC, IK and CS verified the data. All authors shared final responsibility for the decision to submit for publication.

Declaration of interests

The authors declare they have no actual or potential competing conflict interests.

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Data sharing statement

Data is available on request. In order to meet ethical requirements for the use of confidential patient data, requests must be approved by the Nepal Health Research Council (NHRC) and the Regional Committee for Medical and Health Research Ethics in Norway. Requests for data should be sent to the authors, by contacting NHRC (http://nhrc.gov.np), or by contacting the Department of Global Health and Primary Care at the University of Bergen (post@igs.uib.no).

References

1 Local Burden of Disease Child Growth Failure C. Mapping child growth failure across low- and middle-income countries. Nature 2020; 577(7789): 231-4.
2 Green R, Miller JW. Vitamin B12 deficiency. Vitam Horm 2022; 119: 405-39.
3 Stabler SP. Vitamin B12 deficiency. N Engl J Med 2013; 368(21): 2041-2.
4 Scott JM, Molloy AM. The discovery of vitamin B(12). Ann Nutr Metab 2012; 61(3): 239-45.
5 Rosenblatt DS, Whitehead VM. Cobalamin and folate deficiency: acquired and hereditary disorders in children. Semin Hematol 1999; 36: 19-34.
6 Bala R, Verma R, Verma P, et al. Hyperhomocysteinemia and low vitamin B12 are associated with the risk of early pregnancy loss: A clinical study and meta-analyses. Nutr Res 2021; 91: 57-66.
7 Rogne T, Tielemans MJ, Chong MF, et al. Associations of Maternal Vitamin B12 Concentration in Pregnancy With the Risks of Preterm Birth and Low Birth Weight: A Systematic Review and Meta-Analysis of Individual Participant Data. American journal of epidemiology 2017.
8 Kvestad I, Hysing M, Shrestha M, et al. Vitamin B-12 status in infancy is positively associated with development and cognitive functioning 5 y later in Nepalese children. Am J Clin Nutr 2017; 105(5): 1122-31.
9 Strand TA, Taneja S, Kumar T, et al. Vitamin B-12, folic acid, and growth in 6- to 30-month-old children: a randomized controlled trial. Pediatrics 2015; 135(4): e918-26.
10 Strand TA, Taneja S, Ueland PM, et al. Cobalamin and folate status predicts mental development scores in North Indian children 12-18 mo of age. Am J Clin Nutr 2013; 97(2): 310-7.
11 Srinivasan K, Thomas T, Kapanee AR, et al. Effects of maternal vitamin B12 supplementation on early infant neurocognitive outcomes: a randomized controlled clinical trial. Matern Child Nutr 2017; 13(2).
12 Thomas S, Thomas T, Bosch RJ, et al. Effect of Maternal Vitamin B12 Supplementation on Cognitive Outcomes in South Indian Children: A Randomized Controlled Clinical Trial. Matern Child Health J 2019; 23(2): 155-63.
13 D'Souza N, Behere RV, Patni B, et al. Pre-conceptional Maternal Vitamin B12 Supplementation Improves Offspring Neurodevelopment at 2 Years of Age: PRIYA Trial. Front Pediatr 2021; 9: 755977.
14 Siddiqua TJ, Ahmad SM, Ahsan KB, et al. Vitamin B12 supplementation during pregnancy and postpartum improves B12 status of both mothers and infants but vaccine response in mothers only: a randomized clinical trial in Bangladesh. Eur J Nutr 2016; 55(1): 281-93.
15 WHO. WHO recommendations on antenatal care for a positive pregnancy experience: World Health Organization; 2016.
16 Behere RV, Deshmukh AS, Otiv S, Gupte MD, Yajnik CS. Maternal Vitamin B12 Status During Pregnancy and Its Association With Outcomes of Pregnancy and Health of the Offspring: A Systematic Review and Implications for Policy in India. Front Endocrinol (Lausanne) 2021; 12: 619176.
17 Antony AC, Vora RM, Karmarkar SJ. The silent tragic reality of Hidden Hunger, anaemia, and neural-tube defects (NTDs) in India. The Lancet Regional Health-Southeast Asia 2022; 6: 100071. The Lancet Regional Health-Southeast Asia 2022; 6.
18 Allen LH, Miller JW, de Groot L, et al. Biomarkers of Nutrition for Development (BOND): Vitamin B-12 Review. J Nutr 2018; 148(suppl_4): 1995S-2027S.
19 Chandyo RK, Ulak M, Sommerfelt H, Schneede J, Ueland PM, Strand TA. Nutritional Intake and Status of Cobalamin and Folate among Non-Pregnant Women of Reproductive Age in Bhaktapur, Nepal. Nutrients 2016; 8(6).

20 Strand TA, Ulak M, Hysing M, et al. Effects of vitamin B12 supplementation on neurodevelopment and growth in Nepalese Infants: A randomized controlled trial. PLoS Med 2020; 17(12): e1003430.
21 Strand TA, Ulak M, Kvestad I, et al. Maternal and infant vitamin B12 status during infancy predict linear growth at 5 years. Pediatr Res 2018; 84(5): 611-8.
22 Chandyo RK, Ulak M, Kvestad I, et al. The effects of vitamin B12 supplementation in pregnancy and postpartum on growth and neurodevelopment in early childhood: Study Protocol for a Randomized Placebo Controlled Trial. BMJ Open 2017; 7(8): e016434.
23 Bayley N. Bayley Scales of Infant and Toddler Development: Bayley-III. San Antaonio, Texas: Harcourt Assessment; 2006.
24 Campbell SK. The Test of Infant Motor Performance: Test User's Manual Version 3.0 for the TIMP Version 5: Infant Motor Performance Scales, LLC; 2012.; 2012.
25 Fedosov SN, Brito A, Miller JW, Green R, Allen LH. Combined indicator of vitamin B12 status: modification for missing biomarkers and folate status and recommendations for revised cut-points. Clin Chem Lab Med 2015; 53(8): 1215-25.
26 Altman DG. Practical statistics for medical research: Chapman and Hall, London; 1991.
27 Ministry of Health and Population, Nepal, New ERA; and ICF. 2017. Nepal Demographic Health Survey 2016. Kathmandu, Nepal, Ministry of Health Nepal.
28 Hannibal L, Lysne V, Bjorke-Monsen AL, et al. Biomarkers and Algorithms for the Diagnosis of Vitamin B12 Deficiency. Front Mol Biosci 2016; 3: 27.
29 Kvestad I, Hysing M, Ranjitkar S, et al. The stability of the Bayley scales in early childhood and its relationship with future intellectual abilities in a low to middle income country. Early Hum Dev 2022; 170: 105610.
30 Brito NH, Fifer WP, Amso D, et al. Beyond the Bayley: Neurocognitive Assessments of Development During Infancy and Toddlerhood. Dev Neuropsychol 2019; 44(2): 220-47.

Table 1. Maternal and household characteristics at enrolment according to intervention arms

General characteristics	Vitamin B_{12}	Placebo
	n = 400	**n = 400**
Age (years), mean (SD)	27·7 (3·8)	27·5 (4·2)
Gestational week at enrolment, mean (SD)*	11·0 (2·8)	11·1 (2·8)
History of previous abortion or miscarriage	73 (18)	60 (15)
Folic acid supplementation	287 (72)	278 (70)
Iron supplementation	76 (19)	69 (17)
Calcium supplementation	74 (19)	68 (17)
BMI (kg/m^2), mean (SD)	23·8 (3·0)	23·6 (3·0)
>25 BMI, kg/m^2	141 (35)	128 (32)
Vegetarian	4 (1)	4 (1)
Completed secondary school or above (≥11 grade)	229 (57)	226 (57)
Salaried job (private or government sector)[†]	117 (29)	121 (30)
Nuclear (parents and children) family	145 (36)	133 (33)
Family own land	262 (66)	277 (69)
Family resides in rented house	102 (26)	96 (24)
Kitchen and bedroom in the same room	109 (27)	105 (26)
Monthly household income (in 1000 NPR), median (IQR)[‡]	30 (20-50)	30 (20-50)
Family receive remittance from abroad	43 (11)	42 (11)
Haemoglobin and biomarker concentrations	**n = 397**	**n = 399**
Haemoglobin (g/dl), mean (SD)[§]	12·4 (1·1)	12·4 (1·1)
Haemoglobin <11.3 g/dl (anaemia, altitude adjusted)	56 (15)	54 (14)
Cobalamin (pmol/L), mean (SD)	194 (80)	187 (72)
Cobalamin <148 pmol/L	126 (31·5)	137 (34·2)
Cobalamin <221 pmol/L	279 (69·7)	290 (72·5)
Total homocysteine (µmol/L), geometric mean, gmsdf[‖]	6·1 (1·4)	6·1 (1·3)
Methylmalonic acid (µmol/L), geometric mean, gmsdf[‖]	0·20 (1·90)	0·21 (1·79)
Folate (nmol/L), mean (SD)	64·1 (52·3)	69·8 (74·6)
Combined vitamin B_{12} indicator (3cB12), mean (SD)[¶]	0·07 (0·63)	0·03 (0·59)

All numbers n (%) unless otherwise stated, *Gestational week was calculated based on last menstruation period (4 mothers did not know last menstruation period), [†]Salaried jobs defined as jobs with regular monthly payment including government or private sectors, [‡]Nepali Rupees, 1 NPR=0·008 USD (as of June 2022) (no information on household incomes from 5 families), [§]55 missing due to unavailability of Hemocue cuvettes, [‖]geometric mean standard deviation factor which is the exponential of the SD of the mean of the log-transformed values, [¶]combined indicator of cobalamin status based on cobalamin, total homocysteine and methylmalonic acid

Table 2. Primary and secondary outcomes in infants

	Vitamin B_{12}	Placebo	
Primary outcomes:			
Anthropometry	**n = 366**	**n = 363**	**diff. (95% CI)**
Length for age z-score at 12 months	-0·57 (1·03)	-0·55 (1·03)	-0·02 (-0·16, 0·13)
Neurodevelopment	**obs·* = 727**	**obs·* =731**	**diff. (95% CI)**
Bayley-III cognitive composite at 6 and 12 months[†]	97·7 (10·5)	97·1 (10·2)	0·5 (-0·6, 1·7)
Key secondary outcomes:			
Anthropometry at 6 months	**n = 363**	**n = 370**	**diff. (95% CI)**
Length for age z-score	-0·5 (1·0)	-0·4 (1·0)	-0·1 (-0·2, 0·1)
Length (cm)	65·6 (2·3)	66·0 (2·3)	-0·3 (-0·6, 0·02)
Weight for age z-score	-0·20 (1·10)	-0·13 (1·10)	-0·07 (-0·23, 0·09)
Weight for length z-score	0·22 (1·07)	0·25 (1·06)	-0·03 (-0·19, 0·12)
Weight (kg)	7·5 (1·0)	7·6 (1·0)	-0·1 (-0·3, 0·03)
BMI z-score	0·11 (1·09)	0·15 (1·08)	0·04 (-0·20, 0·12)
Anthropometry at 12 months	**n = 366**	**n = 363**	**diff. (95% CI)**
Length (cm)	73·6 (2·7)	73·8 (2·7)	-0·2 (-0·6, 0·2)
Weight for age z-score	-0·32 (1·06)	-0·26 (1·04)	-0·07 (-0·22, 0·09)
Weight for length z-score	-0·07 (1·07)	0·01 (1·03)	-0·08 (-0·23, 0·08)
Weight (kg)	9·0 (1·2)	9·2 (1·2)	-0·1 (-0·3, 0·04)
BMI z-score	0·01 (1·06)	0·09 (1·02)	-0·08 (-0·23, 0·07)
Neurodevelopment (Bayley-III scores) at 6 months	**n = 363**	**n = 370**	**diff. (95% CI)**
Cognitive composite score	100·1 (10·1)	98·9 (10·7)	1·1 (-0·4, 2·6)
Language composite score	88·3 (8·0)	88·9 (7·5)	-0·5 (-1·6, 0·6)
Motor composite score	99·8 (13·1)	99·6 (13·7)	0·2 (-1·8, 2·1)
Socio-emotional composite score	100·6 (17·5)	101·2 (16·6)	-0·6 (-3·1, 1·8)
Neurodevelopment (Bayley-III scores) at 12 months	**n = 364**	**n = 361**	**diff. (95% CI)**
Cognitive composite score	95·4 (10·4)	95·3 (9·2)	0·03 (-1·4, 1·5)

Language composite score	77·2 (12·2)	76·7 (11·5)	0·5 (-1·2, 2·5)
Motor composite score	93·8 (11·1)	94·0 (12·0)	-0·2 (-1·9, 1·5)
Socio-emotional composite score	100·1 (16·9)	100·3 (15·9)	-0·3 (-2·7, 2·1)
Test of Infant Motor Performance (TIMP), 8-12 weeks	**n = 357**	**n = 355**	**diff. (95% CI)**
TIMP total score	76·2 (10·4)	78·0 (9·1)	-1·8 (-3·3, -0·4)
TIMP score below average range, n (%)	181 (51)	136 (38)	12·4 (5·1, 19·6)
Haemoglobin and biomarker concentrations at 6 months	**n = 127**	**n = 129**	**diff. or GMR[‡] (95% CI)**
Haemoglobin (g/dl)[¥]	11·2 (1·1)	11·3 (1·1)	-0·1 (-0·3, 0·1)
Haemoglobin <11.3 g/dl (anaemia, altitude adjusted), n (%)	199 (53)	188 (50)	3·1 (-3·4, 10·3)
Cobalamin (pmol/L)	222 (79)	192 (60)	31 (13, 48)
Total homocysteine (µmol/L), geometric mean (gmsdf)[§]	7·3 (1·3)	10·8 (1·5)	1·5 (1·4, 1·6)
Methylmalonic acid (µmol/L), geometric mean (gmsdf)[§]	0·25 (1·84)	0·42 (2·10)	1·7 (1·4, 2·0)
Folate (nmol/L)	51·3 (21·5)	66·1 (25·8)	-14·8 (-20·6, -8·9)
Combined vitamin B_{12} indicator (3cB12)[¶]	-0·2 (0·6)	-0·8 (0·7)	0·6 (0·5, 0·8)
Haemoglobin and biomarker concentrations at 12 months (six months after end of supplementation)	**n = 134**	**n = 131**	**diff. or GMR[‡] (95% CI)**
Haemoglobin (g/dl)[β]	11·1 (1·2)	11·2 (1·2)	-0·1 (-0·2, 0·1)
Haemoglobin <11.3 g/dl (anaemia, altitude adjusted), n (%)	202 (56)	186 (51)	4·6 (-2·7, 11·8)
Cobalamin (pmol/L)	225 (90)	217 (92)	8 (-15, 30)
Total homocysteine (µmol/L), geometric mean (gmsdf)[§]	6·9 (1·3)	8·4 (1·4)	1·2 (1·1, 1·3)
Methylmalonic acid (µmol/L), geometric mean (gmsdf)[§]	0·32 (2·1)	0·43 (2·1)	1·4 (1·1, 1·6)
Folate (nmol/L)	47·8 (20·1)	49·7 (20·2)	-1·9 (-6·8, 2·0)
Combined vitamin B_{12} indicator (3cB12)[¶]	-0·3 (0·7)	-0·6 (0·7)	0·3 (0·2, 0·5)

All numbers in mean (SD) unless otherwise stated, *total observations at 6 and 12 months of age, [†]generalized estimating equation with two timepoints (6 and 12 months), [‡]geometric mean ratio (an effect size of 1·5 indicates that the concentration is 50% higher in the placebo group than in the vitamin B_{12} group), [¥] n=375 and n=377 in vitamin B12 and placebo arm respectively, [§]geometric mean standard deviation factor which is the exponential of the SD of the mean of the log-transformed values , [β] n=362 and n=363 in vitamin B12 and placebo arm respectively, [¶]combined indicator of cobalamin status based on cobalamin, total homocysteine and methylmalonic acid.

Table 3. Pregnancy and birth outcomes, and maternal biochemical response during pregnancy and 6 months postpartum

	Vitamin B_{12}	Placebo	
Pregnancy and birth outcomes	**n=377**	**n=383**	**diff. (95% CI)**
Gestational length (weeks)	38·4 (1·8)	38·5 (1·9)	-0·1 (-0·3, 0·3)
Preterm delivery (<37 weeks), n (%)	37 (10)	33 (9)	1·3 (-2·8, 5·4)
Congenital anomaly, n (%)[*]	12 (3)	6 (2)	-1·6 (-3·8, 0·5)
Birthweight (g)	2990·2 (456·0)	3048·1 (446·8)	-57·0 (-121·8, 7·8)
<2500g, n (%)	39 (10)	32 (8)	-1·8 (-6·0, 2·4)
<2000g, n (%)	9 (2)	5 (1)	-1·1 (-3·1, 0·9
>4000g, n (%)	7 (2)	3 (1)	-0·8 (-2·4, 0·7)
Haemoglobin and biomarker concentrations at 8 months during pregnancy	**n=186**	**n=184**	**diff. or GMR[†] (95% CI)**
Haemoglobin concentration (g/dl)	11·6 (1·3)	11·6 (1·2)	0·1 (-0·1, 0·3)
Cobalamin (pmol/L)	243 (72)	160 (51)	83 (70, 95)
Cobalamin <148 pmol/L, n (%)	11 (6)	85 (46)	-40·3 (-48·2, -32·3)
Total homocysteine (µmol/L), geometric mean (gmsdf)[‡]	5·6 (14)	6·5 (1·4)	1·2 (1·0, 1·8)
Methylmalonic acid (µmol/L), geometric mean (gmsdf)[‡]	0·19 (1·62)	0·32 (1·70)	1·7 (1·6, 1·9)
Folate (nmol/L)	20·3 (13·9)	21·3 (12·5)	-1·0 (-3·7, 1·8)
Combined vitamin B_{12} indicator (3cB12)[§]	0·3 (0·5)	-0·3 (0·6)	0·7 (0·6, 0·8)
Haemoglobin and biomarker concentrations at 6 months postpartum	**n=127**	**n=129**	**diff. or GMR[†] (95% CI)**
Haemoglobin concentration (g/dl)	13·2 (1·1)	13·3 (1·0)	-0·1 (-0·3, 0·1)
Cobalamin (pmol/L)	268 (102)	182 (49)	86 (67, 106)
Cobalamin <148 pmol/L, n (%)	9 (7)	31 (24)	-16·9 (-25·5, -0·1)
Total homocysteine (µmol/L), geometric mean (gmsdf)[‡]	6·8 (1·3)	8·5 (1·4)	1·2 (1·2, 1·3)
Methylmalonic acid (µmol/L), geometric mean (gmsdf)[‡]	0·17 (1·55)	0·27 (1·73)	1·6 (1·4, 1·8)
Folate (nmol/L)	18·7 (9·1)	17·7 (7·9)	1·0 (-1·0, 3·1)
Combined vitamin B_{12} indicator (3cB12)[§]	0·3 (0·5)	-0·3 (0·5)	0·6 (0·5, 0·8)

All numbers in mean (SD) unless otherwise stated, [*]The following congenital anomalies were identified during first visit after delivery; congenital heart disease (atrial septum defect, patent ductus arteriosus, cleft palate, polydactyly and other (undescended testes, laryngomalacia, pes planus, hypoplastic phalanges, [†]geometric mean ratio factor (an effect size of 1·2 indicates that the

concentration is 20% higher in the placebo group than in the vitamin B_{12} group), [‡]geometric mean standard deviation factor which is the exponential of the SD of the mean of the log-transformed values, [§]combined indicator of cobalamin status based on cobalamin, total homocysteine and methylmalonic acid

Table 4. Severe adverse pregnancy and neonatal outcomes

		Vitamin B_{12} n=400	Placebo n=400
	n		
Miscarriage	15	11	4
Medical termination*	12	8	4
Still birth	6	1	5
Hospitalization of the mother†	117	68	49
Infant death (total)	6	2	4
Due to neonatal sepsis		2	2
Due to extreme prematurity			1
Due to complex congenital heart disease			1

*Due to severe anomaly detected during USG (such as holoprosencephaly, cystic hygroma and hydrops fetalis, anencephaly, gross hydrocephalus etc.). †Mainly due to threatened abortion, urinary tract infection, oligohydramnios, hypertension, hyperemesis gravidarum, eclampsia or pre-eclampsia and decreased foetal heart sound

Figure legends:

Figure 1

Title: Trial flow-chart of a study investigating the effect of daily vitamin B_{12} supplementation during pregnancy and postpartum on growth and neurodevelopment in 800 Nepalese mother-infant dyads

Figure 2

Title: The response of vitamin B_{12} supplementation during pregnancy and post-partum on the distribution of plasma cobalamin, total homocysteine (tHcy), and methylmalonic acid (MMA) in mothers and infants at different time points

Legend: Distribution of plasma cobalamin and log transferred tHcy and MMA concentrations at four time points (mothers: baseline, 8th month of pregnancy and 6 months after delivery; infants: 6 and 12 months of age) by treatment group. Solid lines represent the vitamin B_{12} arm and dotted lines the placebo arm.

Appendix

Contents

Table S1. Variable characteristics (categorical/continuous) and source of information for baseline maternal, demographic, and socio-economic characteristics and for pregnancy and birth outcomes in a study investigating the effect of daily vitamin B_{12} supplementation during pregnancy and postpartum on neurodevelopment and growth in 800 Nepalese pregnant mother-infant dyads

	Categorical/ continuous	Responsible	Source of information
Baseline maternal characteristics			
Maternal age	Years	Physician/ Supervisor	Mother's report
Body mass index (BMI)	kg/m2	Physician/ Supervisor	Height and weight measured at the clinic
Gestational weeks at enrolment	Weeks	Physician/ Supervisor	Based on last menstruation period reported by mother
Primipara	yes/no	Physician/ Supervisor	Mother's report
Previous history of abortion	yes/no	Physician/ Supervisor	Mother's report
Haemoglobin concentration	g/dL	Lab technician	Measured on Hemocue
Anaemia (<11 g/dl)	yes/no		Based on Hemocue data
Folic acid supplementation	yes/no	Physician	Mother's report
Iron supplementation	yes/no	Physician	Mother's report
Calcium supplementation	yes/no	Physician	Mother's report
Demographic and socio-economic characteristics			
Mothers who completed secondary school or above (11 grade and above)	Years	Field Worker	Mother's report
Mothers with salaried jobs (private or government sector)*	Categorical	Field Worker	Mother's report
Vegetarian mother	yes/no	Field Worker	Mother's report
Nuclear family	Categorical	Field Worker	Parental report
Family that owns land	Categorical	Field Worker	Parental report
Family resides in rented house	Categorical	Field Worker	Parental report
Kitchen and bedroom in the same room	yes/no	Field Worker	Parental report
Monthly household income, NPR	years	Field Worker	Parental report
Receiving remittance from abroad	yes/no	Field Worker	Parental report
Weekly visit outcomes (Daily information)			
Taking supplement	yes/no	Field Worker	Mother's report

Taking folic acid/ Iron/calcium	yes/no	Field Worker	Mother's report
Reason for not taking supplements	Categorical	Field Worker	Mother's report
Vomiting after supplementation	yes/no	Field Worker	Mother's report
Any sign/symptoms (fever, diarrhoea, oedema, abdominal pain etc.)	Categorical	Field Worker	Mother's report
Visit to health facility	yes/no	Field Worker	Mother's report
Use of antibiotics	yes/no	Field Worker	Mother's report/hospital record
Hospitalization	yes/no	Field Worker	Mother's report/hospital record
Food items consumed (egg, meat, fish, curd, milk /fruits/nuts)	yes/no	Field Worker	Mother's report
Alcohol	yes/no	Field Worker	Mother's report
Other nutrient supplements	yes/no	Field Worker	Mother's report
Anthropometric measurement			
Weight of infants	Kilograms	Field Worker/ Supervisors	Measured weight at clinic or home
Length of infants	Centimetres	Field Worker/ Supervisors	Measured length/ height at clinic or home
Pregnancy and birth outcomes			
Spontaneous abortion[†]	yes/no	Physician	Hospital record
Medical termination of pregnancy	yes/no	Physician	Hospital record
Still birth[‡]	yes/no	Physician	Hospital record
Gestational length, weeks, mean (SD)	Weeks	Physician	Based on last menstruation period reported by mother
Preterm delivery (<37 weeks)	yes/no	Physician	Hospital record
Congenital anomaly[§]	yes/no	Physician	Hospital record
Preeclampsia/eclampsia	Categorical	Physician	Hospital record
Birth weight	Grams	Physician/ Supervisor	Hospital record

*Salaried jobs defined as the jobs with regular monthly based payment including government or private sectors. Reference groups include housewives, agriculture, carpet worker, daily wage earner, business, and foreign employment, [†]Before 20 weeks of gestation without any intervention, [‡]still delivery after 28 weeks of gestation, [§]as per the record from hospital discharge sheet after delivery. List of anomalies reported are congenital heart disease (atrial septum defect, patent ductus arteriosus (5) and complexed (1)), cleft palate (3), polydactyly (3) and other (undescended testes, laryngomalacia, pes planus, hypoplastic phalanges).

Table S2. Information on daily compliance in a study investigating the effect of daily vitamin B_{12} supplementation during pregnancy and postpartum on neurodevelopment and growth in 800 Nepalese pregnant mother-infant dyads

	Weekly visits before delivery						Weekly visits after delivery					
	Vitamin B_{12}			Placebo			Vitamin B_{12}			Placebo		
	N*=400			N*=400			N*=400			N*=400		
	n†	obs‡	%	n†	obs‡	%	n†	obs‡	%	n†	obs‡	%
Mother available during weekly visit												
Yes	8,913	11,376	78·3	9047	11,712	77·2	7,237	10,196	71·0	7,196	10,278	70·0
By phone	1,761	11,376	15·5	1815	11,712	15·5	2,415	10,196	23·7	2,492	10,278	24·2
No contact	702	11,376	6·2	850	11,712	7·3	544	10,196	5·3	590	10,278	5·7
Days taking supplement	70,603	72,465	97·4	71915	73,860	97·4	60,887	64,927	93·8	61,067	65,183	93·7
Days not taking supplement	1,862	72,465	2·6	1,945	73,860	2·6	4,040	64,927	6·2	4,116	65,183	6·3
Reason for not taking B12 supplement												
Illness	283	1,817	15·6	218	1,915	11·4	331	3,975	8·3	291	4,026	7·2
Forgot	823	1,817	45·3	1060	1,915	55·4	2,066	3,975	52·0	2,040	4,026	50·7
Travelling	131	1,817	7·2	133	1,915	6·9	183	3,975	4·6	273	4,026	6·8
Does not like	55	1,817	3·0	66	1,915	3·4	79	3,975	2·0	69	4,026	1·7
Supplement not available§	192	1,817	10·6	86	1,915	4·5	472	3,975	11·9	478	4,026	11·9
Other reasons including delivery)	333	1,817	18·3	352	1,915	18·4	844	3,975	21·2	875	4,026	21·7
Days vomiting after supplement intake	49	70,650	0·1	89	71,950	0·1	22	60,901	0·0	17	61,076	0·0

*Number of women, †number of days, ‡total number of days observed, §mainly during COVID-19 pandemic and lockdown period due to unscheduled travelling outside study area.

Table S3. Reported frequency of illness in a study investigating the effect of daily vitamin B_{12} supplementation during pregnancy and postpartum on neurodevelopment and growth in 800 Nepalese pregnant mother-infant dyads

Morbidities and symptoms reported by mothers	Weekly visits before delivery						Weekly visits after delivery					
	Vitamin B_{12}			Placebo			Vitamin B_{12}			Placebo		
	N*=400			N*=400			N*=400			N*=400		
	n†	obs‡	%	n†	obs‡	%	n†	obs‡	%	n†	obs‡	%
Vaginal bleeding	542	72,438	0·7	496	73,847	0·7	7,132	64,883	11·0	7,140	65,150	11·0
Oedema of leg	2,805	72,442	3·9	3,481	73,847	4·7	934	64,880	1·4	1,164	65,147	1·8
Fever	403	72,429	0·6	479	73,839	0·6	340	64,886	0·5	530	65,154	0·8
Diarrhoea	65	72,439	0·1	128	73,840	0·2	58	64,887	0·1	91	65,138	0·1
Vomiting	1,107	72,439	1·5	1,095	73,840	1·5	16	64,887	0·0	27	65,138	0·0
Cough and cold	1,953	72,439	2·7	2,263	73,840	3·1	1,677	64,887	2·6	1,557	65,138	2·4
Abdominal pain	2,722	72,439	3·8	3,116	73,840	4·2	774	64,887	1·2	730	65,138	1·1
Headache	434	72,439	0·6	802	73,840	1·1	307	64,887	0·5	350	65,138	0·5
Vertigo	134	72,439	0·2	127	73,840	0·2	77	64,887	0·1	111	65,138	0·2
Skin problems	415	72,439	0·6	154	73,840	0·2	99	64,887	0·2	37	65,138	0·1
Visit to health facility	1,518	72,420	2·1	1,640	73,845	2·2	249	64,847	0·4	256	65,083	0·4
Antibiotic use	718	72,120	1·0	778	73,466	1·1	2,390	64,241	3·7	2,382	64,577	3·7
Hospitalization§	710	72,156	1·0	540	73,489	0·7	807	64,254	1·3	541	64,602	0·8
Refer to hospital by Field worker	53	72,151	0·1	55	73,478	0·1	28	64,234	0·0	29	64,583	0·0

*Number of women, †total number of days, ‡total number of days observed, §mainly for the threatened abortion, gestational diabetes, decreased foetal movement, oligohydramnios, urinary tract infection, eclampsia and pre-eclampsia etc.

Table S4. Daily intake of food items and antenatal supplementation in a study investigating the effect of daily vitamin B_{12} supplementation during pregnancy and postpartum on neurodevelopment and growth in 800 Nepalese pregnant mother-infant dyads

	Weekly visits before delivery						Weekly visits after delivery					
	Vitamin B_{12}			Placebo			Vitamin B_{12}			Placebo		
	N*=400*			N*=400			N*=400			N*=400		
	n†	obs‡	%	n†	obs‡	%	n†	obs‡	%	n†	Obs‡	%
Intake of egg	31,242	72,138	43·3	31,733	73,481	43·2	32,818	64,250	51·1	32,879	64,604	50·9
Intake of meat	27,033	72,141	37·5	27,153	73,478	37·0	32,797	64,252	51·0	33,143	64,601	51·3
Intake fish	2,933	72,144	4·1	2,690	73,478	3·7	961	64,240	1·5	959	64,596	1·5
Intake curd	10,330	72,129	14·3	11,147	73,472	15·2	1,338	64,241	2·1	1,520	64,593	2·4
Milk/ milk tea drinking	42,282	72,139	58·6	45,382	73,484	61·8	38,285	64,252	59·6	39,228	64,603	60·7
Any Fruits	43,185	72,145	59·9	45,098	73,479	61·4	16,578	64,249	25·8	16,710	64,601	25·9
Lentil/pulses	42,289	72,147	58·6	43,834	73,486	59·6	35,736	64,255	55·6	35,076	64,607	54·3
Any alcohol§	2,818	72,149	3·9	3,093	73,482	4·2	4,629	64,253	7·2	5,724	64,601	8·9
Readymade sweet, malted milk powder such as Horlicks	10,119	72,148	14·0	11,986	73,486	16·3	12,852	64,251	20·0	12,961	64,601	20·1
Nuts or others	27,728	69,977	39·6	29,329	71,278	41·1	14,037	64,209	21·9	14,919	64,597	23·1
Supplement intake												
Folic acid	7,471	72,451	10·3	7,231	73,852	9·8	284	64,901	0·4	155	65,163	0·2
Iron	63,693	72,452	87·9	64,926	73,853	87·9	21,305	64,896	32·8	21,391	65,164	32·8
Calcium	63,947	72,439	88·3	65,243	73,842	88·4	22,000	64,892	33·9	21,682	65,160	33·3
Any other vitamins	3,850	72,121	5·3	3,479	73,482	4·7	1,814	64,255	2·8	1,586	64,600	2·5
Other nutrient supplements	696	72,155	1·0	550	73,487	0·7	197	64,251	0·3	106	64,602	0·2

*Number of women, †total number of days, ‡total number of days observed, §mainly home-made local rice beer

Table S5. Median (IQR) biomarker concentration in mothers and infants at 5 time points in a study investigating the effect of daily vitamin B_{12} supplementation during pregnancy and postpartum on neurodevelopment and growth in 800 Nepalese pregnant mother-infant dyads

		Vitamin B_{12} (N=400)	**Placebo (N=400)**
	n	Median (IQR)	Median (IQR)
Mother			
Cobalamin (pmol/L)			
Baseline	796	177·7 (138·1, 230·3)	175·6 (132·3, 229·0)
8 months of pregnancy	370	237·2 (200·3, 273·7)	152·7 (125·9, 191·0)
6 months postpartum	255	247·0 (186·4, 323·2)	172·3 (149·7, 202·8)
Total homocysteine (mmol/L)			
Baseline	796	5·9 (5·1, 7·0)	5·9 (5·0, 7·2)
8 months of pregnancy	370	5·4 (4·6, 6·6)	6·3 (5·0, 7·8)
6 months postpartum	256	6·7 (5·8, 7·8)	7·9 (6·6, 9·8)
Methylmalonic acid (mmol/L)			
Baseline	796	0·18 (0·13, 0·29)	0·20 (0·13, 0·32)
8 months of pregnancy	370	0·18 (0·13, 0·25)	0·32 (0·22, 0·46)
6 months postpartum	256	0·16 (0·12, 0·22)	0·26 (0·18, 0·39)
Folate (nmol/L)			
Baseline	796	57·8 (33·9, 76·2)	57·2 (34·9, 75·0)
8 months of pregnancy	370	17·0 (12·0, 24·5)	19·0 (13·4, 24·5)
6 months postpartum	256	17·9 (12·9, 21·6)	16·9 (12·4, 19·7)
Infant			
Cobalamin (pmol/L)			
6 months of age	255	208·6 (170·5, 249·1)	183·6 (153·3, 213·7)
12 months of age	265	193·0 (160·3, 278·5)	184·2 (156·8, 266·7)
Total homocysteine (mmol/L)			
6 months of age	256	7·1 (6·1, 8·7)	10·4 (8·3, 13·5)
12 months of age	266	7·0 (5·8, 8·3)	8·1 (6·6, 10·1)
Methylmalonic acid (mmol/L)			
6 months of age	256	0·23 (0·15, 0·37)	0·37 (0·24, 0·61)
12 months of age	266	0·29 (0·19, 0·49)	0·37 (0·27, 0·67)
Folate (nmol/L)			
6 months of age	256	47·4 (37·2, 60·4)	61·2 (45·0, 80·5)
12 months of age	266	44·3 (33·8, 57·0)	44.2 (37·3, 58·7)

Table S6a. Subgroup analyses. Primary and secondary outcomes in infants of mothers with adequate baseline vitamin B12 status (3cB_{12} >the lowest 33rd percentile)*

	N	Vitamin B_{12} (N=269)	Placebo (N=262)
Anthropometry			
6 months			
Length-for-age z-score	484	-0·5 (0·9)	-0·4 (1·0)
Weight-for-age z-score	484	-0·2 (1·1)	-0·1 (1·1)
Weight-for-length z-score	484	0·2 (1·1)	0·3 (1·1)
BMI z-score	484	0·1 (1·1)	0·2 (1·1)
12 months			
Length-for-age z-score	483	-0·5 (1·0)	-0·5 (1·1)
Weight-for-age z-score	483	-0·4 (1·1)	-0·2 (1·1)
Weight-for-length z-score	483	-0·1 (1·1)	0·0 (1·1)
BMI z-score	483	-0·1 (1·1)	0·1 (1·0)
Neurodevelopment			
Bayley-III scores, 6 months			
Cognitive composite score	484	100·4 (10·3)	99·4 (10·9)
Language composite score	484	88·1 (8·0)	89·1 (7·4)
Motor composite score	484	99·4 (13·2)	99·6 (13·8)
Socio-emotional score	484	101·0 (18·0)	101·4 (17·9)
Bayley-III scores, 12 months			
Cognitive composite score	481	95·6 (10·6)	95·8 (9·3)
Language composite score	481	76·7 (11·4)	76·5 (11·2)
Motor composite score	481	93·2 (11·0)	94.4 (12·0)
Socio-emotional score	481	100·7 (17·1)	100·7 (16·2)
Test of Infant Motor Performance (TIMP), 8–12 weeks			
TIMP total score	475	75·5 (9·9)	77·9 (9·2)
TIMP scores below average	475	128 (53·3%)	90 (38·3%)
Infant haemoglobin			
Haemoglobin concentration (g/dL), 6 months	501	11·2 (1·0)	11·2 (1·1)
Haemoglobin concentration (g/dL), 12 months	478	11·1 (1·2)	11·1 (1·2)
Pregnancy and birth outcomes			
Still birth†	508	No obs	1 (0·4)
Gestational length at birth, (weeks), mean (SD)‡	508	38·5 (1·5)	38·5 (1·9)
Preterm delivery (<37 weeks)	508	21 (8·3%)	23 (9·0%)
Congenital anomaly§	508	9 (3·6%)	5 (2·0%)
Birth weight baby (gm)	507	2990.0 (440·4)	3075·6 (454·9)
Birth weight<2500 gm	507	24 (9·5%)	17 (6·7%)
Birth weight<2000 gm	507	5 (2·0%)	4 (1·6%)
Birth weight>4000 gm	507	3 (1·2%)	2 (0·8%)
Maternal haemoglobin			
Haemoglobin concentration (g/dL), 8 months of pregnancy	508	11·7 (1·3)	11·6 (1.1)
Haemoglobin concentration (g/dL), 6 months postpartum	508	13·2 (1·2)	13·3 (1.0)

*Combined indicator of cobalamin status based on cobalamin, total homocysteine and methylmalonic acid, †still delivery after 28 weeks of gestation, ‡Gestational week was calculated based on last menstruation period, §The following congenital anomalies were identified during first visit after delivery; congenital heart disease (atrial septum defect, patent ductus arteriosus, cleft palate, polydactyly and other (undescended testes, laryngomalacia, pes planus, hypoplastic phalanges)

Table S6b. Subgroup analyses. Primary and secondary outcomes in infants of mothers with poor baseline vitamin B_{12} status ($3cB_{12}$ in the lowest 33rd percentile)*

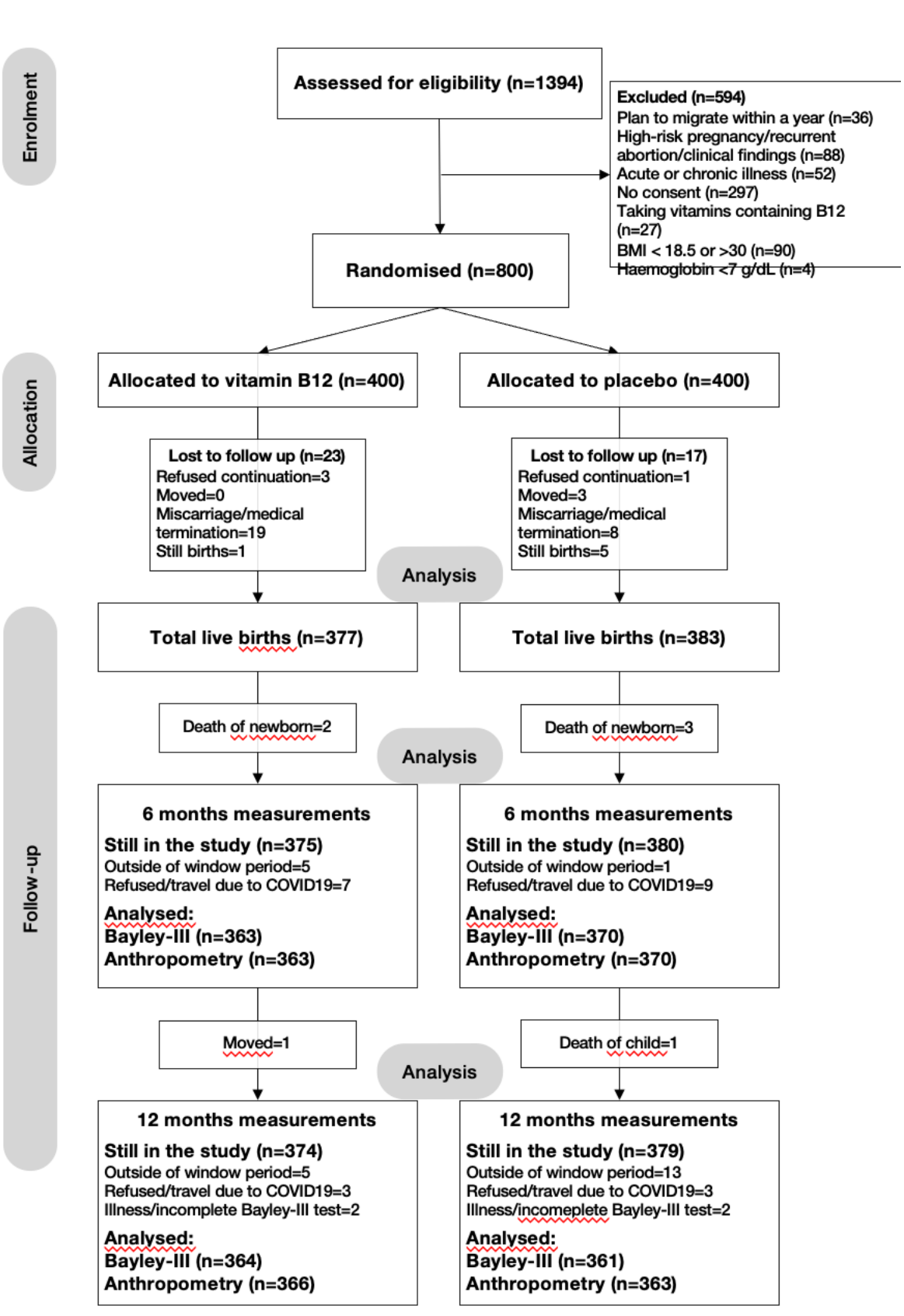
	N	Vitamin B_{12} (N=128)	Placebo (N=137)
Anthropometry			
6 months			
Length-for-age z-score	245	-0·5 (1·1)	-0·5 (0·9)
Weight-for-age z-score	245	-0·2 (1·1)	-0·2 (1·0)
Weight-for-length z-score	245	0·3 (1·0)	0·2 (1·0)
BMI z-score	245	0·1 (1·0)	0·1 (1·0)
12 months			
Length-for-age z-score	242	-0·6 (1·1)	-0·6 (1·0)
Weight-for-age z-score	242	-0.2 (1·1)	-0·3 (1·0)
Weight-for-length z-score	242	0·1 (1·0)	0·0 (1·0)
BMI z-score	242	0·2 (1·0)	0·1 (1·0)
Neurodevelopment			
Bayley-III scores, 6 months			
Cognitive composite score	245	99·3 (9·9)	97·9 (10·3)
Language composite score	245	88·7 (8·1)	88·5 (7·7)
Motor composite score	245	100·5 (12·7)	99·5 (13·6)
Socio-emotional composite score	245	99·6 (16·6)	100·8 (13·8)
Bayley-III scores, 12 months			
Cognitive composite score	240	94·9 (9·9)	94·4 (9·2)
Language composite score	240	78·3 (13·8)	77·1 (12·0)
Motor composite score	240	95·1 (11·2)	93·3 (12·2)
Socio-emotional composite score	240	98·9 (16·4)	99·7 (15·4)
Test of Infant Motor Performance, 8-12 weeks			
TIMP total score	234	77.3 (11·2)	78·0 (8·8)
TIMP scores below average	234	53 (46·1%)	46 (38·7%)
Infant haemoglobin			
Haemoglobin concentration (g/dL), 6 months	247	11·2 (1·0)	11·2 (1·1)
Haemoglobin concentration (g/dL), 12 months	243	11·2 (1·2)	11·3 (1·3)
Pregnancy and birth outcomes			
Still birth†	255	1 (0·8%)	4 (3·0%)
Gestational length at birth, (weeks), mean (SD) ‡	255	38·2 (2·2)	38·5 (1·8)
Preterm delivery (<37 weeks)	255	16 (13·1%)	10 (7·5%)
Congenital anomaly§	255	1 (0·8%)	2 (1·5%)
Birth weight baby (gm)	253	2978·3 (509·9)	2984·5 (437·7)
Birth weight<2500 gm	253	16 (13·1%)	16 (12·2%)
Birth weight<2000 gm	253	5 (4·1%)	2 (1·5%)
Birth weight>4000 gm	253	3 (2·5%)	1 (0·8%)
Maternal haemoglobin			
Haemoglobin concentration (g/dL), 8 months of pregnancy	253	11·5 (1.2)	11·5 (1·3)
Haemoglobin concentration (g/dL), 6 months postpartum	253	13·2 (0.9)	13·3 (0·9)

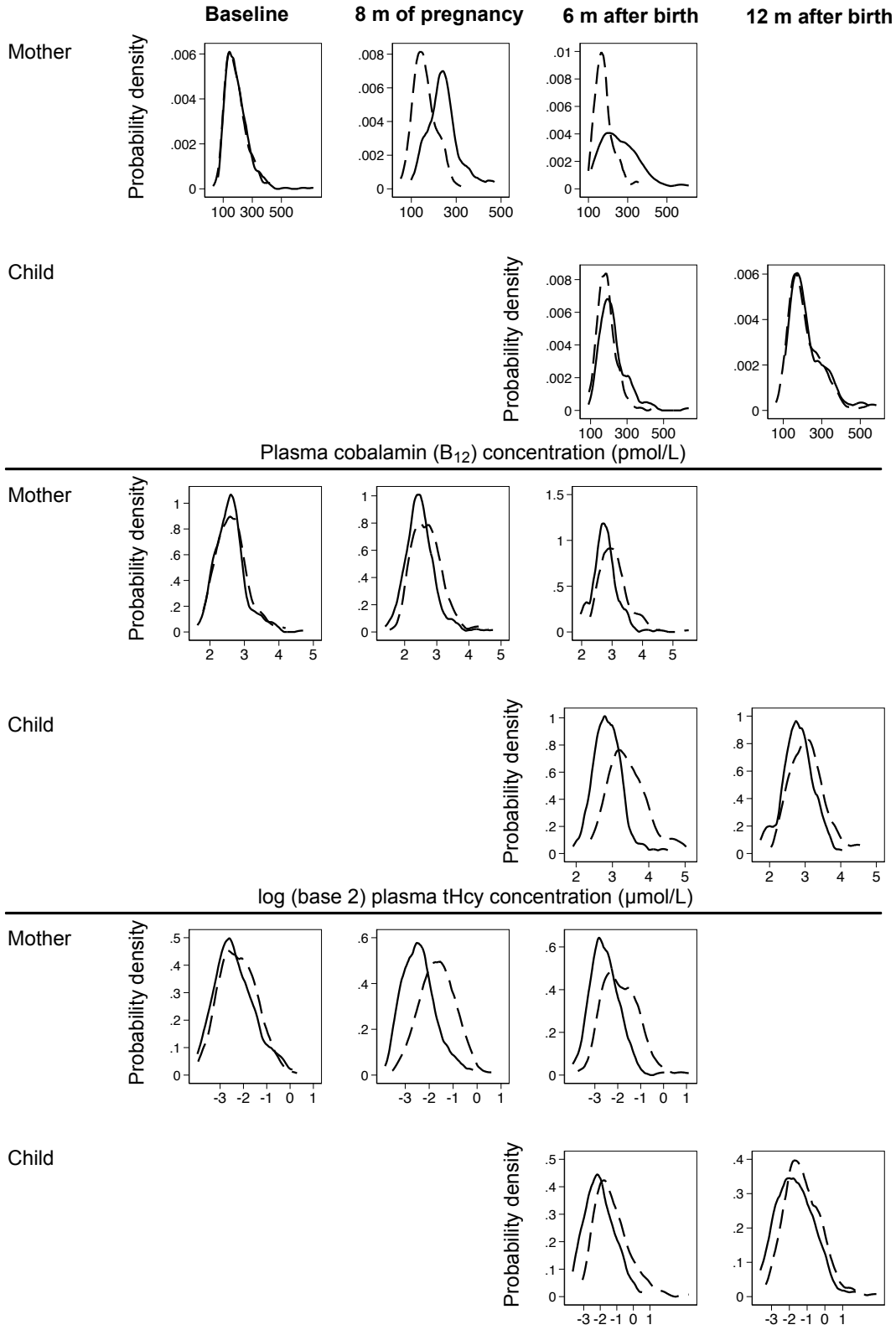
*Combined indicator of cobalamin status based on cobalamin, total homocysteine and methylmalonic acid, †still delivery after 28 weeks of gestation, ‡Gestational week was calculated based on last menstruation period, §The following congenital anomalies were identified during first visit after delivery; congenital heart disease (atrial septum defect, patent ductus arteriosus, cleft palate, polydactyly and other (undescended testes, laryngomalacia, pes planus, hypoplastic phalanges)

Table S7. Severe adverse events reported in a study investigating the effect of daily vitamin B12 supplementation during pregnancy and postpartum on neurodevelopment and growth in 800 Nepalese pregnant mother-infant dyads

		Vitamin B_{12} (N=400)	Placebo (N=400)
	n		
Miscarriage	15	11	4
Medical termination*	12	8	4
Still birth	6	1	5
Hospitalization of the mother†	117	68	49
Infant death (total)	6	2	4
Due to neonatal sepsis		2	2
Due to extreme prematurity			1
Due to complex congenital heart disease			1

*Due to severe anomaly detected during USG (such as holoprosencephaly, cystic hygroma and hydrops fetalis, anencephaly, gross hydrocephalus etc.). †Mainly due to threatened abortion, urinary tract infection, oligohydramnios, hypertension, hyperemesis gravidarum, eclampsia or pre-eclampsia and decreased foetal heart sound


Enrolment
Assessed for eligibility (n=1394)
Excluded (n=594)
Plan to migrate within a year (n=36)
High-risk pregnancy/recurrent abortion/clinical findings (n=88)
Acute or chronic illness (n=52)
No consent (n=297)
Taking vitamins containing B12 (n=27)
BMI < 18.5 or >30 (n=90)
Haemoglobin <7 g/dL (n=4)
Randomised (n=800)
Allocation
Allocated to vitamin B12 (n=400)
Allocated to placebo (n=400)
Lost to follow up (n=23)
Refused continuation=3
Moved=0
Miscarriage/medical termination=19
Still births=1
Lost to follow up (n=17)
Refused continuation=1
Moved=3
Miscarriage/medical termination=8
Still births=5
Analysis
Follow-up
Total live births (n=377)
Total live births (n=383)
Death of newborn=2
Death of newborn=3
Analysis
6 months measurements
Still in the study (n=375)
Outside of window period=5
Refused/travel due to COVID19=7
Analysed:
Bayley-III (n=363)
Anthropometry (n=363)
6 months measurements
Still in the study (n=380)
Outside of window period=1
Refused/travel due to COVID19=9
Analysed:
Bayley-III (n=370)
Anthropometry (n=370)
Moved=1
Death of child=1
Analysis
12 months measurements
Still in the study (n=374)
Outside of window period=5
Refused/travel due to COVID19=3
Illness/incomplete Bayley-III test=2
Analysed:
Bayley-III (n=364)
Anthropometry (n=366)
12 months measurements
Still in the study (n=379)
Outside of window period=13
Refused/travel due to COVID19=3
Illness/incomeplete Bayley-III test=2
Analysed:
Bayley-III (n=361)
Anthropometry (n=363)


Baseline
8 m of pregnancy
6 m after birth
12 m after birth
Mother
Child
Probability density
Plasma cobalamin (B_{12}) concentration (pmol/L)
log (base 2) plasma tHcy concentration (µmol/L)
log (base 2) plasma MMA concentration (µmol/L)