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Vitamin B₁₂ status in infancy and the effect of a vitamin B₁₂ injection in infants with subclinical vitamin B₁₂ deficiency: study protocol for a register-based randomised controlled trial

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ABSTRACT

Introduction Vitamin B₁₂ (cobalamin) is crucial for optimal child development and growth, yet deficiency is common worldwide. The aim of this study is twofold; (1) to describe vitamin B₁₂ status and the status of other micronutrients in Norwegian infants, and (2) in a randomised controlled trial (RCT), investigate the effect of vitamin B₁₂ supplementation on neurodevelopment in infants with subclinical vitamin B₁₂ deficiency.

Methods and analysis Infant blood samples, collected at public healthcare clinics, are analysed for plasma cobalamin levels. Infants with plasma cobalamin <148 pmol/L are immediately treated with hydroxocobalamin and excluded from the RCT. Remaining infants (cobalamin ≥148 pmol/L) are randomly assigned (in a 1:1 ratio) to either a screening or a control group. In the screening group, baseline samples are immediately analysed for total homocysteine (tHcy), while in the control group, the baseline samples will be analysed after 12 months. Screening group infants with plasma tHcy >6.5 μmol/L, are given an intramuscular injection of hydroxocobalamin (400 μg). The primary outcomes are cognitive, language and motor development assessed using the Bayley Scales of Infant and Toddler Development at 12 months of age.

Ethics and dissemination The study has been approved by the Regional Committee for Medical and Health Research Ethics (ref: 186505). Investigators who meet the Vancouver requirements will be eligible for authorship and be responsible for dissemination of study findings. Results will extend current knowledge on consequences of subclinical vitamin B₁₂ deficiency during infancy and may inform future infant feeding recommendations.

Trial registration number NCT05005897.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study is a population-based randomised controlled trial with an innovative design making the results generalisable for the infant population in Norway.
⇒ Effects on child neurodevelopment will be assessed using a comprehensive neurodevelopmental test battery.
⇒ The lack of blinding is a limitation of the study.
⇒ Exclusion of infants with low cobalamin concentrations can bias the effect estimates towards a null effect.

INTRODUCTION
Vitamin B₁₂

Vitamin B₁₂ (cobalamin) deficiency is common in many low-income and middle-income countries.[1][2] There is also evidence that deficiency and subclinical deficiency may be common in developed countries, particularly among specific groups of the population, such as vegans, vegetarians and the elderly.[3] Poor vitamin B₁₂ status has been described in Norwegian infants and young children.[4–7] Observational studies link vitamin B₁₂ status to neurodevelopment, growth and other outcomes.[8–11] Four randomised controlled trials (RCT) have investigated the effect of vitamin B₁₂ supplementation on neurodevelopment and growth in children.[6][7][12][13] Of these, two facility-based RCTs among Norwegian infants born with low birth weight or with developmental delay, demonstrated that a single intramuscular injection of 400 μg hydroxocobalamin substantially improved short-term motor development.[6][7] The two other studies were population-based RCTs in India and Nepal. In the Indian study, where growth and neurodevelopment were secondary outcomes, daily supplementation with 1.8 μg vitamin B₁₂ for 6 months resulted in small, borderline significant, beneficial

effects on neurodevelopment and growth.[12 13] The study in Nepal enrolled 600 infants who were supplemented with 2 µg vitamin B_{12} or a placebo daily for 1 year. While vitamin B_{12} supplementation had a substantial effect on direct and indirect markers of B_{12} status,[14] no effects were observed for growth, neurodevelopment or other clinical outcomes.[14–16] There are many potential reasons for the heterogeneity of these results. Notably, there was a substantial difference in the doses and mode of administration; the two Norwegian studies provided a single intramuscular injection dose of 400 µg,[6 7] while the studies in India and Nepal delivered daily, oral doses at around two to three times the recommended dietary allowance for 6 and 12 months, respectively.[12 13]

Measuring vitamin B_{12} status

Total plasma cobalamin (bound to transcobalamin and haptocorrin) is the most commonly used marker of vitamin B_{12} status.[17] However, the functional markers, total homocysteine (tHcy) and methylmalonic acid (MMA), are considered more sensitive.[18] Homocysteine re-methylation to methionine requires vitamin B_{12} and folate, and vitamin B_{12} deficiency leads to elevated plasma tHcy concentrations.[19] Vitamin B_{12} also functions as a cofactor in the enzyme, methylmalonyl CoA mutase, which links circulating concentrations of MMA to vitamin B_{12} status.[19] There is currently no consensus on an accepted plasma cobalamin cut-off value for vitamin B_{12} deficiency although many use <148 pmol/L.[20] Similarly, there is no agreed cut-off for plasma tHcy levels indicative of vitamin B_{12} deficiency, but tHcy >6.5 µmol/L has been used in research to define impaired cobalamin function among infants.[6 7] Although plasma MMA is generally thought to be a more specific functional indicator of vitamin B_{12} status, compared with tHcy, MMA is typically elevated and highly variable during infancy and, accordingly, not well suited for evaluating B_{12} status during this period.[21 22]

Micronutrients and breast feeding

It is widely accepted that human milk alone provides almost all necessary nutrients for the first 6 months of life.[23] Globally, exclusive breast feeding and breast feeding duration is associated with reduced prevalence and severity of infectious diseases, gut inflammation and cognitive abilities.[24 25] There is also compelling evidence that human milk may have long-term positive health effects for both the child, as well as the mother, for example, by reducing risk of breast cancer.[25 26] However, there are also reports suggesting that the status of micronutrients, including vitamin B_{12}, is lower in exclusively and predominantly breastfed children compared with non-breastfed children.[7] Both the concentrations and bioavailability of vitamin B_{12} are higher in dairy products compared with human milk.[27 28] While a positive association has been observed between maternal vitamin B_{12} intake and human milk concentration in marginally nourished women,[29] there is a paucity of evidence in well-nourished women. One Norwegian study has described an association between maternal vitamin B_{12} status and infant status,[30] but another

study found no association between maternal vitamin B_{12} intake and human milk B_{12} concentrations.[31] Vitamin B_{12} concentrations in human milk are highest in colostrum and decrease during the lactation period.[29] If the infant's mother has had inadequate absorption or intake of vitamin B_{12} during pregnancy, the infant will have considerably lower vitamin B_{12} stores and therefore be at higher risk of deficiency.[29] Emerging evidence suggesting a link between breast feeding, micronutrient status and in particular mild vitamin B_{12} deficiency, could challenge current breastfeeding recommendations.[32] Breastfed infants may be more likely to have subclinical vitamin B_{12} deficiency that could potentially affect their development and other health outcomes. From this perspective, to recommend a prolonged period (4–6 months) of exclusive or predominant breast feeding may not be justified in a Norwegian context. Thus, there is a need to more robustly investigate infant vitamin B_{12} status, the effect of breast feeding and the potential implications of subclinical vitamin B_{12} deficiency during infancy for development and long-term health.

The aim of this study is twofold; (1) to describe vitamin B_{12} status and the status of other micronutrients in Norwegian infants, and (2) in an RCT, investigate the effect of vitamin B_{12} supplementation on neurodevelopment in infants with subclinical vitamin B_{12} deficiency. Due to ethical reasons, it is not possible to randomise those with known subclinical vitamin B_{12} deficiency into a placebo group. Accordingly, many nutritional RCTs enrol participants who are not deficient and whose participation dilutes the effect of the intervention increasing the risk of a type II error (ie, causing clinically relevant effects sizes to be overlooked). Moreover, since this study will provide high doses and parenteral administration, we will target only those with biochemical signs of subclinical vitamin B_{12} deficiency and not all infants.

Additional exposures and outcomes, such as early markers of chronic disease, exposure to environmental contaminants, inflammation, epigenetics and long-term consequences will also be addressed in the observational cohort study.

Aims and primary objectives

Aim 1

Describe vitamin B_{12} status and the status of other micronutrients in Norwegian infants and explore risks for poor status in an observational design.

Primary objectives

1. Measure vitamin B_{12} status using plasma cobalamin and tHcy in Norwegian infants.
2. Describe risk factors for subclinical vitamin B_{12} deficiency in Norwegian infants.

Aim 2

Within an RCT design investigate the effect of providing a high-dose vitamin B_{12} injection (400 µg) to infants with subclinical vitamin B_{12} deficiency on neurodevelopment.

Primary objective

1. Among infants with subclinical vitamin B_{12} deficiency (defined as plasma tHcy >6.5 µmol/L) investigate the effect of a single intramuscular injection of 400 µg hydroxocobalamin, given when the child is 6–12 weeks old, on Bayley Scale of Infant and Toddler Development—3rd edition scores at 12 months of age.

Secondary objectives

1. Investigate the effect of the intervention on other (neuro)developmental outcomes such as eye-tracking, cardiac vagal tone and Ages and Stages Questionnaire (ASQ) at 6 ad 12 months of age.
2. Measure the effect of the intervention on linear growth at 12 months.
3. Measure the status of several nutrients in pregnancy and during the first year of life, including markers of folate, iron (ferritin, transferrin receptor), iodine (thyroglobulin) and vitamin D.
4. Measure the status of several nutrients in human milk.
5. Measure the status of iodine and thyroid function in mothers and their infants.
6. Investigate the extent to which different micronutrients deficiencies and thyroid dysfunction are associated with poor growth and neurodevelopment.
7. Evaluate the relationship between the status of several nutrients and inflammation and immune function in mothers and their infants.
8. Investigate the long-term effect of 400 µg hydroxocobalamin injection on neurodevelopment, growth and markers of non-communicable diseases during childhood.

METHODS

Study setting, recruitment and participants

This protocol paper is based on the study protocol V.1.2 dated 22 June 2021. All versions of the protocol will be available on request. We keep a log of the amendments to the protocol which will also be made publicly available. The study is organised through Innlandet Hospital Trust (IHT), Norway, in collaboration with Inland Norway University of Applied Sciences, Department of Psychology, and Public Healthcare Clinics. The project is linked with a birth registry cohort entitled the Innlandet Health Registry for Offspring and Parents (IHOP) and a maternal and child research biobank (MoBI biobank). The study population consist of infants born in Innlandet County in Norway. Parents are informed and recruited to IHOP during their routine pregnancy ultrasound scan at around 18 weeks of gestation. We obtain informed consent from both parents.

After birth, at the infant's 6–12 week visit to the public healthcare clinic, the study staff invite all mother–infant pairs to the observational cohort study and the vitamin B_{12} RCT, and a new informed consent is obtained. For the RCT part of the study, we have some additional eligibility and exclusion criteria. In addition to availability

of informed consent, to participate the families must plan to reside in the defined study area for the next 12 months. Infants with severe systemic illness requiring hospitalisation, growth retardation or severe congenital malformations are excluded from the RCT. Furthermore, infants with vitamin B_{12} deficiency defined by plasma cobalamin concentration <148 pmol/L are excluded from the RCT, treated immediately for vitamin B_{12} deficiency and included only in the observational cohort study.

Study design and intervention

For Aim 1 and many of the secondary objectives, we use an observational design where all participants are included in the cohort study. For Aim 2, we implement an alternative RCT design, as described in figure 1. Prior to randomisation and as part of the screening process a blood sample is drawn from all infants in the study which is analysed for plasma cobalamin concentration. Table 1 describes the definitions we use in this study.[33] Those with vitamin B_{12} deficiency (defined as plasma cobalamin <148 pmol/L) are immediately treated with an intramuscular injection of hydroxocobalamin (400 µg) and excluded from the RCT, but remain in the observational cohort study. Infants with plasma cobalamin >148 pmol/L are randomised (in a 1:1 ratio) to either the screening or the control group. In the screening group, blood samples are analysed for plasma tHcy immediately, while in the control group samples are analysed 12 months after enrolment in the RCT. If plasma tHcy is elevated (tHcy >6.5 µmol/L) in the screening group, the infant is treated with an intramuscular injection of 400 µg hydroxocobalamin. This design allows us to study the effects of the intervention in infants with subclinical vitamin B_{12} deficiency (tHcy >6.5 µmol/L) without violating the principle of clinical equipoise, while simultaneously ensuring that infants with vitamin B_{12} deficiency (plasma cobalamin <148 pmol/L) receive treatment.

Rationale for choosing plasma tHcy to define subclinical vitamin B_{12} deficiency

A plasma tHcy concentration of >6.5 µmol/L was chosen since this level was previously suggested as a cut-off for defining subclinical vitamin B_{12} deficiency in infants.[34] This concentration represented the 97.5 percentile in infants who were given a single intramuscular injection of 400 µg of hydroxocobalamin at 6 weeks of age, to render them B_{12} replete.[4]

Rationale for choosing 400 µg dose of hydroxocobalamin

The 400 µg dose of hydroxocobalamin has been used in previous RCTs among infants without any observed adverse effect.[7 34] Nonetheless, we will closely follow all the children who receive intramuscular injection to ensure optimal safety and detailed reporting of any adverse events. Study personnel administer hydroxocobalamin injections at participating healthcare clinics.
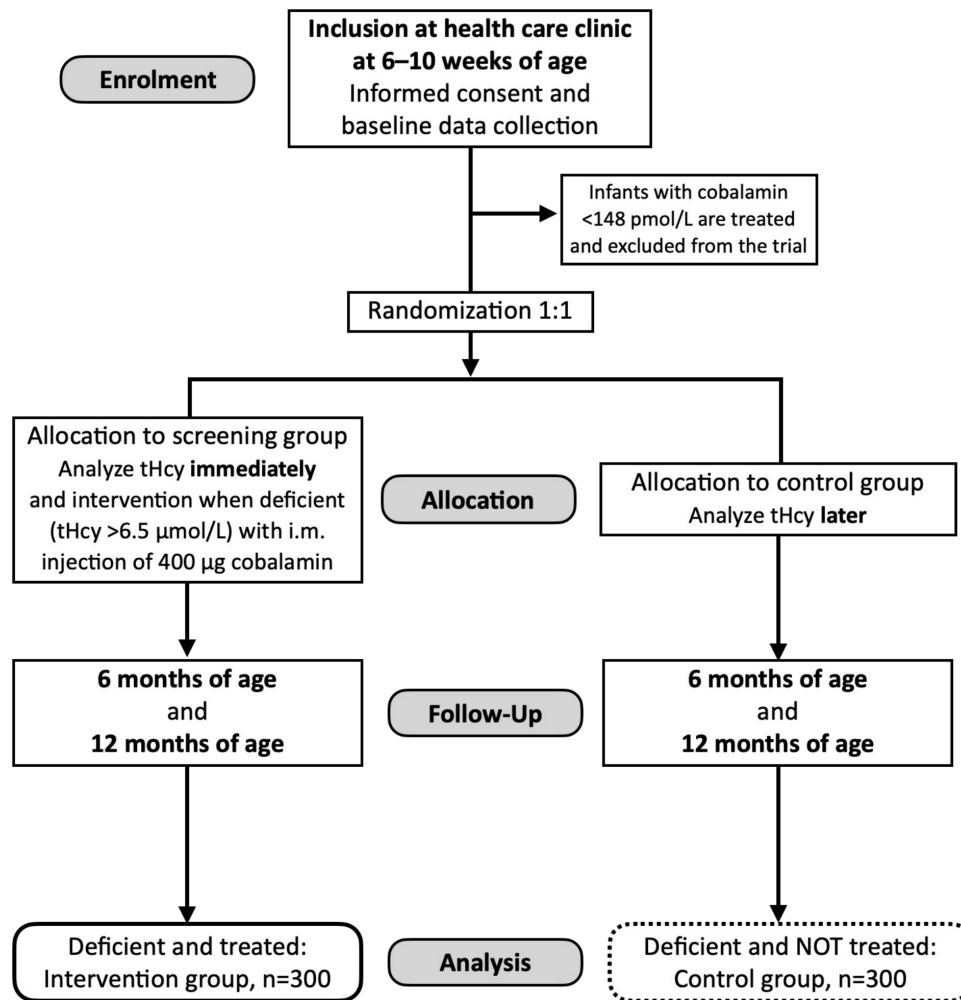
Figure 1 Study flow chart of the randomised controlled trial with vitamin B_{12} supplementation to children with subclinical vitamin B_{12} deficiency. i.m., intramuscular injection; tHcy, total homocysteine.

Randomisation, concealment of allocation and blinding

Randomisation is performed by an independent researcher otherwise not involved in the study using a computer-generated randomisation list. Infants are randomised in a 1:1 ratio in random block sizes between 2 and 8. The allocation is concealed from the study team, and only the researcher responsible for the analyses of plasma tHcy has access to the randomisation list. Primary outcome assessors are blinded from treatment arm, and parents are requested to not reveal to the assessors whether their infant has received an injection.

**Table 1** Vitamin B_{12} status definitions used in the present study[33]

Vitamin B_{12} deficiency	Plasma cobalamin <148 pmol/L.
Subclinical vitamin B_{12} deficiency	Plasma tHcy >6.5 μmol/L and plasma cobalamin ≥148 pmol/L.
Adequate vitamin B_{12} status	Plasma tHcy ≤6.5 μmol/L and plasma cobalamin ≥148 pmol/L.

tHcy, total homocysteine.

Sample size justification

We anticipate following 85% of the enrolled participants with signs of subclinical vitamin B_{12} deficiency until the main outcome is assessed at 1 year. For an effect size of 0.25 SD, results from 500 infants are required to achieve a statistical power of 80%. We will therefore target 600 infants (500/(1–0.15)=588). Sample sizes or different scenarios for the various effects are shown in figure 2. The power to detect differences between the study groups are larger for larger effect sizes. In our estimations we assume a two-sided significance level of 0.05. The calculations were performed using Stata V.16 using the 'power twomeans' command.

In this study we will compare cognitive development assessed at 1 year, between infants with subclinical vitamin B_{12} deficiency at baseline treated with hydroxocobalamin (n=300) versus infants with untreated subclinical vitamin B_{12} deficiency (n=300).

Previous studies from Norway have indicated that approximately 8% of infants have vitamin B_{12} deficiency, and 46–79% have subclinical deficiency.[6 35 36] We will use the same biomarker and cut-off to define subclinical
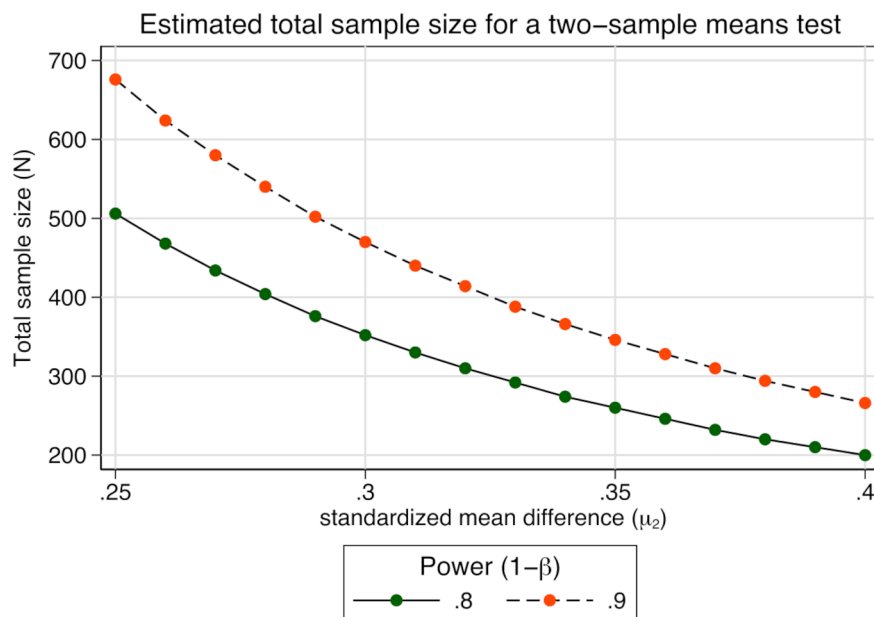
Figure 2 Estimated required total sample sizes for the randomised controlled trial according to different standardised mean differences.

vitamin B_{12} deficiency, but anticipate the proportion of infants may be different in our population-based sample. To reach the goal of including 600 infants with subclinical vitamin B_{12} deficiency, we have chosen to target 2400 infants. This will be sufficient even if only one-third of screened infants have signs of subclinical vitamin B_{12} deficiency (tHcy>6.5 μmol/L).

Study procedure and outcomes
Data collection procedures are described in table 2. The first participant was enrolled in the RCT in November 2021, and we plan to continue to enrol participants for at least 2 years. Data collection of the primary outcomes will be completed approximately 1 year after the last enrolment. The participants are enrolled through public healthcare centres, and we also use these facilities to ensure minimum dropouts throughout the study period. Our study team, which consists of medical doctors, midwives, clinical nutritionists, nurses and other certified health personnel, will maintain contact with the participants, again optimising study participants' retention. We also use a digital tool recording all scheduled activities in this project.

Blood sampling, processing and analysis
Phlebotomy trained study personnel collect a venous blood sample from enrolled infants at the routine 6–12 week visit to the public healthcare clinics. A maximum of 2.6 mL K2EDTA blood is drawn from the dorsal metacarpal veins (the back of the hand) by open venous sampling.[37] If unable to collect a venous blood sample, capillary blood is collected using a heel lance procedure. A maximum of four attempts are made before the child is excluded from the study. An oral sugar solution is administered before blood sampling for analgesic effects. Within 30 min of blood sampling, the K2EDTA blood sample is centrifuged,

plasma is harvested and stored at 4°C in cryogenic storage tubes which are shipped to IHT at Lillehammer for storage and further processing. The samples are stored long-term at −80°C in the MoBI biobank at IHT, except for an aliquot of plasma which is analysed for cobalamin on the same day as blood collection using accredited methods at the clinical laboratory at IHT. A frozen aliquot from each participant is shipped to an external laboratory (bevital.no) where cobalamin, tHcy, folate as well as other relevant biomarkers reflecting micronutrient status and inflammation activities are measured (figure 1).

Assessment of child development
The neurodevelopmental assessments are administered by trained testers supervised by experienced psychologists. In addition, parents are asked to complete questionnaires assessing child development. In this study we wanted to assess neurodevelopment as late as possible to improve reliability.[38 39] The following tests are administered:

Bayley Scale of Infant and Toddler Development—3rd edition (Bayley-3). Bayley-3 is a comprehensive assessment tool of early child development in infants and toddlers aged 1–42 months.[40] The Bayley-3 has a Norwegian validated version; it is administered directly with the child and provides comprehensive information on various aspects of development, such as cognition, language, fine and gross motor abilities. The Bayley-3 is considered by many to be the gold standard for developmental assessment of infants and young children and is widely used to evaluate the effect of interventions in early childhood.[40] Bayley-3 is administered at 12 months.

Eye tracking is a sensitive and specific method for investigating subtle delays or deficits in development[41] using the eye's reflection of near infrared light to assess ocular motor control and the efficacy of processing visual

Table 2 Schedule for events in the register-based adaptive randomised trial

	Pregnancy			Months in infancy											
	1	2	3	1	2	3	4	5	6	7	8	9	10	11	12
Informed consent	*				†										
Data collection in pregnancy	x		x												
Demographics	x														
Maternal samples (blood and urine)	x		x												
Anthropometry	x		x												
Baseline data collection for the RCT					x										
Infant samples (blood and urine)‡					x										x
Maternal samples (blood, urine and human milk)					x										
Anthropometry of infant					x				x			x			x
Dietary assessments (24-hour recall and/or FFQ)					x										x
Maternal mental health§					x										
Allocation (screening or control group)					x										
Intervention (hydroxocobalamin injection)					**										
Neurodevelopment¶									x						x

*Information about the study and first consent during the first ultrasonography.
†At approximately 4 weeks postpartum we ask eligible mother–infant pairs to participate in the RCT at the healthcare clinics.
‡One blood sample is drawn at 6–12 weeks of age. Children with identified vitamin B_{12} deficiency or subclinical vitamin B_{12} deficiency will repeat the blood sample at 12 months.
§Edinburgh Postnatal Depression Scale.
¶Parent-reported Ages and Stages Questionnaire at 6 and 12 months, test-team administrated Bayley Scales of Infant and Toddler Development, eye-tracking and heart rate variability at 12 months of age.
**Infants in the screening group with elevated plasma tHcy (>6.5 µmol/L) are treated with an intramuscular injection of hydroxocobalamin (400 µg) at 6–12 weeks of age.
FFQ, Food Frequency Questionnaire; RCT, randomised controlled trial; tHcy, total homocysteine.

information.[42] The method has been validated for use in children aged 2 months and older.[43] It can assess infants' visual acuity, visuospatial orientation as well as attention to social cues. These skills provide building blocks for more complex cognitive systems and have been found to predict later neurodevelopment.[44] Three tests of early cognitive function are administered: A visual search task to assess attention and visual orientation abilities, a learning task to assess visuospatial memory functioning across modalities and a social interaction task to assess social cognition. In addition, basic ocular motor functions (saccades and smooth pursuit) are tested by showing alternating and moving objects.

Heart rate variability or cardiac vagal tone is an index of the parasympathetic nervous system and can serve as a psychophysiological marker for emotion regulation in both children and adults.[45] Measuring heart rate changes during stimulus presentation is an effective way to assess the alerting and attentional systems of infants.[41] Vagal tone is influenced by the prefrontal cortex and has been associated with emotion, cognitive and social adaptability.[45] As such, vagal tone can serve as an important indicator of early neurodevelopment. Vagal tone activity is measured by recording infants' cardiac activity while watching a short video.

Ages and stages questionnaire—3rd edition (ASQ-3). The ASQ-3 is an easily administered checklist of child development standardised for children aged 1–66 months.[46] The questionnaires contain age-appropriate questions with 2–3 months intervals. Each questionnaire contains 30 items assessing five domains: communication, gross motor, fine motor, problems solving and personal social. In addition to the ASQ-3, the Ages and Stages Questionnaire: Social and Emotional (ASQ:SE) is administered to assess social and emotional difficulties. To assess developmental trajectories, we ask parents to fill out the ASQ-3, and ASQ:SE at 6 months and 12 months.

Other study variables

We collect additional data on socioeconomic status, maternal micronutrient status during pregnancy, obstetric history, breast feeding and maternal mental health. Infant weight and length will be measured at scheduled controls at the public healthcare clinics at baseline (6–12 weeks), 6 months, 9 months and at 12 months of age. We also administer a 24-hour dietary recall and food frequency

questionnaires to gather information on maternal dietary habits and infants' food and drink intake.

Training and quality control

The study team arranges Bayley-3 training workshops for testers responsible for assessing children in the study. The testers are trained and standardised in performing the Bayley-3 ahead of starting the study assessment procedures. To ensure good interobserver agreement, 10% of all sessions are scored by two testers.

Data management and analysis

The collected data is stored on services for sensitive data (TSD) and the research server at IHT. Both servers are approved for storing and managing sensitive data and personal information. Only selected researchers have access to the data through the institutions mentioned in this application. Data will not be made public, but access to the data can be granted through a formal application process ensuring privacy and integrity of data. The statistical software packages Stata and R will be used to analyse the data. A detailed plan of analysis for the main and secondary outcomes will be made prior to data analysis. Data cleaning, definition of outcome variables, exclusion of cases, as well as programming of scripts in the statistical packages, will also be performed before the analysis-files are merged with the randomisation lists. The intervention groups will be coded so the researchers do not know the group identities until the main analyses are completed. The analyses will be planned and undertaken in a joint workshop attended by the researchers involved. All analyses will initially be undertaken on an intention-to-treat-basis. All randomised participants with complete data on primary outcomes will be included in the analyses. For the observational cohort design, we will also begin by planning the analyses where the research questions, exposures and outcomes are clearly defined. We will use multiple regression models for dichotomous and continuous outcome variables adjusting for relevant confounders. We will also explore the relation between the relevant exposure variables and outcomes in nonlinear models such as generalised additive models.

Monitoring

The data monitoring committee consists of three members independent from the study team. They are scheduled to meet twice each year where they are informed about adverse events and the study progress. They can also request an interim analysis before the required sample size has been met.

Patient and public involvement

Public healthcare nurses participate throughout the study through regular meetings with the research team. Through this collaboration we have the opportunity to receive valuable feedback on various aspects concerning the conduct of the study, such as the enrolment procedures, relevant information to be provided ahead of study start, as well as practicalities in the study implementation.

We will also discuss our findings and get valuable input on the interpretation and communication of results.

ETHICS AND DISSEMINATION
Ethical considerations

The study is undertaken in accordance with the Helsinki Declaration and Oviedo Convention including clinical trial registration. Relevant ethical approval has been obtained from the regional Human Research Ethics Committee (REK; ref: 186505). Informed written consent is collected from all participants prior to participation. Participants are informed that participation in the study is voluntary and can be terminated at any time. All participants are given time and encouraged to ask questions and raise potential concerns. The study results and data collected are strictly confidential and only unidentifiable data will be used for evaluations. The biological samples are stored in the registered biobank 'MoBI-Biobank' (Biobank # 3792 Mor og Barn i Innlandet) which has been approved by REK. To reduce discomfort for the infant, the sampling of venous blood is performed by trained and experienced health personnel. All neurodevelopmental tests are performed in an age adequate way without stress for the infants. Even though no clinical evaluation of child development is performed during the study, the use of neurodevelopmental tests makes it possible to detect developmental delays. In the case that serious delays that are not caused by a pre-known health condition are detected, children will be referred to appropriate health professionals for further evaluation. If severe deficiencies or other signs of illness are detected among the enrolled children, they will be referred to the study physician who is a paediatrician. Infants receiving hydroxocobalamin intervention are monitored closely after the injection to ensure they do not develop any reactions. A scientific advisory group consisting of representatives from the user group, paediatricians, nutritionists and epidemiologists otherwise not involved in the project has been established prior to recruiting the first child. The advisory group provides advice on the design, dose, inclusion and exclusion criteria. Investigators who have contributed to conceptualising and conducting the study, as well as being involved in the data analyses and manuscript writing will be eligible for authorship and be responsible for dissemination of the study findings.

DISCUSSION

This proposed study will report the vitamin B_{12} status of up to 2400 infants and investigate the effect of injection of hydroxocobalamin (vitamin B_{12} supplementation) at 6–12 weeks on child developmental outcomes at 12 months. The lack of or limited effect of B_{12} supplementation in previous RCTs may be due to too low doses, short supplementation duration or timing of the intervention. The two previously mentioned small RCTs in clinical populations found substantial beneficial effects

of high-dose hydroxocobalamin. However, it is difficult to justify high-dose interventions among infants with no clinical or biochemical signs of insufficiency or deficiency. Further if infants with mild deficiency or clinical signs of poor status are identified and randomised to the placebo arm, we will violate the principle of clinical equipoise. It should also be noted that the measurement of vitamin B_{12} status is challenging, with no single biomarker capable of being used as an unequivocal indicator of B_{12} status or deficiency. Although total plasma cobalamin is the most commonly used marker of vitamin B_{12} status, it lacks sensitivity as a functional marker of B_{12} status compared with tHcy, MMA or holotranscobalamin.[47] However, while tHcy may be a more sensitive marker, it can lack specificity, since tHcy concentrations can be influenced by factors other than vitamin B_{12} status, including folate status, age, smoking and genetics.[48] While this project will use only plasma cobalamin and tHcy to screen at baseline, we will also measure multiple biomarkers involved in one-carbon metabolism providing the opportunity to investigate other potential functional biomarkers of vitamin B_{12} status in infants, such as cystathionine.

The balance between avoiding the provision of high-doses of hydroxocobalamin to B_{12} replete individuals and randomising vitamin B_{12} deficient individuals to a placebo group, was the reason we chose a screening design for this RCT. However, REK initially did not approve the protocols based on the principle of clinical equipoise. We appealed their decision and following an additional review, REK upheld their decision. Following an appeal to the National Committee for Medical and Health Research (NEM), the study was approved in June 2021. Due to the initial concern raised by REK, we added the first screening step in order to screen out (exclude) and treat all infants with plasma cobalamin concentration <148 pmol/L.

This project will contribute to better understanding of the relationship between nutrition, growth and neurodevelopment, which in turn may influence public healthcare and nutrition policies. The widely accepted recommendation of exclusive breast feeding for 6 months is challenged by claims that exclusive breast feeding beyond 3–4 months increases the risk of certain micronutrient deficiencies, which may impact health and development.[32] Through our involvement in decision-making public bodies, we will ensure rapid dissemination of our study results to relevant local and national health authorities.

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