

# **Biological risks and neurodevelopment in young North Indian children**

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## **Abstract**

### **Background**

Children growing up in poverty face multiple biological and psychosocial risks, some of which are primarily found in low to middle income countries. Unfavorable biological circumstances, such as inadequate nutrition and childhood illnesses put children at risk for compromised development. Early child development is a precursor for later cognitive functioning and may have lasting consequences for academic achievement and lifelong quality of life. There is a need to identify modifiable risk factors for poor development in order to break the vicious cycle of poverty and poor development.

### **Objectives**

The objectives of the current thesis are threefold. First, to examine the effect of two recommended daily allowances of vitamin B12 and/or folic acid for six months on neurodevelopment. Second, to identify other relevant risk factors for poor neurodevelopment in young North Indian children; and, finally, to assess the feasibility of the screening tool Ages and Stages Questionnaire 3<sup>rd</sup> ed. (ASQ-3) administered by field supervisors to collect reliable data on neurodevelopment in this North Indian field setting.

### **Methods**

In a randomized double blind trial, children six to 30 months were given vitamin B12 and/or folic acid or placebo daily for six months. At the end of the study, we measured neurodevelopment in 422 children by ASQ-3 administered directly with the child. During the six months study period, we collected data on childhood illnesses bi-weekly. Information regarding socioeconomic status was collected at baseline, and information regarding stimulation and learning opportunities was collected at end

study. Applying linear and logistic regression models, we measured the effect of vitamin B12 and/or folic acid on neurodevelopment, and identified other predictors of developmental status. We also assessed the psychometric properties of the ASQ-3 in the current setting.

## **Results**

Compared to placebo, children who received both vitamin B12 and folic acid had 0.45 (95% CI 0.19, 0.73) and 0.28 (95% CI 0.02, 0.54) higher SD-units in the domains of gross motor and problem solving functioning, respectively. The effect was highest in selected subgroups consisting of stunted children, those with evidence of folate and vitamin B12 deficiency and in those who were younger than 24 months at the end of the study. With the exception of a significant improvement in gross motor scores by vitamin B12 alone, supplementation of either vitamin alone had no effect on any of the outcomes measures. In the multiple regression models, we were able to explain 30.6% of the variation in the total ASQ-3 score. Height for age and weight for height z-scores were positively associated with the total ASQ-3 score, while the number of days with diarrhea was negatively correlated. Variables defined as stimulation and learning opportunities explained most of the variation (25.9%). Our results also indicate that it is possible to collect reliable data on neurodevelopment in this field setting using ASQ-3.

## **Conclusion**

Vitamin B12 and folate deficiency, stunting and prolonged diarrhea illness may be important modifiable biological risks adverse neurodevelopment in young children in the current setting. Adequate stimulation and responsive care are crucial for healthy development. Interventions targeting young children growing up in poverty in LMIC should integrate various modifiable risk factors including nutrition, hygiene and early child stimulation to maximize developmental potential in children.

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## List of publications

- Paper I Kvestad, I., Taneja, S., Kumar, T., Bhandari, N., Strand, T. A., & Hysing, M. (2013). The assessment of developmental status using the Ages and Stages questionnaire-3 in nutritional research in north Indian young children. *Nutrition journal*, *12*(1), 50.
- Paper II Kvestad, I., Taneja, S., Kumar, T., Hysing, M., Refsum, H., Yajnik, C.S., Bhandari, N., & Strand, T.A. (2015). Vitamin B12 and Folic Acid Improve Gross Motor and Problem-Solving Skills in Young North Indian Children: A Randomized Placebo-Controlled Trial. *PLoS ONE*, *10*(6).
- Paper III Kvestad, I., Taneja, S., Hysing, M., Kumar, T., Bhandari, N., & Strand, T. A. (2015). Diarrhea, stimulation and growth predict neurodevelopment in young north Indian children. *PLoS ONE*, *10*(3).

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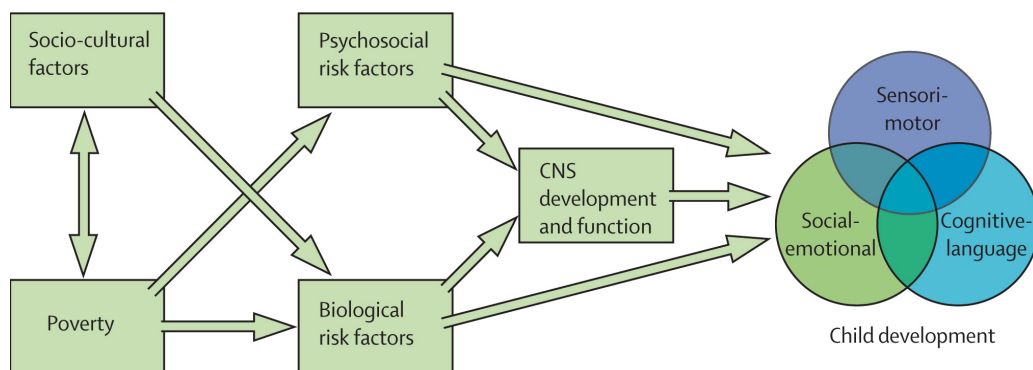
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# 1 Introduction

## 1.1 Background

Poverty is a marker for several biological and psychosocial risks that affect early child development (**Figure 1**) (1). Some of these risks are primarily seen in low to middle income countries (LMICs) (1-4). Optimal development for young children has a range of immediate and long-term positive consequences (5). Early child development is a precursor for later cognitive functioning with lasting consequences for academic achievement, and children that do not fulfill their potential due to poverty related risks early in life are less likely to be productive adults (6-8). To maximize developmental potential through secured health, well-being and that all young children develop according to their full potential should be an obligation in itself for the global community (9).



*Figure 1. Pathways from poverty to poor child development from Walker et al.(1)*

Inadequate nutrition and childhood illnesses are biological risks that can compromise early child development (1, 3). Approximately 26% (165 million) of the children in the world suffer from poor linear growth (10). Micronutrient deficiencies are widespread in the context of poverty (11) and the burden of childhood morbidity is

high in LMICs (12). The effect these biological risk factors exert on the central nervous system (CNS) is accompanied by the influence of several psychosocial risk factors that may further compromise development. The impact of both of these clusters of risks on child development is determined by various intermediate factors such as timing of the exposure, dosage and cumulative effects (3, 13). To fully understand the impact of these factors on child development, the socio-cultural context must be taken into consideration. In the context of poverty in LMICs, there is a need to identify modifiable risk factors to break the vicious cycle of poverty, illness and impaired development (7, 14, 15).

## 1.2 Clarification of concepts

For the current thesis the concept of *neurodevelopment* will be used to describe the functional outcome of the CNS, and thus as a phenomenon that is closely linked to processes in the developing brain. The term is understood to encompass various domains of development, and the developmental changes at different ages.

In the framework of this thesis, it may be useful to distinguish between the structural and the functional outcomes of the development in the CNS. In the following, the structural correlate of child development will be referred to as the *developing brain* encompassing brain growth and maturity in interaction with environmental factors giving rise to behavioral outcomes (16). The functional outcome is the observable behavior in the child that may be captured through various child development assessment tools. In infants and young children, the term *developmental status* is frequently used when referring to their global capacity captured in assessment tools (17). This is a descriptive term referring to performance at given milestones for the current age. Furthermore, due to the rapid growth and development in the early ages, the concept of developmental status underscores the idea that assessments only provide a snapshot of the child's current functioning. Closer follow-ups are needed to capture developmental trajectories and fully understand development in infancy and

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toddlerhood (17, 18). In the course of brain development, the emerging skills in question will change, and these changes will have consequences for what domains that can be assessed. In early infancy, for example, simple motor responses and vocalizations are easily measured, while with maturation, more functional motor behavior and advanced observable CNS skills such as executive functions and language can be more easily assessed (17).

Recently, there has been extensive interest in the study of early child development in children compromised by the consequences of poverty in LMICs. In this field, the concept of *early child development* has been widely used to describe children's development in the period from early infancy through preschool years (1, 6, 7, 19). The specific age ranges applied vary in the literature. Some define, the period up to five to six years, while others restrict the period of early child development to approximately two years, based on the widely used notion of 1000 significant days from conception through 24 months (2, 20, 21). The definition of relevant developmental domains also differs. Some authors apply a wide definition, including physical growth and well-being, cognitive development, and socio-emotional development (7). Others define the concept somewhat narrower, for example as the three related, but still distinct developmental domains of sensorimotor, cognitive-language and social-emotional function (**Figure 1**) (1, 6, 19). Furthermore, studies on child development and risks often restrict the definition of early child development to the assessment of single domains and processes such as the study of motor development, executive functioning, specific memory processes or socio-emotional abilities.

### 1.3 The developing brain

The first years of life from gestation and onwards is a period of rapid brain growth and development through neurogenesis, cell migration, neuronal differentiation, and pruning of cells and their interconnections (17). These developmental processes are

finely tuned with genetically predetermined sequences building on each other under the influence of the environment (16, 22, 23). Thus, the unique brain architecture is generated prenatally based on the genetic framework in close interaction with environmental influences. The high-level neural circuits that give rise to complex mental processes build on this architecture, refined and modified by individual experiences and inputs from the surrounding environment over an extended period of CNS maturation lasting into adolescence (13, 16). Since low-level circuits mature early in life and high-level circuits mature later, age-appropriate experiences are essential for the developing brain, where different types of experiences are crucial at different age periods (16).

Due to massive and sequential growth, many argue that the first years of life are a period in which the brain is particularly susceptible to environmental input, with enhanced capacity to respond and modify itself according to exposures (16, 24, 25). The brain develops through a combination of experience-expectant and experience-dependent mechanisms. The former reflects the universal development occurring across settings (i.e. through sensory stimulation such as visual, auditory and tactile, and linguistic input), whereas the latter depends on input from the surrounding environment to emerge (i.e. quality and quantity of stimulation) (26). If the presence or absence of an exposure is associated with irreversible changes to the brain, the period is characterized as a critical period for development (16, 27). Critical periods have sharply defined time windows, and are linked to specific neurodevelopmental domains (27). For the visual system and gross motor abilities, critical periods have been suggested early in life, whereas less is known for more complex neurobehavioral domains (23, 27). Sensitive periods are periods with increased susceptibility to exposure from the environment. In contrast to critical periods, sensitive periods have broader time windows and are not necessarily linked to irreversible brain impairments.

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Timing is a key factor in determining the impact of an exposure to the developing brain (22, 24). The relationship between timing of exposures to the brain and the neurodevelopmental outcomes is complex, and various exposures may be linked to various sensitive periods (23). In research on early brain injury, for example, insults to the brain prior to age three are associated with poorer outcomes and more global cognitive deficits, whereas insults later in childhood are associated with faster recovery and outcomes closer to normal developmental expectations (25). Similar effects of timing have been reported for example in early onset epilepsy (28), and in relation to the influence of nutrition (24). With regard to nutrition, the impact on neurodevelopment will vary according to the type of the nutritional deficiency, and to what extent these coincide with essential timeframes in the developing brain (24, 29). Some micronutrients exert their impact at very specific points of time. For instance folate is crucial for the CNS in the third week of pregnancy during the closing of the neural tube (30), whereas for other key micronutrients, the significance of timing has not yet been firmly established (31).

In the context of poverty, threats to healthy brain development tend to cluster together and the literature consistently shows that the higher the number of risk factors surrounding the child, the more detrimental for the developing brain (3, 4, 21). Dosage refers to both the intensity of exposures (or the lack of one), and to the numbers of risks encountered (4). For nutrition, for example, the impact on the developing brain may depend upon the severity of the nutritional deficiency. At the same time, micronutrient deficiencies in malnourished populations generally co-occur, and it is likely that the deficiency of one key micronutrient is accompanied by several other deficiencies, representing multiple threats to the developing brain (32).

## 1.4 Biological risks

### 1.4.1 Inadequate nutrition

The developing brain depends on adequate nutrition for healthy development. Although important throughout the life-span, nutritional input is of greatest importance during gestation and the first years of life involving rapid brain growth (13, 31). Nutritional deficits in these first years may have serious consequences for the developing brain. Similarly this early period also represents a window of opportunity for reversibility and repair if the necessary nutritional supplements are provided (31).

Nutritional deficiencies influence children's development both directly and indirectly. Directly, adequate nutrition is necessary for the development of brain structures, as well as the level and effect of neurotransmitters (33). Indirectly, adequate nutrition may be a prerequisite for the child's ability to engage with and profit from the environment. A malnourished child might be fussy and irritable, or weak and apathetic. These consequences of malnutrition may compromise neurodevelopment through alternations in how the child relates to the environment and explores his or her surroundings, and carries with it the risk of not receiving adequate stimulation from the caregiver. These indirect effects of malnutrition are often referred to as functional isolation (33-35).

Nutritional deficits are generally studied through poor growth and micronutrient deficiencies. In the literature, both poor growth and micronutrient deficiencies have been linked to adverse neurodevelopmental outcomes.

#### *Poor growth*

Poor growth is often expressed as being underweight [weight-for-age z-score  $<-2$  (WAZ)], wasted [weight-for-height z-score  $<-2$  (WHZ)] or stunted [height-for-age z-score  $<-2$  (HAZ)]. The concept of being underweight does not distinguish between

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wasting and stunting. As a result, when used as the only measure in areas where the prevalence of stunting but not of wasting is high, there is a risk of underestimating the prevalence of malnutrition (36). Wasting indicates poor ponderal growth, reflecting a relatively short-term process with the lack of uptake and conversion of nutrients due to factors such as food insecurity and illnesses. Stunting refers to chronic undernutrition affecting linear growth. Stunting has a complex etiology, and the underlying mechanisms and reasons are less clear than for wasting (10). Stunting is not restricted to food insecure populations, and nutritional interventions have only modest effects on growth (37). Stunting may be viewed as an inflammatory disease occurring as a consequence of multiple causes of enteropathy seen in poverty settings, such as diarrhea, acute malnutrition and micronutrient deficiencies (36). In short, stunting can be perceived as a result of a catabolic state either caused by malnutrition or chronic illness, where the “anabolic drive” needed for growth is suppressed.

A recent review of 68 studies involving childhood stunting and neurodevelopment reports a robust positive association between linear growth and cognitive and motor development (38). The review indicates that the largest effects on neurodevelopment occur when the growth restriction appears before the age of two. Several studies suggest that stunting occurring in early childhood predicts educational outcomes in later childhood and adolescence (39-41). However, being closely related to poverty, a wide range of co-existing risk factors associated with stunting have an impact on neurodevelopment (31, 38). Thus, confounding variables will always be a challenge when studying stunting and neurodevelopmental outcomes.

The mechanisms linking stunting to impaired neurodevelopment are not fully understood (36). Indirect effects referred to as functional isolation may help explain some of the impact of chronic undernutrition on neurodevelopment. In addition, the CNS grows rapidly in early childhood, and the brain has a major demand for energy. Lack of energy in this period is likely to affect the development of the CNS (36). The

importance of the degree of nutrient deficiency for neurodevelopmental outcomes has been described (13). It seems like the developing brain is compromised in case of severe nutrient deficiency, but is spared if the deficiency is mild. The phenomenon of protecting brain growth when overall growth is impaired is referred to as brain sparing, and has been described in animal studies (42).

### *Micronutrient deficiency*

Micronutrient deficiencies are widespread in the context of poverty. Whereas most nutrients are important for growth and development, some are particularly important for the developing brain and healthy neurodevelopment (43). In general, the literature presents solid evidence for the importance of iron and iodine in the developing brain (3, 13, 34). Iron deficiency is common worldwide and a widely accepted risk factor for adverse brain development. It has been discussed however, whether the relationship between iron status and neurodevelopment only becomes apparent when the deficiency is severe and causes anemia (44). There is sound evidence linking severe prenatal iodine deficiency to mental retardation in the children through maternal hypothyroidism (31). The effect of moderate to mild iodine deficiency on neurodevelopment is less clear (45).

Micronutrients such as n-3 polyunsaturated fatty acids (n-3 PUFA), folate, zinc and vitamin B12 are linked to brain development, but the evidence is less clear. Van de Rest (2012) concludes in a review that although some studies show an effect of n-3 PUFA on certain cognitive domains, there is little evidence supporting an overall beneficial effect (30). The role of folate in the closing of the neural tube in early pregnancy is widely accepted, and the supplement of folic acid is recommended from around conception in order to secure sufficient levels in early gestation (46). In the Norwegian population-based MOBA study, folic acid supplement during pregnancy was associated with postnatal neurodevelopment. The lack of folic acid supplement during pregnancy was associated with severe language deficits (47), and an increase in the prevalence of autism spectrum disorders (48). For Zinc, supplementation trials



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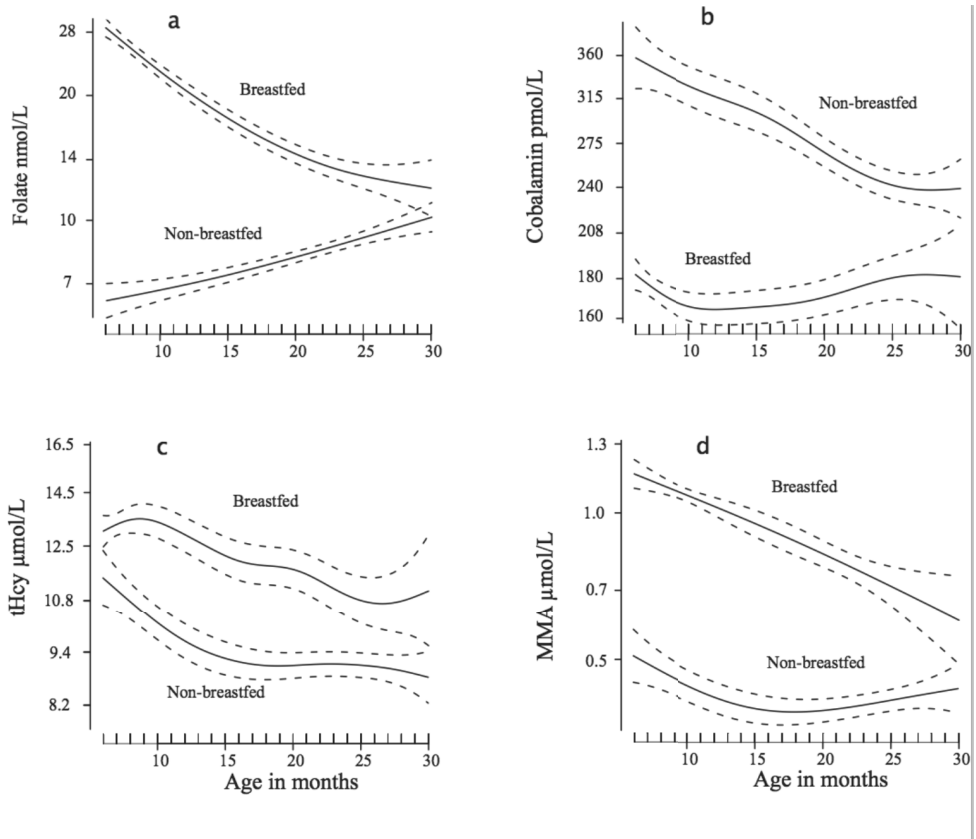
have given inconsistent results (31). For example, a study set in North India showed only marginal effects from zinc supplementation on the developmental scores in infants 12 to 18 months (49), whereas a study in Bangladesh showed positive effect of zinc supplementation in exploratory behavior in infants 12 months of age (50).

The neurodevelopmental effects of a given nutrient are based on the metabolic effects in a given brain region, the timing of the deficiency and the degree and duration of the deficiency (24, 29, 31). Furthermore, there are likely individual thresholds for each nutrient when deficiencies have an adverse effect on the developing brain. These thresholds are not known for all nutrients (31). For example, the negative consequences of severe acute vitamin B12 deficiency in early childhood are thoroughly described, but less is known about the impact of long-term marginal vitamin B12 levels in children (51).

### *Vitamin B12*

The main source of vitamin B12 is animal products, such as meat, poultry, eggs and milk. Animal source foods are often expensive, and thus vitamin B12 deficiency is associated with poverty and is common in many LMICs (51-53). Some studies however, have demonstrated that marginal vitamin B12 status is common also among children in a general population in Norway (54, 55). For the infant, the main source of vitamin B12 is through the mother's diet during pregnancy and in the period of exclusive breastfeeding. Thus, low maternal vitamin B12 concentrations are linked to low concentration in infants both before and after birth (52, 56). In South Asia where poverty is widespread and many do not eat animal products due to cultural and religious reasons, it has been demonstrated that more than half of women and children have biochemical evidence of poor vitamin B12 status (55, 57, 58). Furthermore, infants who are exclusively breastfed have lower vitamin B12 levels than infants who are not (**Figure 2**), which is also a finding among Norwegian infants (57, 58).

In the brain, folate and vitamin B12 are important for cell division and are involved in common biological processes (46). Therefore, folate needs to be taken into account when studying the impact of vitamin B12 on neurodevelopment. Elevated plasma total homocysteine (tHcy) is a marker of both vitamin B12 and folate deficiency, whereas increased plasma methylmalonic acid (MMA) indicates functional vitamin B12 deficiency only (59, 60).



*Figure 2.* The association between age and plasma vitamin B12, folate, tHcy and MMA according to whether the child was breastfed or not in North Indian young children (based on data from ref. 57).

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Among adults and the elderly, having low levels of vitamin B12 is related to cognitive impairments, dementia and depression (61). In the developing brain, vitamin B12 is essential for myelination (51, 62). Myelin is the lipid material that covers the axons in the CNS, and its main purpose is to increase the speed at which impulses travel along the nerve fiber (63). The process of myelination of the CNS starts during gestation, is concentrated through the first year of life, and continues throughout puberty (22). Alterations in the myelination of the brain have consequences for multiple systems. Consequently, the impact of vitamin B12 deficiency may be seen in a variety of CNS systems and neurodevelopmental outcomes (51).

#### *Studies of vitamin B12 and neurodevelopment in early childhood*

Various sources have documented the significance of vitamin B12 in the developing brain. The literature is mainly based on case studies and observational studies of children or their mothers suffering from vitamin B12 deficits. Several randomized controlled trials (RCT) have also been carried out involving the supplement of multiple micronutrients (MMN) both in mothers during pregnancy and in children, where vitamin B12 is included as part of the micronutrient packages. In this review of literature the relevant RCTs involving MMN with vitamin B12 to either pregnant women or to infants/young children have been included. For the studies involving children, the search has been restricted to studies distributing the supplement (RCT) or measuring vitamin B12 levels (observational design) in young children less than three years old (**Table 1**).

In a review of case studies of vitamin B12 deficit in infants, Dror and Allen (2008) report symptoms such as failure-to-thrive, eating problems, apathy and developmental regression. In most cases, recovery is rapid following treatment, however, some studies report of continuing delays after treatment (62). There are few observational studies on the association between vitamin B12 status and neurodevelopment in young children. Five studies were identified investigating the

association between maternal vitamin B12 status and neurodevelopment in offspring, providing inconsistent evidence of the significance of maternal vitamin B12 status on neurodevelopment. Three of these studies are set in Pune, India (64-66), the fourth in Mexico (67), while the fifth is placed in Massachusetts, USA (68). The studies in India use plasma concentration during pregnancy as the measure of vitamin B12 levels, whereas the latter assesses maternal dietary intake during pregnancy. Two of the Indian studies investigated the association between maternal vitamin B12 status (28 and 30 weeks of gestation) and cognitive functioning in their nine-year-old offspring with contradictory findings. One study reported worse performance on a test of sustained attention and memory digit span in children of vitamin B12 deficient mothers (64). In a second study, there was no consistent association between maternal vitamin B12 and cognitive performance among their nine-year olds (66). The third study from the same population measured vitamin B12 status at 28 and 34 weeks pregnancy in 123 women, and in their children at two years of age (65). The children were assessed by a general developmental status and motor test and a social maturity test. At two years, the vitamin B12 status of the children was associated with maternal vitamin B12 status during pregnancy. Furthermore, their mental and social development was positively associated with maternal vitamin B12 status independent of their current status. No associations were seen between vitamin B12 deficiency and motor development in this study. In the study set in Mexico, maternal dietary deficiency of vitamin B12 during the first trimester was negatively associated with the mental development index of the Bayley Scales of Infant Development (Bayley) 2. ed. during the first year of life (67). The study from the USA found a slight inverse relationship between intake of vitamin B12 at second trimester and the cognitive scores (68).

We have identified two observational studies investigating early childhood vitamin B12 status and neurodevelopmental outcomes (69-71). In a Dutch observational study, children who were raised on macrobiotic diets had poorer growth and worse performance on cognitive tests in adolescence independent of their current vitamin

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B12 status (70). In the same group of children, poorer language development and delayed gross motor milestones were reported in early childhood compared to a control group (69). The association between early postnatal vitamin B12 status and neurodevelopmental outcomes was also demonstrated in a North Indian cohort of young children, where low levels of vitamin B12 consistently were associated with lower scores on the mental development index scores on the Bayley 2. ed. (71). A third observational study has been described, but results have not been formally published in a peer-reviewed journal (72). In this study, 12-month-old children in Guatemala with inadequate vitamin B12 levels had impaired motor functioning compared to children with adequate vitamin B12 levels.

Few RCTs involving supplementation of vitamin B12 during pregnancy or in infants and young children younger than three years with neurodevelopment as the primary outcome have been identified. No RCTs were identified involving maternal vitamin B12 supplementation as a single micronutrient. However, six studies were identified that provided MMN to one or more of the study groups, in which vitamin B12 was included (73-78). Although the results from these studies must be interpreted with care on the specific effect of vitamin B12, the studies are included in this report. The earliest of these studies was conducted in rural Taiwan in the late 1960s early 1970s and reported higher motor scores on the Bayley 1<sup>st</sup> ed. in children of mothers who received high calorie and protein supplement also including MMN during pregnancy compared with placebo (73). The vitamin B12 dose in this MMN was 1 µg, and children were tested during their first year of life. The second study was in HIV-1 infected mothers in Tanzania who received 50 µg vitamin B12 daily as part of a MMN resulting in a significant increase in motor scores on the Bayley scales (75). In the last four studies, all in Asian countries, the vitamin B12 dose in the MMN was 2.6 µg, and the MMN was compared with supplement of iron and folic acid (74, 76-78). In three of these studies, children were assessed at age 42 months or younger (74, 76, 77). In the fourth study, which was in Nepal, a follow-up assessment of cognition and motor abilities at age 7-9 years reported no effect of maternal MMN on any of the

cognitive scores compared to placebo (78). Whereas one study carried out in China reports an overall benefit of MMN on mental development on the Bayley scales at 12 months (77), the two remaining studies find effects of MMN in susceptible subgroups such as in women with low BMI in Bangladesh (76), and in malnourished and anemic mothers in Indonesia (74) in children aged 7 and 42 months respectively.

Five RCTs involving supplementation of vitamin B12 in infant and young children were identified. In four of these trials, children from six to 12 months received vitamin B12 as part of an MMN supplementation in fortified milk, powder or syrup (0.7 µg) (79), in porridge (0.25 µg) (80), in tablets and butter (0.5 µg) (81) or in a liquid mixture in one out of five treatment groups (50), for 4 months, 26 weeks and 6 months respectively. All studies show improvements in the MMN groups on measures of neurodevelopment. The first study included observational measures of physical activity and exploration (79), the two next on gross motor milestones (80, 81) and the latter on Bayley 2<sup>nd</sup> ed. and the Behavior Rating Scale (50). In one of these trials however, differences were found between the control group and all the groups that received MMN, independent of the inclusion of 0.5 µg vitamin B12 (81). Furthermore, in one trial there were no differences between the group that received iron and zinc and the group who received the MMN with vitamin B12 (50). A fourth trial has been conducted in Norwegian infants who were initially referred to a pediatric outpatient clinic (POPC) due to eating difficulties (82). All children, six weeks old, had biochemical signs of vitamin B12 deficiency, and received an injection of a large dose of vitamin B12 (400 µg) or placebo. After one month, results on a gross motor test showed positive effect of the injection (82).

In the aforementioned eleven RCTs, ten studies provided vitamin B12 supplementation as part of a MMN. It is therefore not possible to disentangle the effect of vitamin B12 from that of the other micronutrients or possible synergistic effects. In all the studies, the dose of vitamin B12 varied from low to high, and there was no information about vitamin B12 status in the study participants at baseline or at

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end study. Eight of the identified RCTs reported positive effects on neurodevelopmental outcomes in groups that received vitamin B12 supplementation as part of an MMN, and one study reported positive effects on neurodevelopmental outcomes in infants that received vitamin B12 alone. Two of these trials were conducted in vulnerable groups, such as HIV-1 mothers and offspring (75), and infants referred to POPC with signs of vitamin B12 deficiency (82). Two trials did not find an overall effect of supplementation performed in a general population, but an effect in vulnerable subgroups of mothers with low-BMI (76) and in mothers who were malnourished and with anemia (74). A common finding in the RCTs involving young children is the impact on gross motor development. Taken together, the findings in these studies provide some evidence of the beneficial effects of MMN where vitamin B12 is included, and of vitamin B12 alone in susceptible infants on gross motor outcomes. However, whether the effects in the MMN studies are driven by vitamin B12 in isolation cannot be answered through these studies.

*Table 1. Studies investigating vitamin B12 and neurodevelopmental outcome in early childhood.*

<b>Author</b>	<b>Country</b>	<b>Sample size</b>	<b>Sample description</b>	<b>Age at testing</b>	<b>Outcome measure</b>	<b>Results</b>
<b>Randomized Controlled Trials</b>						
<i>During Pregnancy</i>						
Joos et al. (1982)	Taiwan	294	Pregnant women received high calorie and protein supplement, also containing MMN (1.0 µg vitamin B12) or placebo throughout the pregnancy.	250 days old	Bayley 1 <sup>st</sup> ed.	Higher motor scores compared to placebo in the high calorie, protein and MMN supplementation group.
McGrath et al. (2006)	Tanzania	327	Pregnant HIV-1 infected women from between 12 to 27 weeks gestation received a daily dose of MMN-supplement (50 µg vitamin B12) or placebo for a minimum of 18 months.	6, 12 and 18 mo	Bayley 2 <sup>nd</sup> ed.	Significant increase on the motor score in the MMN supplementation group.



Tofail et al. (2008)	Bangladesh	2853	Six groups of pregnant women: early (8-10 wk gestation) or usual (approx. 17 wk gestation) supplementation with food and MMN (2.6 µg of vitamin B12), 30 mg FE +400 µg folic acid or 60 mg FE + 400 µg folic acid.	7 mo	2 problem-solving tests, motor index of the Bayley 2 <sup>nd</sup> ed. and a behavior rating.	No overall effects in any of the groups. Small benefits of early food and MMN in children of low-BMI mothers but not in high-BMI mothers.
Li et al. (2009)	China	1305	Three groups of pregnant women: folic acid, folic acid and iron, and MMN (2.6 µg vitamin B12) supplementation daily during pregnancy.	3, 6 and 12 mo	Bayley 2 <sup>nd</sup> ed.	Significant improvements in the mental development scale at 12 months in the MMN supplementation group compared to the other groups. No effect on the motor scores.
Christian et al. (2010)	Nepal	676	Four groups of pregnant women: iron/folic acid, iron/folic acid/zinc, iron/folic acid/zinc/MMN (2.6 µg vitamin B12) or placebo (vitamin A alone) supplement daily from early pregnancy to 3 months postpartum.	7 to 9 yrs	UNIT, tests of executive functioning, MABC and finger tapping test.	Overall better test scores in the group that received folic acid and iron compared to placebo. No effect in the other intervention groups.

Prado et al. (2012)	Indonesia	487	Two groups of pregnant women: daily iron/folic acid or MMN (2.6 µg of vitamin B12).	42 mo	Tests of fine and gross motor development, language abilities, visuospatial abilities, visual attention, executive function and socio-emotional development.	No overall effect of MMN compared to iron/folic acid in any developmental domain. Effect of MMN on gross motor abilities and visual attention/spatial abilities in undernourished mothers, and on visual attention/spatial abilities in anemic mothers.
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*During infancy/early childhood*

Black et al. (2004)	Bangladesh	221	Infants 6 mo were randomized to 5 groups; iron, zinc, iron/zinc, MMN (including vitamin B12) or riboflavin weekly for 6 mo. Pre and post testing.	6 and 12 mo	Bayley 2 <sup>nd</sup> ed. and the Behavior Rating Scale.	Positive effect of supplementation of iron and zinc and the MMN on motor development.
Faber et al. (2005)	South Africa	361	Infants 6-12 mo were randomized to receiving MMN (0.25 µg vitamin B12) or placebo for 26 weeks in a fortified/unfortified porridge. Pre and post testing.	12-18 mo	Mothers report of gross motor milestones.	Positive effect of MMN on motor development.

Adu-Afarwuah et al. (2007)	Ghana	313	Infants 6 mo randomized to 3 groups of MMN, in 2 groups the MMN contained 0.5 µg vitamin B12. Supplement was provided for 6 mo and compared with a control group.	12 mo	Mothers report on 4 gross motor milestones: standing and walking with or without assistance.	Positive effect on the ability to walk in all supplement groups compared with the control group.
Aburto et al. (2010)	Mexico	188	Infants 4-12 mo receiving milk-based fortified-food supplement, MMN-powder or MMN-syrup for 4 mo compared to a control group (no MMN). All intervention groups include 0.7µg vitamin B12.	8-12 mo	Direct observation of activity and exploration.	Positive effect of MMN on the level of activity and exploration
Torsvik et al. (2013)	Norway	79	Children in a clinical sample with signs of vitamin B12 deficiency divided in 2 groups: placebo and 400 µg vitamin B12 injections.	10 wks	Alberta Infant Motor scale.	Positive effect of vitamin B12 on motor abilities one month after injection.

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## Observational studies

### *During pregnancy*

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Bhate et al. (2008)	India	108	Two groups of children were selected based on maternal plasma vitamin B12 concentration at 28 weeks gestation: low maternal plasma vitamin B12 (<77pM) and high maternal concentration (>224pM).	9 yrs	Raven, Visual recognition, Color trial test and Digit span test.	Children in the group with high maternal concentration had better performance on sustained attention and short-term memory. No differences between groups in the other tests.
Garcia et al. (2009)	Mexico	253	Maternal vitamin B12 and folate intake was assessed through dietary questionnaires during first trimester.	1,3,6 and 16 mo	Bayley 2 <sup>nd</sup> ed.	Dietary deficiency of vitamin B12 was negatively associated with mental development index.
Veena et al. (2010)	India	536	Maternal plasma concentration of folate, vitamin B12, tHcy were measured at 30 wk gestation.	9-10 yrs	Kauffman assessment battery and additional tests of cognitive functioning.	No consistent associations of maternal vitamin B12 and tHcy concentration with cognitive performance at age 9-10.

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Bhate et al. (2012)	India	123	Maternal plasma concentration of vitamin B12 and folate were measured at 28 and 34 wk gestation.	2 yrs	DASII and Vineland social maturity scale.	Maternal vitamin B12 and folate concentration were positively associated with mental and social development at 2 years.
Villamor et al. (2013)	USA	1210	Maternal intake of vitamin B12, folate, choline, betaine and methionine was assessed during the first and second trimesters of pregnancy.	3 yrs	Peabody Picture Vocabulary test III and the Wide Range Assessment of Visual Motor Abilities test.	Positive association between folate intake during first trimester and scores on the PPVTIII. A weak inverse association between vitamin B12 intake during second trimester and the PPVTIII score.

*During infancy/early childhood*

Dagnelie et al. (1994)	Nederland	110*	Infants raised on a macrobiotic diet (53) compared with a control group (57).	4-18 mo	Pediatric checklist; sitting and head balance, locomotion, fine motor, speech and language.	Children on a macrobiotic diet had lower scores on gross motor and language development.
Lowman et al. (2000)	Nederland	93*	Children raised on macrobiotic diet to the age of 6 yrs, with previously low vitamin B12 status (<229, 31), normal status (>229, 17) compared to a control group (24).	10-18 yrs	Raven, Block design, picture completion, digit symbol, digit span, word recall, word fluency, Pegboard, TAART.	Significant association between early vitamin B12 levels and scores on cognitive tests in later childhood/adolescence.

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Strand et al. (2013)	India	650	Children from an urban neighborhood in New Delhi.	12-18 mo	Bayley 2 <sup>nd</sup> ed.	Folate and vitamin B12 plasma concentration was significantly associated with scores on the mental development index.
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\* *results based on the same study*

### 1.4.2 Childhood illnesses

Diarrhea and pneumonia are the main causes of childhood mortality and morbidity in LMICs, with the greatest proportion of severe episodes occurring in Southeast Asia and Africa (83, 84). The quality of care provided for children burdened with these illnesses is often poor, and a large proportion of deaths associated with these illnesses occur during the first years of life (12, 83-85).

There are several plausible pathways from childhood infections to adverse neurodevelopmental outcomes. The indirect effects of functional isolation described earlier may apply in the case of childhood illnesses, which put the affected child in risk of delayed development due to the behavioral consequences of the infections.

Cytokines are inflammatory signaling proteins (86). Chronic cytokine activation is associated with alterations in biological processes needed for the development of brain structures. This activation has been linked to impaired neurodevelopment through cerebral white matter injuries (31, 86-88). Pneumonia and acute lower respiratory infections (ALRI) are widespread among children in poverty settings in LMICs. The burden of inflammation caused by these diseases in children may be substantial, and can result in a catabolic state and cytokine activation, which is associated with less growth and impairments of the developing brain.

Furthermore, during gastrointestinal illnesses, the associated inflammation may aggravate the inhibition of absorption of certain nutrients in the intestinal tract (89). Early childhood diarrhea may thus hinder the access of critical key nutrients in times of massive brain growth, which would result in adverse effects on the developing brain and subsequent neurodevelopment. A high burden of diarrhea has been linked to poor neurodevelopment (90-93). Reports in sub-samples from a prospective cohort study in Brazil demonstrate associations between early childhood diarrhea and cognitive functioning at age 6-10 years, school readiness and performance, as well as

later verbal fluency (90, 91, 93). The cohort study that these results are based on involved 189 children with complete diarrhea surveillance recorded three times a week during their two first years of life (91). The reports from the study have been criticized however, for lack of power to detect differences, as well as for not adjusting for potential confounding variables such as growth, socioeconomic status and other health related factors (94). As diarrhea is closely linked to growth and nutritional status, it has been questioned whether there is a causal link between diarrhea and neurodevelopment independently of malnutrition (94). A recent meta-analysis including four studies of early childhood diarrhea and subsequent neurodevelopment concluded that when taking stunting into account, there was no measurable association between diarrhea prevalence and scores on cognitive tests (95).

## 1.5 Early life psychosocial risk factors

Most research on psychosocial risk factors in child development has been carried out in high-income countries. However, in recent reviews on risks and neurodevelopment in LMICs, inadequate stimulation has been presented as one of the main risk factors for impaired neurodevelopment in children growing up in poverty (1-3). While biological-based interventions such as nutrition have been shown to have relatively small effect sizes on neurodevelopment, the effects of interventions involving cognitive and language stimulation are stronger (2).

The caregiving context is critical in protecting and socializing children for healthy development (20). Responsive parenting and care is the capacity to recognize and follow the child's signals, being emotionally supportive of his or her needs and acting in a developmentally appropriate manner towards the child's behavior (96). Although there are cultural differences in the concept of responsive care, studies repeatedly show that parental responsiveness is linked to healthy development across developmental domains in high income countries as well as in LMICs (96). Through



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data in the Multiple Indicator Cluster Survey (MICS), which is a multinational household survey carried out in 28 LMICs, the researchers found support for two modalities of positive caregiving that lay the foundation for neurodevelopment; the cognitive and the socio-emotional (97). The researchers argue that although shaped by culture, these modalities are common across cultures. The mother-child cognitive interaction promotes the child's learning about the world which predicts mental and verbal development. The caregiver's socio-emotional sensitivity motivates the child to connect with others, giving rise to interpersonal competencies in the children (97). Similarly, in their review, Walker et al. (2007) identified three aspects of parenting in LMICs that promote healthy cognitive and socio-emotional development; cognitive stimulation, caregiver's sensitivity and responsiveness, and caregiver's affect (1).

Findings from the MICS-study suggest that overall mothers in all the 28 participating countries engage in more socio-emotional activities (i.e. play, sing songs, take child outside) than cognitive activities (i.e. read books, storytelling, naming/counting/drawing) (97). More than half of the mothers reported to have played with their children during the last 3 days, while only one third or fewer had read books or told stories. HDI is a measure of the general standard of living in a country and an indication of the available support that may promote human development (98). Mothers in countries labeled as low on an index of the social and economic status, the Human Development Index (HDI), engaged in less cognitive caregiving activities than the grand mean (97). Similarly, mothers in countries high in HDI engaged in more activities than the grand mean. There were no similarly consistent patterns in socio-emotional caregiving and the HDI-status. In other studies from LMICs, it is suggested that only 10-40% of households have materials that can be used for stimulation and learning situations with children, and only 11-33% families report playing with their children (1, 5).

Parental practices are influenced by parental knowledge of child development in general, and of when it is appropriate to provide opportunities for stimulating

activities (99). Parents' knowledge of child development and the acquisition of skills differ between cultures (99). A study in urban Turkey indicates that mothers believe that skill and activities occur later than the normative age, and few mothers knew that sight, social smile, vocalization and brain development started in early infancy (99). Education and the number of children in families were related to mothers' knowledge, the higher education and the lower number of children, the better knowledge of child development. Interventions involving early stimulation and responsive caregiving have repeatedly showed potential to enhance parenting skills in order to make a more stimulating environment for their children (19). For instance, in a Turkish controlled trial, caregivers received the WHO program Care for Development intervention or standard health care (100). In this study, more families in the intervention groups had optimal scores on a standardized home observation (the Home Observation for the Measurement of the Environment (HOME Inventory) (101)) one month later compared with the control group. In a cluster-randomized trial in Pakistan the Care for Development program enhanced positive caregiving behavior such as parenting skills and emotional availability (102), which gave a positive effect on developmental outcomes as measured by the Bayley 3<sup>rd</sup>. ed. (103).

Poverty and economic hardship lead to parental psychological distress which influence their parenting practices (104). Parental responsiveness and the quality of the home environment are influenced by their psychological well-being, which in turn influence their children's development. Recent studies from South Asia document high prevalence of maternal postnatal depressive symptoms (105, 106). Maternal depression is associated with poor growth and high prevalence of diarrheal disease (107, 108). In reviews maternal depression is presented as a major risk factor for adverse neurodevelopment (1, 3). In a study from Jamaica, mothers of undernourished children had poorer psychosocial functioning. In this study, depressive symptoms and low self-esteem was related to less stimulation provided by these mothers to their children. The poor psychosocial functioning was partly explained by a stressful environment (109). In a study in Bangladesh, maternal

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depressive symptoms were associated with less responsiveness towards their children and fewer stimulating activities in the home (110). When the depressive symptoms were linked with perceived irritability in the infants, this led to fewer acquisitions of skills in the infants from they were six to 12 months old. Multiple risk factors account for the high levels of maternal depressive symptoms in LMICs (111, 112). Rates of poverty, economic distress and low education are associated with maternal depression, and in LMIC settings these are more extreme than in high-income countries (111). Other factors that have been linked to maternal depression in LMICs are low social support, domestic violence and large families, as well as factors such as having a family member with mental illness, a child with developmental disabilities, an unplanned/unwanted child and to giving birth to a female child (105, 111).

A relevant perspective in the discussion of the impact of poverty on early neurodevelopment is the investment perspective. In the investment perspective, neurodevelopment is viewed as linked to the families' ability to invest resources such as material and time in the children (104, 113). Poverty leads to less access to resources that help children develop such as stimulating toys, books and games, quality child care and schools, and quality time for caregivers to spend with their children (104). Factors such as unsafe environments, and family stress factors such as family violence and maternal depression are also widespread in the context of poverty (1, 3, 104). All these factors are important in order to understand how poverty affects early child development. Whereas family stress factors are thought to account for social emotional outcomes to a higher degree, it is argued that the caregivers lack of ability to invest in their children through toys, books and quality schooling is of greater importance for neurodevelopment, school readiness and academic achievements (104). The investment model and the family stress variables interact, and both are thought to be critical in describing the impact poverty has on children in high-income countries (104). These perspectives may also be relevant for understanding the mechanisms influencing children growing up in poverty in LMICs.

## 1.6 Developmental assessment tools for research in low-to-middle-income countries

Accurate and careful measurements of outcomes are among the fundamental design issues in epidemiological and clinical studies (114). There are several valid choices to be made when deciding upon feasible assessment tools for study settings in LMICs. Characteristics of the study such as research question, several age factors and available tools and personnel in the study setting should guide the selection of appropriate assessment tools. The approaches to the assessment differ in terms of the amount of involvement of the child, and comprehensiveness of the tools.

### 1.6.1 Study characteristics

#### *Research question*

The overarching hypothesis and study objectives should be linked to the process of selecting appropriate neurodevelopmental outcome measures for a particular study (29, 43). Existing empirical evidence and theory shed light on which domains to be examined, guiding the choice of appropriate assessment tools (115). In the area of nutrition, for example, there may be hypotheses regarding areas in the brain that are affected by a specific nutritional deficit. By careful selection of assessment tools, the effects of these deficits may be captured (24, 43, 116). Lack of findings in a study could be due to methodological factors and the inadequate choice of assessment tools, rather than a lack of differences in the targeted population (117).

#### *Age factors*

There are several age factors that need to be considered in the selection of appropriate assessment tools for a specific study. Age at insult effects is important in the understanding of which areas that may be affected by an exposure (or the lack of one) (25). Age at testing and the developmental stage of the child must also be taken into

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account to ensure the use of tools that are engaging for the child, but also sensitive enough to capture effects (32). In young children, for example, it may be difficult and in some cases not possible, to collect reliable and valid data on complex cognitive skills. While in other developmental areas such as the gross motor domain, the collection of data may be less challenging and can be covered by a wide variety of assessment tools (17, 116).

### *Available and valid tests for the study population*

The wide variety of assessment tools for children is primarily developed in high-income countries, more specifically in USA and Western Europe. Consequently, the validity of these tools has been assessed in the framing of westernized thinking and expectations towards normal development. When transferred to a new cultural setting these tools may not necessary be valid. Parenting practices forming the child's environment are to a large extent guided by cultural ideas and ideals of child development (118). Skills that promote competence in one culture may be irrelevant for the expression of competence in others (119). Children and parents are embedded in their cultural systems, and all developmental advances are influenced by the opportunities the children get to develop skills, the attitudes, knowledge and expectations of the caregivers (98). One consequence of these cultural factors may be, for example, cultural variations in what age skills occur, variations in children's familiarity with certain materials and toys, and differences in when daily life skills are considered appropriate (116, 120). Thus, the assessment of neurodevelopment in children across cultures is challenging, and requires knowledge of child development as a universal construct, and of the specific cultural context.

When performing developmental assessments in LMICs, preferable assessment tools should be developed within the specific context (118). In most LMICs, these are rare. There are, however, examples like the Baroda development screening test for infants created in India (121), and the Kilifi Developmental Inventory developed in Kenya (122). Furthermore, some tests originally from high-income countries have been

formally translated and carefully adjusted to new cultural contexts. For example, the official validated Indian version of the Wechsler Intelligence Scale for Children 4<sup>th</sup> ed. (WISC-IV) was launched in India in 2013 ([www.pearsson.in](http://www.pearsson.in)), and should be a clear option for the measurement of intellectual capacity in children six years and older in an Indian context.

The most common approach in the study of mental phenomenon in LMICs is to translate and adjust existing assessment tools for the research project (2, 116, 118). With a careful process of translation and adjustments following standard guidelines, it is possible to embrace the understanding of child development as a universal process and at the same time encompass the uniqueness given by cultural expressions of development (114, 116). Thus, in the process of translation and adjustments of tools, both knowledge of child development in general, and of the particular cultural setting for the study site in specific, is of equal importance to ensure validity of data.

The possibility of replication and extension of results is imperative for high quality research, and thus transparency in procedures including careful documentation of the translations and adjustments of assessment tools is necessary (123). Furthermore, psychometric qualities of the assessment tools should be assessed and documented within the present context (118). This process can be time consuming since additional adjustments may be required to optimize the validity and reliability following the initial piloting process (116). Relevant measures of reliability are test-retest reliability and inter-rater agreement measures, as well as the assessment of internal consistency through measures such as Cronbach's alpha. The assessment of the construct validity of a scale involves the evaluation of whether the tool measures what it is intended to measure (114). Methods such as exploratory and confirmatory factor analysis may be appropriate. Furthermore, the assessment of concurrent validity would provide evidence for the validity if the test correlates well with a previously validated tool, preferable a gold standard measure (114). In convergent validity, the support is found through the correspondence with factors known to be related to the construct,

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whereas in discriminatory validity there is no correspondence to factors known to be unrelated (114). In addition to being reliable and valid in a new cultural setting, a test must have discriminatory power where the distribution of scores enables the test to distinguish between children of various abilities (116).

Since tools are commonly standardized in the setting in which they have been constructed, the normative scores and cut-offs are based on statistical calculations in the standardization sample (e.g. normative groups in the UK or in the USA) (118). In other words, the concept of normality is reflected through the accomplishments of children in the country of origin. Based on the previous discussion, these norms may not be compatible for children in other cultures. In a research setting, however, it may be feasible to use an assessment tool to compare study groups even though no formally validated norms are available for the study population. The scores may then be used as a measure of relevant development in the study sample independent of normative scores and cut-offs (118). When using the test to compare study groups, the differences in performance are the only interest. Due to a lack of normative scores and cut-offs, there is no information about whether the children are delayed or not.

### *Available personnel*

Available personnel at the study site are a critical factor in the planning of a study, and for the selection of assessment tools (115). Some tools have requirements about who can perform the tests, such as for the Bayley scales and the Wechsler tests. Other scales and tools are not followed by such recommendations. The Ages and Stages Questionnaires (ASQ), for example, may be filled out by nonprofessionals and caregivers (124). In general, the administration of some tests is more challenging than others. For the more advanced tests (e.g. the Bayley scales and Wechsler tests), knowledge and experience in child development are an advantage, and can optimize the quality of data. In study settings where professionals are scarce, less advanced tools involving less complex administration are often the primary choice in order to secure valid and reliable data. However, for research purpose, systematic

administration through standardized procedure is imperative. This involves shared agreements between testers and supervisors, and shared understanding of critical factors in the assessment. Thus, some amount of training of personnel is necessary irrespective of the complexity of the tool to ensure validity and reliability of the collected data (115).

### **1.6.2 Approaches to the assessment**

Direct assessment, indirect assessment, and structured observations are three main approaches to the neurodevelopmental assessment of children (118). Direct assessment with the child is often considered to be the gold standard for the collection of data on neurodevelopment, and is thought to provide unbiased information about the child's skills and abilities (118). To ensure validity and reliability of the data, thorough training and standardization of testers is critical (115). Furthermore, testers should preferably be personnel with competence and expertise in the field of child development. Thus, direct assessments are both time and resource consuming, and in certain settings not possible to implement.

In indirect assessments, data are based on questionnaires, scales or checklists completed by informants close to the child (118). This approach is often considered to be a more efficient method for data collection than direct assessment. However, the use of secondary informants risks biasing the information collected (125, 126). Furthermore, due to high levels of illiteracy, informants in LMICs may not be able to fill in the questionnaires by themselves, and they may lack knowledge and experience with questions about child development (118). Thus, trained field workers frequently administer the questionnaires, requiring a minimal amount of training and quality controls to ensure reliable data.

In structured observational methods, information about child development is collected through standardized observations (i.e. through direct observations or through video recordings) (118). These methods provide valuable and more objective



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information about phenomenon that might not be easily accessible in other approaches, such as for example mother-child interaction (127). However, observational methods require thorough training and standardization of experienced personnel to secure valid and reliable data, and the processing of data may be time consuming (118). In addition several neuroimaging techniques are available that may identify unique changes to the developing brain in early childhood. Techniques such as EEG, ERP, and MRI may be efficient methods adding to the picture of the possible effects of exposures to the developing brain (43).

A second choice is between global or specific measures. Comprehensive tools such as the Bayley scales for young children (18) and the Wechsler tests from 2 years and onwards (128, 129) are often considered the gold standard for the assessment of neurodevelopment. These tools provide detailed information about the child's global capacity, and are mainly based on direct assessment with the child. Scores (global development score for the younger children and a general intelligence measure for children older than two) are standardized, and comparisons can be done with a high level of precision when norms from the current population are available (118). Global measures may be particularly useful in early infancy when specific cognitive abilities are difficult to measure separately. For older children, specific measures may have several advantages in the study of the specific effects of biological risks or as a valuable addition to the global measures (116). In specific measures, specific skills are targeted, for instance motor development in the Movement assessment battery for children (130), or executive functioning by subtests such as the Statue and Inhibition in the NEPSY-battery (131). These types of tests allow for targeted assessments following specific hypothesis (116, 117).

Screening tools are frequently used in child assessments and can provide brief information on development with little time and resources (123). Screening tools may rely on both direct assessment e.g. the Denver Developmental Screening Test (132), or as questionnaires to secondary informants e.g. the ASQ (124). In child

development, these tools identify children at risk of delay through predetermined cut-offs (118). For many screening tools, norms and validation of cut-off scores are not available for LMICs, and thus these should not be used to determine developmental delay in these contexts (118).

## 1.7 Study design

Based on clearly formulated hypotheses, RCTs are set up to test the effects of interventions in experimental designs. In the hierarchy of evidence, RCTs are considered the most scientifically rigorous method to answer specific research questions concerning effects of an intervention (133). In addition to important factors such as sufficient sample size and accuracy of measurement tools, there are several quality indicators in an RCT design that can maximize the precision and accuracy of findings (134). Biases refer to any factors or processes in the design or execution of a study that tend to systematically bias the results or conclusions resulting in under- or overestimation of the effects (133, 135). Confounding variables are factors other than the exposure variables that are correlated with the independent outcome and that consequently may bias the estimates (135). The main purpose of an RCT is to limit the effect of known and unknown confounders that may bias the data, and thus to make causal inferences between an intervention and a defined outcome with a high degree of confidence (134).

A core principle in RCTs is randomization. Random allocation to study groups ensures that all factors that may have an effect on the outcome are randomly distributed in the study groups, minimizing systematic differences that may bias the data (136, 137). Allocation concealment is that the person that randomizes the study participants to intervention groups does not know the next group allocation. This reduces the risk for selection bias and heightens the effect of the randomization process (133). Blinding is when the participants do not know their group assignment, and double-blinding is when group allocation is blinded for both participants and study

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personnel (137). Blinding reduce the information bias. Furthermore, to evaluate the quality of a RCT, the rate of attrition is of importance. A high degree of incompleteness in a study represents a possible selection bias. In a study, one should attempt to have as complete follow-up as possible, and as much information on the lost to follow-ups as possible (136).

In observational designs one makes inferences based on associations between systematic observations. The key difference between experimental and observational designs is that observational designs lack control over the exposures under investigation. The consequences is the risk of confounding if these are not sufficiently accounted for (134). However, confounding variables should not be confused with mediating factors, and that a factor lie in the causal pathway between the exposure and the outcome variable (135). In case of causal pathways, the variable provides additional information about the associations under study, and not an alternative explanation as is the case with confounding. Furthermore, the results from observational designs are limited to demonstrate associations, and thus there will always be an issue of causality. Reverse causality between the dependent and independent variable is also possible.

Although the primary choice of method to assess the effect of an intervention, RCTs are not always feasible to conduct. Well-conducted RCTs are both time and resource consuming. Second, RCTs are not possible and/or advisable to conduct in some situations due to the nature of the research question (133). There are conditions under which to conduct an intervention trial would be inappropriate due to practical (i.e. no willing participants) and/or ethical reasons (i.e. possible harmful to either intervention or placebo group). Thus, although RCTs provide the strongest form of evidence to base sound inferences, some research issues call for other approaches.

## 1.8 Aims of the thesis

The aim of the thesis is threefold:

### **Aim I**

To measure the effect of two recommended daily allowances of vitamin B12 and/or folic acid for six months on neurodevelopment in young North Indian children.

### **Aim II**

Identify predictors for neurodevelopment in young North Indian children.

### **Aim III**

To assess the feasibility of the screening tool ASQ-3 administered directly with the child to measure neurodevelopment in young children in a field setting in North India.

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## 2 Methods

### 2.1 Overview of the study

Data on neurodevelopment was collected in a sub sample of children participating in the RCT: *Routine administration of folic acid and vitamin B12 to prevent childhood infections*, a randomized double-blind placebo-controlled preventive field trial, with a factorial design (138). The RCT was conducted in the urban neighbourhoods of Tigri and Dakshinpuri in New Delhi, India and was a collaboration between the Society for Applied Studies (SAS) in New Delhi, India, Center for International Health at the University of Bergen and Innlandet Hospital Trust. Enrollment for the main study started in January 2010 and the follow-up ended in September 2011. Children aged six to 30 months were randomized into four study groups: placebo, folic acid only, vitamin B12 only and vitamin B12 and folic acid (in the following referred to as vitamin B12/folic acid), and received daily supplementation for 6 months. During the 6 months period, children had biweekly morbidity visits by trained field workers. Growth was assessed at enrollment and at the end of the study. Blood samples were collected at enrollment in all children and in 20% of the children at the end of the study (**Figure 3**).

Neurodevelopment was assessed by the ASQ-3 at the end of the study in 422 children. The children were among the 440 who were last enrolled and randomized to the main study (n=1000). Enrollment of these children lasted from November 2010 to March 2011, and neurodevelopmental assessment and the collection of information on stimulation and learning opportunities, was conducted at the end of the study from May through September 2011.

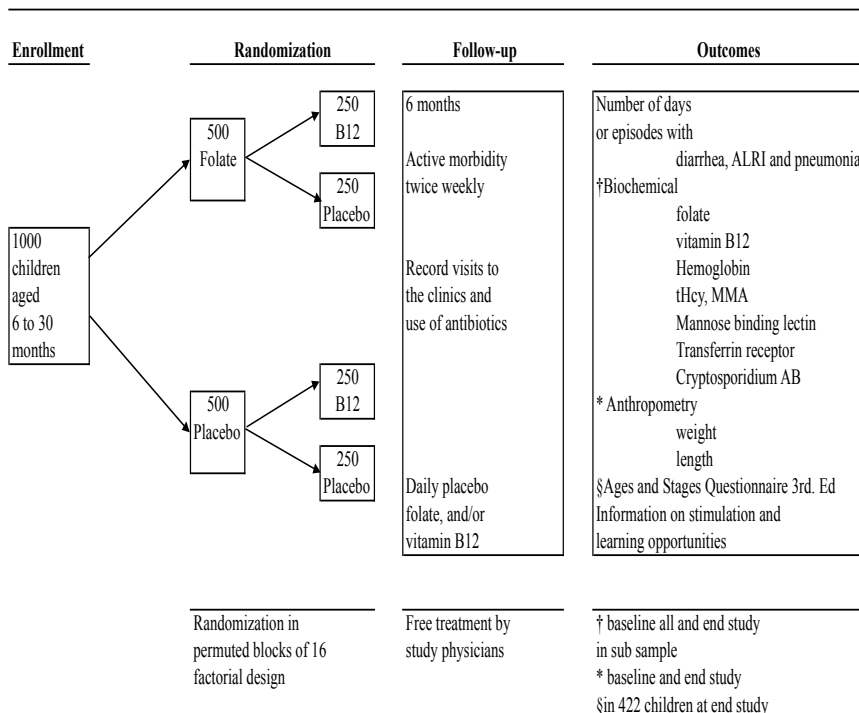


Figure 3. Study design.

## 2.2 Study setting and population

Tigri and Daksinpuri are urban neighborhoods of New Delhi, India, with approximately 300 000 inhabitants at the time of the study. The main investigators have over many years carried out a number of trials in these neighborhoods and are well known to its inhabitants. Families at the study sites are from low- to middle income economic settings and available data from the last decades show consistently high prevalence of malnutrition, childhood illness and micronutrient deficiencies among young children in the area (57, 139, 140). In the adult population, there is on average five years of schooling among women and nine years among men. In previous

studies approximately 35% of the mothers report being non-literate (49, 139). Approximately 30-40% of children under five are stunted (138, 141). The average incidence of diarrhea in children less than 30 months is seven episodes per child a year, where about 5% of these episodes are persistent (139). Vitamin B12 and folate deficiency are reported to be common, ranging from 28-48% depending on cut-off, breastfeeding status and age (57). Table 2 shows the high prevalence of vitamin B12 and folate deficiency among young children in the study population related to age and breastfeeding status.

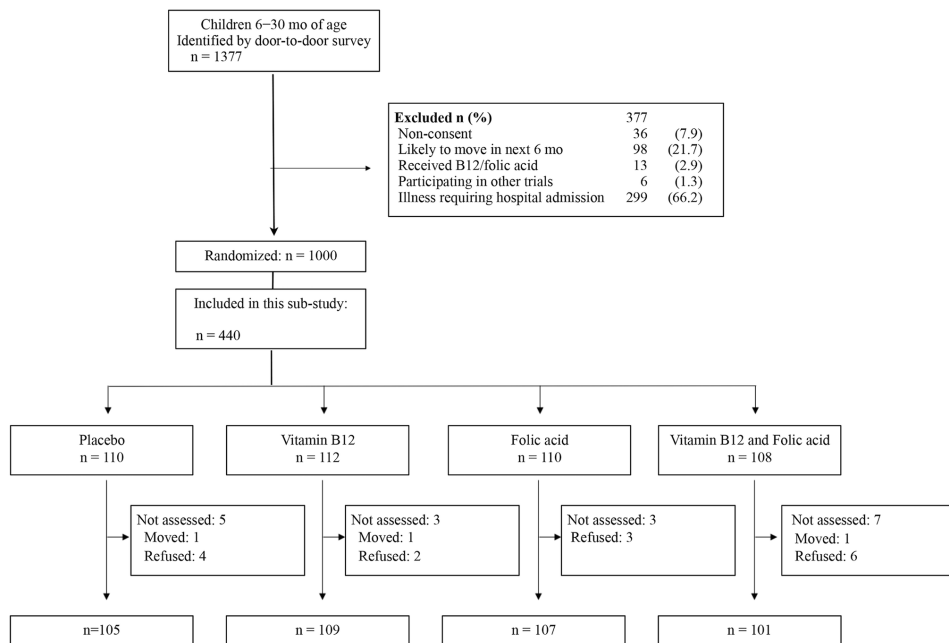
*Table 2.* Plasma concentration of vitamin B12, folate, total homocysteine and methylmalonic acid in young Indian children. Table indicates high prevalence of vitamin B12 and folate deficiency at the field site (57).

	Breastfed				Not Breastfed			
	6 – 11 months		12 – 30 months		6 – 11 months		12 – 30 months	
<b>Median and IQR</b>								
Vitamin B12, pmol/L	184	120 - 263	172	124 - 253	334	235 - 463	261	194 - 348
Folate, nmol/L	20.2	11.7 – 34.4	11.3	7.4 – 17.6	5.3	3.4 – 7.7	6.5	4.7 – 9.2
Total Homocysteine, $\mu$ mol/L	12.6	9.2 – 18.1	11.3	8.7 – 15.2	10.7	8.2 – 13.9	9.1	7.4 – 11.2
Methylmalonic acid $\mu$ mol/L	1.03	0.54 – 2.08	0.74	0.42 – 1.36	0.45	0.31 – 0.71	0.38	0.26 – 0.59

## 2.3 Study sample

One thousand children 6 to 30 months of age in families planning to reside in the area for the study period and with caregivers available for informed written consent, were included in the main study. Children with severe systemic illnesses, and/or

malnutrition requiring treatment and/or hospitalization were excluded, as were children who already took B vitamins. Children with severe anemia and ongoing acute infections were temporarily excluded, referred for treatment and then enrolled if possible after recovery. With no neurodevelopmental assessment prior to enrollment, only those children whose caregivers reported of developmental disabilities at baseline were excluded from the study.



*Figur 4.* Flow chart of the randomized, placebo controlled trial in 6 – 30 months old North Indian children.

The neurodevelopmental assessment was included in the study after the main study had started, and thus we were only able to include 440 of the 1000 children in the main study for neurodevelopmental assesment. Of these 440 children, three children were not available for assessment and 15 did not wish to participate. Hence a total of 422 children were assessed by the ASQ-3 at end study (**Figur 4**). There were no



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additional inclusion and exclusion criteria for the neurodevelopment sub study, other than a new consent from the caregivers.

## 2.4 Procedure

### 2.4.1 Enrollment, randomization and intervention

Participants in the study were recruited through a door-to-door survey where eligible children were identified and brought to the study clinic for the initial screening. When the study physicians and supervisors had completed the screening procedures, caregivers were read an information sheet and requested to sign an informed consent form. In case of non-literates, an impartial witness witnessed the consent. All witnesses were registered in a list.

Information on socioeconomic status of the families was gathered at enrollment, height and weight was taken, and baseline blood sample collected. Participants were then allocated to one of four intervention groups; Placebo, Vitamin B12, Folic acid and Vitamin B12/folic acid by block randomization in groups of 16. A scientist at the University of Bergen, who was otherwise not involved in the study, provided the randomization scheme using Stata Version 10 (StataCorp, College Station, TX, USA) linking the unique child identification number with the intervention group. One group of field workers visited the homes daily to provide supplements for the participating children. To secure blinding, placebo and supplement were similar in appearance and taste and were offered to the children according to the serial number provided by the producer (NUTRISET, Ltd, Malaunay France). The treatment allocation was masked both for the families and the study team throughout the data collection period.

Based on earlier results in the same population demonstrating that vitamin B12 and folate deficiency is common (57), approximately twice the recommended daily allowances of vitamin B12 and folic acid doses were given to the children. The lipid

based paste was chosen because of its acceptability in similar populations and because it is a feasible way to provide vitamins and minerals to child populations without degradation or contamination. Children were given one spoon (5 g) if they were 6 to 11 months, and two spoons (10 g) if they were 12 months and above. In addition to the relevant vitamins, each 10 g of supplement contained 54.1 kcal total energy, 0.7 g proteins and 3.3 g fat. For the groups that were assigned to receive B vitamins, the 10 g of supplement contained 150 µg folic acid or 1.8 µg vitamin B12, or the combination of both. For children less than 12 months, the 5 g supplement contained half of the doses compared with the dose for the older children. For holidays and when families were travelling, the caregivers administered the supplement based on instructions from the study team.

### **2.4.2 Measurements**

*Neurodevelopmental assessment; the Ages and Stages Questionnaire, 3rd ed. (ASQ-3).*

ASQ-3 is a comprehensive screening instrument of developmental status standardized in the USA for children 1-66 months with age-appropriate questionnaires for every one to five month intervals (124). The ASQ-3 consists of five subscales: Communication, Gross motor, Fine motor, Problem solving and Personal social. Each form contains 30 items, six for each subscale, written in a simple language. Some questions are specific for certain age groups, while other items are for a wider age range, and thus repeated in various questionnaires. Each item is answered Yes (10 p), Not Yet (5 p) and No (0 p), and the possible scores range from 0 to 300 in the total scale and 0 to 60 in the five subscales. The questionnaires are designed to be completed by caregivers as self report, but can also be administered directly with the child, referred to as the “home procedure” in the ASQ-3 user guide (142). In this procedure the professional plays an active part in the assessment of the child, providing necessary materials for the assessment of skills during the sessions. The

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“home procedure” is not necessarily performed in the home of the family, but in any given arena where the child, caregiver and the professional can meet.

The US versions of the ASQ-3 come with binary cut-offs to determine the risk of developmental delays. To our knowledge, cut-offs had not been determined for a North Indian population at the time of the study. However, the ASQ-2<sup>nd</sup> ed. has been validated against a developmental assessment tool in North India, and found to have good test characteristics for detecting developmental delay in this setting (143). Both ASQ-2 and ASQ-3 have been used as outcome measures in epidemiological studies worldwide, where both continuous and binary outcomes are reported (144-147).

### **Training**

We prepared for the neurodevelopmental assessment during an 11 days workshop including three field supervisors and the main study physician. The field supervisors were responsible for the work of the field workers in the main study and under supervision of study physicians. Two of the supervisors had degrees at master`s level, but none had formal training in developmental psychology. All had extensive experience in working with families in the local community. I was responsible for the training. During the workshop, the 11 forms relevant for the age range at end study (12 to 36 months) were thoroughly discussed in terms of the overarching developmental ideas that we were studying, the suitability for the study setting and the more practical administration and need for materials and local adjustments. We also discussed how to secure an optimal assessment session and give the child a chance to show his or her best performance. When the workshop was finalized, the 11 relevant forms for the study were translated to Hindi, necessary adjustments had been made, standardized material kits were made and the supervisors were standardized in the administration through assessment exercises in 30 children in the age range.

### **Translation and adaptations**

We translated and adapted the relevant questionnaires to Hindi followed by back translation and validation. All items in the forms were critically reviewed in the group and discussed in terms of possible challenges in the process of translation and suitability for the cultural setting. Throughout the questionnaires, small changes were done to examples and instructions to fit the culture. For instance, objects named in the communication subscales were changed (i.e. from coat to shoe, since children rarely would wear a coat, and from book to toy, since children in general would not be as familiar with books as the more general term “toy”). All together, four items were changed to be suitable for the local setting (**Table 3**).

Three of the adjusted items involved materials that we did not expect children in the neighborhood to have experience with; a mirror, a fork and a zipper coat. These were changed based on the knowledge of early child development and the culture. The fourth item that we changed involved the child’s knowledge of both his/her first and last name, since children in this setting in general do not know their last name. During the workshop we also discussed other items. For example items involving hugging a stuffed toy and participating in simple household chores were extensively discussed in terms of suitability for Indian boys. However, we decided to not change these items, but to be attentive to these items when working on the data.

*Table 3. Changes to items in the Ages and Stages Questionnaire 3<sup>rd</sup> edition to adjust for a North Indian research setting.*

<b>Subscale</b>	<b>Original item</b>	<b>Changed to</b>
Personal Social	<i>While looking at himself in the mirror, does the child offer a toy to his own image?</i>	<i>While looking at himself in the mirror, does your child smile and interact with the reflection?</i>
Personal Social	<i>Does your child eat with fork?</i>	<i>Does your child take chapatti with dal?</i>
Communication	<i>Show you child how a zipper on a coat moves up and down and say, “ See this goes up and down”. Put the zipper to the middle, and as your child to move the zipper down. Return the zipper and ask your child to move the zipper up. Do this several times, placing the zipper in the middle before asking your child to move it up or down. Does your child consistently move the zipper up when you say “up” and down when you say “down”?</i>	<i>Show your child how a magnet can move up and down on a magnet wall, and say” See this goes .....</i>
Communication	<i>When you ask, “What is your name?” does your child say both her first and last name?</i>	<i>When you ask, “What is your name” does your child say her first name? (If the child also has a last name, does it say first and last name?)</i>

## **Procedure**

A field worker brought the children and caregivers to the study clinic for the end study procedures. The ASQ-3 sessions were administered immediately following the main end study procedures. We prepared a room for the ASQ-3 assessments with a carpet on the floor for the assessment to be carried out, and with necessary materials

such as a mirror, a board with magnets and appropriate pictures on the walls. Necessary materials had been gathered in standardized kits. During the sessions, the supervisors intended to answer as many items as possible based on observations. For some items, however, this was not possible. When children did not show the relevant skill during the session, we had to depend on the mother's reporting on whether the child knew the skill or not. For each item, supervisors noted if the skill was observed during a session or not.

Each session lasted for approximately 20 to 30 minutes depending on the child. If necessary, children were allowed some time to play with the material in order to be familiar with the situation and the material. The supervisors used toffees as motivators when called for. After the completed sessions, children were given a small toy and some bread. The field supervisors were trained to give some basic advice and information based on their experiences with the child and caregiver during the ASQ-3 assessment, and some caregivers used the opportunity to discuss their child and its development in the sessions.

Some end-study procedures and ASQ-3 sessions were done in the homes of the children rather than in the clinic if the families were unavailable within walking distance of the clinic. Other families refused to visit the clinic for a second time, but allowed the team to visit them in their home to perform the procedures.

### *Stimulation and Learning opportunities*

During the ASQ-3 sessions, we interviewed the caregivers about the child's home environment and source of stimulation and learning opportunities through an eight item questionnaire (**Table 4**).

Table 4. Questionnaire to the caregivers in the home environment of the child.

1	Number of toys available in the home (3=none, 4=less than 5, 5=5-10, 6=more than 10)
2	Toys with which the child plays (1 = car, ball, doll, 2 = blocks, 3 = puzzles, 4 = others, specify.....)
3	Number of books in the home (3 = none, 4 = less than 5, 5 = 5 -10, 6 = more than 10)
4	Does the child go to anganwadi? (1 = yes, 2 = no)
5	Number of children in the home
6	Number of hours per week child play with other children (including children outside of the home)
7	Caregiver believes the child's behavior can be changed or modified and is influenced by the parent's behavior (1=yes, 2=no). Credit if the caregiver responds that she teaches the child, or the child learns by watching others or being taught by others. No credit should be given if the caregiver has no idea how the child learns things. <i>Ask: "How does your child learn to do things, such as feed himself (for children more than 24 months, add: or dress himself)?"</i>
8	Caregiver consciously encourages developmental advances by doing activities that promotes the child's development (1=yes, 2=no). <i>Ask: "When your child is playing with a toy, do you name the toy or comment on the color, size, or shape of the toy? Or when there is an activity that your child cannot do yet (i.e. walk), do you position (i.e. standing position) the child so that the child can practice and learn the activity?"</i>

Six of the questions (1 to 6 in **Table 4**) were chosen based on an earlier study in the same population (148). One of these questions was on the attendance to anganwadi center. These centers are governmental-funded child care centers, which are part of the public health care system providing pre-school activities as well as a free lunch. Families in the area use these centers differently, and there is no information in the current study whether attendance means that the child is provided with pre-school experiences or free lunch only. Based on experiences and subsequent discussions in the group during the workshop, we selected two questions from the stimulation and teaching dimension of the HOME Inventory (101). One of the questions was about “Mother’s belief that child’s behavior can be modified” and one was about “Mother’s encouragement of developmental advances”. These concepts were discussed with the mother, and the answered Yes/No was given by the field supervisor. The HOME Inventory is a widely used inventory in large population-based studies in LMIC and has previously been used in several supplementation trials across the world including India (149). In the present study, we only used only the two questions described above due to limited time and resources.

### *Growth, childhood illness and biochemical markers*

Weight and height was measured at enrollment and at end study according to standard guidelines. Weight was measured with no clothes on by a portable salter scale, and height with a locally made infantometer.

A second group of trained field workers visited the families in their homes twice a week to gather morbidity data on the child. Diarrhea was defined as passage of  $\geq 3$  loose or watery stools in a 24-hour period. Children were considered to have recovered from a diarrhea episode on the first of two consecutive diarrhea free days. ALRI was defined as cough and fast breathing or lower chest indrawings as assessed by a physician. Clinical pneumonia as either a combination of cough with crepitation or bronchial breathing by auscultations, or as an episode of ALRI associated with at least one of lower chest indrawings, convulsions, not able to drink or feed, extreme lethargy,



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restlessness or irritability, nasal flaring or that the child was abnormally sleepy and difficult to wake. Sick children requiring treatment were referred to the study clinic to be seen by the study physicians and nurses. We believe that the threshold for such referral was low as the parents were encouraged to visit the clinic even with mild symptoms and these visits were free.

Venous blood samples were obtained at baseline for all children, and at end study in a subsample of randomly selected blocks (94 children for the subsample). Three mL of blood was collected into an evacuated tube containing EDTA (BD, Franklin Lakes, NJ, USA). Immediately following blood sampling, plasma was separated from the blood cells by centrifugation at room temperature (450 x g x 10 min), transferred into storage vials and stored at -20 C until analysis. Plasma tHcy was analyzed using commercial kits (Abbott Park, IL, USA) (150). Plasma concentrations of vitamin B12 and folate were determined by microbiological assays using a chloramphenicol-resistant strain of *Lactobacillus casei* and colistin sulfate-resistant strain of *Lactobacillus leichmannii*, respectively (151). In the study analyses we used the following cut-offs for deficiency: <7.5 nmol/L was considered low in folate, < 200 nmol/L for low vitamin B12 levels and >10 µmol/L was considered elevated levels of tHcy (57).

## 2.5 Ethics

The study was approved by the ethics committees of the Society for Essential Health Action and Training (India), Society for Applied Studies (India), Christian Medical College, Vellore (India), and the Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST), July 2008. The trial was first registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT00717730, in July 2008 and in August 2010 at [www.ctri.nic.in](http://www.ctri.nic.in) as CTRI/2010/091/001090.

## 2.6 Statistical analysis

Statistical analysis was conducted using STATA (StataCorp, College Station, TX), version 12 and 13. Within 48 hours following the assessment sessions, data was entered twice by two data clerks and followed by validation. For each child, we summed up the item scores to five total subscale scores, and a total ASQ-3 score. 0.21% of the responses were missing, and for missing items we followed the instructions from the ASQ3 manual (142). Height-for-age, weight-for-age and weight-for-height z scores were calculated using the most recent WHO growth charts (152).

### 2.6.1 Paper I

A detailed plan of analyses was made. The ASQ-3 scores for the total sample are presented as means (SD). We used linear regression and multiple logistic regression to compare the intervention groups: vitamin B12, folic acid and vitamin B12/folic acid against the placebo on a continuous scale. For the multiple logistic regression, scores were categorized on the 25th percentile (decided *a priori*). In the logistic models, we also examined the effects in various predefined subgroups, adjusting for sex, age, breastfeeding status, height-for-age z-scores and mother's encouragement of developmental advances. Finally, we performed the overall analyses adjusting for important baseline factors such as sex, gender, breastfeeding status, height-for-age, weight-for-age z-scores and log transformed family income. We calculated standardized mean differences by dividing the mean differences by the overall SDs of the different outcomes. All analyses were performed following an intention-to-treat protocol.  $P < 0.05$  (two-tailed) was considered significant.

### 2.6.2 Paper II

We measured the association of relevant independent variables with the total ASQ-3 in multiple linear regression models. We selected the variables for the regression

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models as described elsewhere (153), and confirmed the manual model by selecting variables in an automatic stepwise linear regression procedure. For the total ASQ-3 score, we presented the selected variables in groups using a hierarchical (nested) regression approach (154). The variable groups are: stimulation and learning opportunities (number of toys in the family, family owns books, hours of play with children during the week, mothers belief that child's behavior can be modified, mothers encouragement of developmental advances), growth (HAZ and WHZ) and childhood illnesses (number of days with diarrhea and incidence of clinical pneumonia). All regression models included child characteristics (sex, age and breastfeeding status) and annual family income. The scores of the five subscales were highly skewed and categorized on the 25<sup>th</sup> percentile in the multiple logistic regression analysis. The selection of variables followed the same procedure as for main regression analysis. Only variables with  $P > 0.05$  are presented in the table.

### **2.6.3 Paper III**

We calculated the Intra-class correlation coefficient (ICC), when comparing any of the examiners with the gold standard (first author) during the exercises, and when comparing two examiners during the data collection. Pearson product moment correlation coefficient was calculated between the five subscales and the total ASQ-3 score. Standardized alpha values, as appropriate when standard scores are summed to form scale scores (155), were calculated for the total score and for the five subscales for the 11 age intervals. Alpha values greater than 0.80 were considered to indicate high internal consistency, values from 0.60 to 0.80 were considered satisfactory, and alpha values from 0.40 to 0.60 were considered moderately internally consistent.

## 3 Results

### 3.1 Demographics and clinical characteristics

A little less than two thirds of the participating children were between one and two years during the assessment, and half of the total children were girls. Median income in the participating families was 73 000 Indian rupees, 89.3 % report having a color TV, scooter or cooler, and 54% lived in nuclear families. Of the mothers, 23.2% report having no schooling, while 49.6% have completed primary school. Furthermore, 96% are daily wagers, maids or un-employed. Among the fathers, 12.6% have no schooling, while 36% have completed primary and 26.8% middle school. 56.2% of the fathers are non-governmental employers, while 21% are self-employed and 20.6% report being daily wagers or un-employed (**Table 5**).

At baseline 86.3% of the children were still breastfed. 40.1% of the children were stunted, 10% were wasted, while 31% were underweight. From the biweekly visits in the homes there is information about 159 incidents of ALRI and 115 incidents of clinical pneumonia. The children had on average 6.6 days of diarrhea, ranging from 0 to 49 days. A total of 99 children had between 10 and 49 days of diarrhea during the study period. The median level of vitamin B12 at baseline was 276.5 with an interquartile range (IQR) of 181 to 423. For folate, the levels were 10.4 with an IQR of 6.3 to 19, while tHcy had a median level of 10.9 with an IQR of 8.8-15.4 (**Table 6**).

Table 5. Demographic characteristics at baseline.

	N	Mean/%	SD
<b>Child characteristics</b> <sup>1</sup>			
Total	422		
Age in month (mean/SD)		21.6	7.1
6-17 months	259	61.3%	
18-30 months	163	38.7%	
Girls	206	48.8%	
Breastfed	364	86.3%	
<b>Family situation</b>			
<i>Economy</i>			
Annual income in INR <sup>2</sup> (median/range)		73000	12000-870000
Families who own color TV or scooter or cooler	377	89.3%	
<i>Maternal characteristics</i> <sup>3</sup>			
Age (mean/SD)		25.7	5.5
Years of schooling (mean/SD)		7	6.3
No schooling (<5 years)	98	23.2%	
Primary (5 years complete)	209	49.6%	
Middle (10 years complete)	39	9.2%	
Higher (> 10 years)	76	17.6%	
Occupation			
Governmental or non governmental employee	9	2.1%	
Self-employed, daily wager, maid or un-employed	412	96 %	
<i>Paternal characteristics</i>			
Years of schooling (mean/SD)		8.6	4
No schooling (<5 years)	53	12.6%	
Primary (5 years complete)	152	36 %	
Middle (10 years complete)	113	26.8%	
Higher (>10 years)	104	24.6%	
Occupation			
Governmental employee	9	2.1%	
Non-governmental employee	237	56.2%	
Self employed	89	21.1%	
Daily wager or un-employed	87	20.6%	
<i>Household characteristics</i>			
Nuclear family	228	54 %	
Number of children in the family (mean/SD)		3	2.3

<sup>1</sup>Numbers in N/% when not otherwise reported, <sup>2</sup>Indian Rupees, <sup>3</sup>One mother deceased

*Table 6.* Clinical characteristics of children in the cohort.

<b>Growth at baseline (N /%)</b>		
Stunted (height for age z score < -2)	169	40.1 %
Wasted (weight for height z score < -2)	42	10%
Underweight (weight for age z score < -2)	131	31%
<b>Childhood Illnesses from the biweekly home visits throughout the study period</b>		
Number of days with diarrhea (mean/range)		6.6 days
Incidents of Acute lower respiratory infection (incidents/%)	159	37.7%
Incidents of Clinical Pneumonia (incidents/%)	115	27.2%
<b>Plasma concentration of vitamin B12, folate and total Homocysteine at baseline (Median/IQR)</b>		
Vitamin B12	276.5	181 - 423
Folate	10.4	6.3 – 19.2
Total Homocysteine	10.9	8.8 – 15.4

### 3.2 Paper I: Vitamin B12 and folic acid improve gross motor and problem-solving skills in young North Indian children: a randomized placebo-controlled trial.

In Paper I we investigated the effect of vitamin B12 and/or folic acid supplement for six months on neurodevelopment measured by the ASQ-3 in a clinical trial. In this study we found that overall, compared to placebo, children who received both vitamin B12 and folic acid had higher ASQ-3 scores with a mean difference of 12.6 (95% CI -1.1, 26.3) ( $P=0.071$ ), as well as lower odds of being in the lower quartile of the ASQ-3 scores [OR: 0.53 (95% CI 0.29, 0.99) ( $P=0.048$ )]. The effects of vitamin B12 and folic acid supplementation appeared in the subscales of gross motor with a mean difference from placebo of 6.6 (95% CI 2.7, 10.3) ( $P=0.001$ ) and problem solving with 3.8 (95% 0.0, 7.6) ( $P=0.048$ ), with no apparent effect in the other subscales. The logistic regression analysis confirms these results with lower odds for being in the lower quartile of the gross motor ( $P=0.002$ ) and problem solving ( $P=0.026$ ) subscale scores for children in the vitamin B12/folic acid group. The effect of vitamin B12 and folic acid administration was more evident in children who at baseline were stunted [OR: 0.26 (95% CI 0.09, 0.78) ( $P=0.03$ )] who had elevated

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tHcy [OR: 0.38 (95% CI 0.16, 0.92) ( $P=0.032$ )] or who were less than 18 months of age [OR: 0.37 (95% CI 0.17, 0.83)( $P=0.015$ )]. Finally, except for an effect on gross motor functioning when receiving vitamin B12, there was no significant effect of receiving vitamin B12 or folic acid supplement alone. The overall analyses were repeated in adjusted models due to baseline differences in demographic characteristics and growth. The adjusted analyses resulted in small changes to the estimates and the levels of significance, and our findings of effect in the domains of gross motor and problem solving were unaltered by these adjustments.

### 3.3 Paper II: Diarrhea, stimulation and growth predict neurodevelopment in young North Indian children.

The objective of Paper II was to identify predictors of neurodevelopment in the cohort of 422 young North Indian children. To reach this aim we used multiple regression models presented as hierarchical regression. In these models we were able to explain 30.6% of the variation in the total ASQ-3 score by relevant predictors for neurodevelopment. Variables defined as Stimulation and learning opportunities explained most of the variation by 25.9% after adjusting for child characteristics and annual family income. Height for age and weight for height z-scores were positively associated with the total ASQ-3 score, while the number of days with diarrhea was negatively associated. Height for age z-scores was positively associated with the gross motor subscale score only, while days of diarrhea was negatively associated with the subscale scores of fine motor and problem solving.

### 3.4 Paper III: The assessment of developmental status using the Ages and Stages questionnaire-3 in nutritional research in North Indian young children

In paper III we assessed the feasibility of the ASQ-3 in the collection of reliable data on development status in young children in a field setting. The main findings were: *a)* All 422 children completed their session with a total of 0.21% missing items, *b)* Correlation (ICC) between gold standard (main author) and examiner 1, 2 and 3 during training were strong across subscales and total scores, the inter-rater agreement remained strong throughout the study, *c)* Most items were observed during sessions by the examiners in the gross motor, fine motor and problem solving subscales, in the communication and personal social scale more answers were based on caregiver's report, *d)* Total ASQ-3 scores ranged from 30-300 with a mean score of 231.9 (SD=50). For the subscales the scores ranged from 0-60 with mean scores from 44.8-47.8. Across ages there were 18 items that showed no variability (constant items), *e)* The standardized alpha values for the total ASQ-3 scores are satisfying, however the values in the five subscales across ages varied from highly internally consistent to even negative covariance. The communication and gross motor subscales have the best alpha values, while the personal social subscale has the poorest internal consistency. The items that were adapted did not differ in alpha values compared to the non-adapted items.



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## 4 Discussion

The data for the three papers comprising this thesis are collected in an RCT involving 422 North Indian children aged six to 30 months at enrollment. In our study, we found that six months supplementation of vitamin B12 and folic acid benefitted gross motor functioning and problem solving skills. The effects of supplementation were most evident in selected subgroups. With the exception of vitamin B12 only in the gross motor domain, there was no effect of the supplementation of either B-vitamin alone. Furthermore, among the 422 young children, we were able to explain 30.6% of the variability in the total ASQ-3 scores by the identified variables. Growth (HAZ and WHZ) and number of days with diarrhea were important predictors of the ASQ-3 score, while stimulation and learning opportunities was the variable group that explained most of the variation in the total score. Finally, the ASQ-3 proved to be a feasible tool for the assessment of neurodevelopment in young children in the current study setting.

### 4.1 Discussion of findings

#### 4.1.1 The biological risks and the developing brain

##### *Vitamin B12 and folate/folic acid*

The beneficial effect of vitamin B12 and folic acid on neurodevelopment in our study is consistent with previous findings. However, there are not many studies that have described an association between folate status and neurodevelopment (71).

Observational studies have shown that both maternal and early childhood vitamin B12 status were associated with neurodevelopment in early and late childhood (64, 65, 67, 69-71). Comparing the results in our study with those of observational studies are challenging due to differences in study characteristics. The observational studies involve both vitamin status measured in the mothers during pregnancy and in the

child after birth, and the neurodevelopmental assessments are conducted at different ages from early childhood to adolescence with a wide variation in assessment tools. Furthermore, the results in the observational studies are inconsistent. In two of the studies involving maternal vitamin B12 status/dietary intake, there is no association between maternal vitamin B12 and neurodevelopment in their offspring (66, 68). The remaining studies do report a relationship.

Several RCTs involving supplementation of vitamin B12 during pregnancy and in early childhood demonstrate a beneficial effect on neurodevelopment (73-77, 79, 80, 82). However, in all of the studies involving supplementation during pregnancy and in two of the studies in infants, vitamin B12 is provided in a MMN and it is not possible to establish whether the effect of supplementation is driven by the vitamin B12 alone, by the other micronutrients or as a synergy of these nutrients.

Furthermore, in most studies, the doses of vitamin B12 were low. One RCT provided a large dose of vitamin B12 only or placebo. In this study, six-weeks old infants initially referred to a POPC for feeding problems and with signs of deficiency had positive effect of an injection of 400 µg vitamin B12 on motor abilities one month later (82). In the current study we used a factorial design of vitamin B12 and/or folic acid or placebo, enabling the assessment of effects of the B vitamins alone or in combination. The study was conducted in a general Indian population with previous documentation of vitamin B12 and folate deficiency.

The effect on neurodevelopment was seen in children who received both vitamin B12 and folic acid, suggesting a synergistic effect of vitamin B12 and folic acid. These findings were expected. Earlier studies have demonstrated that deficiencies in both vitamin B12 and folate are prevalent among children in the current population (57). These B-vitamins are closely connected, and the metabolic effects of vitamin B12 in the brain might be dependent on the effects of folate (46). There was also an effect of vitamin B12 alone on gross motor functioning. Thus, it may seem that vitamin B12 alone has an effect on neurodevelopment, but that the effect of the vitamin depends

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on concomitant administration of folic acid. The observed effect of vitamin B12 without folic acid on gross motor functioning could be understood as a consequence of several measurement factors. As an age-of-testing factor, due to rapid development in this domain, gross motor assessment tools may be particularly sensitive measures in this age group. The data are collected mainly through observation at the clinic and may be less biased by caregivers' report. Furthermore, the administration involved in the assessment of gross motor abilities is simpler. Finally, gross motor abilities may be easily transferable across cultures. These measurement factors may result in higher effects sizes and higher precision of the estimates. The consequence of enhanced precision may be reflected in lower p-values compared to the other subscales.

Although having used different assessment tools, most of the identified RCTs report effects in the gross motor domain, (73-76, 79, 80, 82). This is in accordance with the current results. It should be noted however, that in some of these studies gross motor was the sole outcome measure, and the results of specific improvements in the gross motor domain may be due to the fact that this is the only domain under study. In the current study, we had a broader assessment covering various developmental domains. We are able to demonstrate an additional effect of the supplements on problem-solving skills. Further research is needed to investigate the impact of vitamin B12 supplement alone on a wide range of developmental domains using assessment tools with high precision.

The effects of supplementation should be discussed in connection with the metabolic effects of the risks in the developing brain (13). Vitamin B12 has been linked to the process of myelination of the CNS (62). The myelination of the neurons starts prenatally, peaks in early childhood and continues throughout childhood into adolescence (63). Since myelination is involved with multiple regions throughout the CNS, one would expect a global impact of the B-vitamin supplementation on neurodevelopment with a possible specific effect on the speed of conductance (51). It has been argued that the process of myelin repair is a slow process, and that the effect

of vitamin B12 and folic acid supplementation is more an expression of increased energy production than restored myelin in the neurons (156). Increased energy production could explain the improvements in gross motor abilities seen in our study and by others. Additional energy may also lead to improved attention, which is an important factor in problem solving, where we also found an effect in our study. Finally, increased energy could lead to improvements in the child's ability to relate to its environment and to profit from stimulation and learning opportunities in his or her surroundings. Thus, improvements in vitamin B12 status may counteract functional isolation of the child.

Our study indicates that there is no effect of vitamin B12 and folic acid on the developmental domains of language, fine motor and personal social abilities. Due to a lack of clarity in the literature, it is challenging to compare our findings with those of previous studies. The lack of findings in these domains may have multiple causes. Our result may reflect a true absent of an effect in these developmental domains. While gross motor functioning and problem solving skills improve from supplementation at the time of exposure, the other developmental domains may not be in a window of opportunity and therefore not affected. Alternatively, these skills may require a longer period of supplement to show any improvement. Furthermore, the process of reversibility in CNS for various developmental domains may have different requirements in terms of time needed to be visible and capture. Thus, differences in these domains may not be seen immediately following the supplementation, but rather at later follow-ups.

The timing of an exposure (or a deficiency) should be considered to understand the effects on the developing brain (13, 34). In our study, children received six months of supplementation from they were six months to they were 36 months depending on age at enrollment. Post hoc analysis revealed that the effect of supplementation was most evident in children who completed supplementation ahead of turning two years. It should be noted, however, that none of the interaction terms with our subgrouping

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variables and vitamin B12 and/or folic acid were statistically significant. There are reports of associations between early vitamin B12 status and neurodevelopmental outcomes later in life (65, 70). Interestingly, these associations are independent of the current vitamin B12 status of the child or adolescent in some studies. This may be an indication that early deficiency is of significance for neurodevelopmental changes, and furthermore, that reversibility in later childhood is less likely despite an increase in vitamin B12 status. However, further research is called for to make conclusions on the impact of timing of the vitamin B12 deficiency and/or supplementation. For instance, follow-ups of children in the current study could provide knowledge on the long-term impact of the early vitamin B12 and/or folic acid supplementation on neurodevelopment, the impact of timing of the exposure, the specific effects on neurodevelopmental outcomes and the possible reversibility of functions.

We found the most apparent effect of supplementation on the neurodevelopmental outcomes of children who at baseline were stunted or who had elevated tHcy. There are multiple causes of linear growth restrictions, and one may be through the lack of vitamin B12 (157). The effect of supplementation in stunted children may be an effect in children with impaired vitamin B12 status who in particular benefitted from vitamin B12 and folic acid. The phenomenon where the micronutrient deficiency that accompanies the chronic undernutrition is understood to be the component compromising neurodevelopment has been described by others (33, 158). tHcy is a biochemical marker of vitamin B12 and folate deficiency, and the effect of supplementation in children with elevated tHcy in our study demonstrate that children who are suffering from vitamin B12 and/or folate deficiency profit the most from supplementation.

Our results on the beneficial effect in vulnerable subgroups are consistent with aforementioned RCTs on maternal and infant supplementation. Four of these studies report effects in susceptible groups of mothers and infants, such as mothers with HIV-1 (75), low-BMI (76), suffering from malnourishment or with anemia (159) and

infants referred to POPC due to eating difficulties with elevated tHcy (82). However, three of these studies are limited by the fact that vitamin B12 is provided as part of MMN (74-76). Furthermore, in two of these the dose of vitamin B12 is low (74, 76), and it is questionable whether such a dose can ensure recovery in grown ups at risk for vitamin B12 deficiency and intestinal illnesses. Similarly, in the current study, we provided twice the recommended daily allowances for the children to secure sufficient intake and proper uptake of the vitamin in a child population at risk for vitamin B12 deficiency and intestinal illness. The results from a study involving Norwegian infants is consistent with our findings, demonstrating effects of a large dose (400 µg) of vitamin B12 injections on motor abilities in children with elevated tHcy (82). It should be noted, however, that in this study plasma tHcy  $\geq 6.5$  µmol/L was considered to reflect impaired vitamin B12 function. This cut-off is considerable lower than the cut-off in the current study (tHcy  $\geq 10$  µmol/L). Our results provide further evidence that the developing brain benefits from supplementation in cases of subclinical deficiencies of vitamin B12. With sufficient vitamin B12 stored in the body, however, the benefit of supplementation on neurodevelopment is likely to be less.

### *Growth and diarrhea*

In the second paper, we demonstrate that growth and diarrhea predicted neurodevelopment, adding to the picture of important biological factors for neurodevelopment in these North Indian children.

Both HAZ and WHZ predicted the total ASQ-3 scores, and there was a linear relationship between the total ASQ-3 scores and HAZ-scores below -2. Being a well-established risk factor for adverse neurodevelopmental outcomes, the association between growth and neurodevelopment was expected. This finding underscores how restricted physical growth is associated with adverse brain development (38).

Stunting is a complex condition with several underlying causes, among which are intestinal illness, chronic inflammation and food insecurity (36, 37). Thus, stunting

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may be seen as an inflammatory disease that occurs due to multiple causes including enteropathy, micronutrient deficiencies, acute malnutrition and diarrhea (36). There may be several plausible pathways that can account for the observed associations between stunting and adverse neurodevelopment. Poor physical growth may lead to poor brain development due to the lack of sufficient energy in a period of massive growth (36). Furthermore, as an inflammatory disease, adverse brain development may be the result of a catabolic state inhibiting growth (31).

Growth status in the present study is based on baseline measurements when the children are between six and 30 months of age. Of the 422 participants, 40.1% were stunted and 10% were suffering from wasting at baseline. The first years of life, characterized by rapid growth, is a period in which the brain is in particularly susceptible to input (16). For instance, when stunting is manifested within the second year of life, the association with later neurodevelopment is more robust than if the growth restriction occurs later (38). Studies have demonstrated that if the growth retardation occurs later than two years of age, this is associated with less impairment in intellectual capacity (94). In our study, we have currently no follow up information on the growth and neurodevelopment of our study participants, and thus no information on possible long-term effects. However, according to the literature, the linear relationship between HAZ at six to 30 months and the total ASQ-3 scores six months later seen in the current study sample, are expected to persist. This put the children at risk for following a trajectory of poorer cognitive abilities later in childhood and less educational achievements (36, 38).

In the current study, each doubling of the number of days with diarrhea was associated with a reduction in the ASQ-3 score by approximately five points. As for stunting, the relationship between days with diarrhea and the total ASQ-3 scores was linear, where the total ASQ-3 score decreased with an increase in days of diarrhea. The association between early childhood diarrhea and neurodevelopment in later childhood has previously been described (90, 91, 93). However, previous studies

have been criticized for not adequately accounting for environmental and health related factors, and for low sample size (94). In the current study, we have information about several potential confounders, including growth, morbidity, socioeconomic status and characteristics of the home environment. In addition, we believe that we have a sufficient sample size to detect differences between groups on neurodevelopmental outcomes.

The children in the study had on average 6.6 days of diarrhea throughout the six-month study period, with a range from zero to 49 days (a total of 99 children had between 10 and 49 days of diarrhea during the study period). High rates of diarrhea impair the absorption function, and a high burden of diarrhea during the early years results in lower access to key nutrients in the period of massive brain growth (89). Furthermore, through inducing a catabolic state and cytokine activation, the inflammatory response as a result of the heavy burdens of diarrhea may lead to less growth and consequently impairments to the developing brain (86). The studies in Brazilian children suggest that the link between early childhood diarrhea and neurodevelopment persists into later childhood (90, 91). Further follow-ups of the current study sample are needed to investigate the link between early childhood diarrhea and long-term neurodevelopmental outcomes.

Based on an observational design, we cannot conclude on causality. Due to the lack of baseline assessments, there is no information on the children's neurodevelopmental status ahead of the follow-up of growth and illnesses. A child with low scores on the ASQ-3 may be more vulnerable for conditions such as undernutrition and diarrhea due to various reasons suggesting that reverse causality can explain our results. For instance, neurodevelopmental delays in children could impair the caregiving system, and a delayed child may receive less responsive care in terms of neglect in feeding, diet and health care. A second explanation could be that the low developmental scores are due to various syndromes with weakened immune system as part of the symptom cluster. Thus, rather than undernutrition and childhood

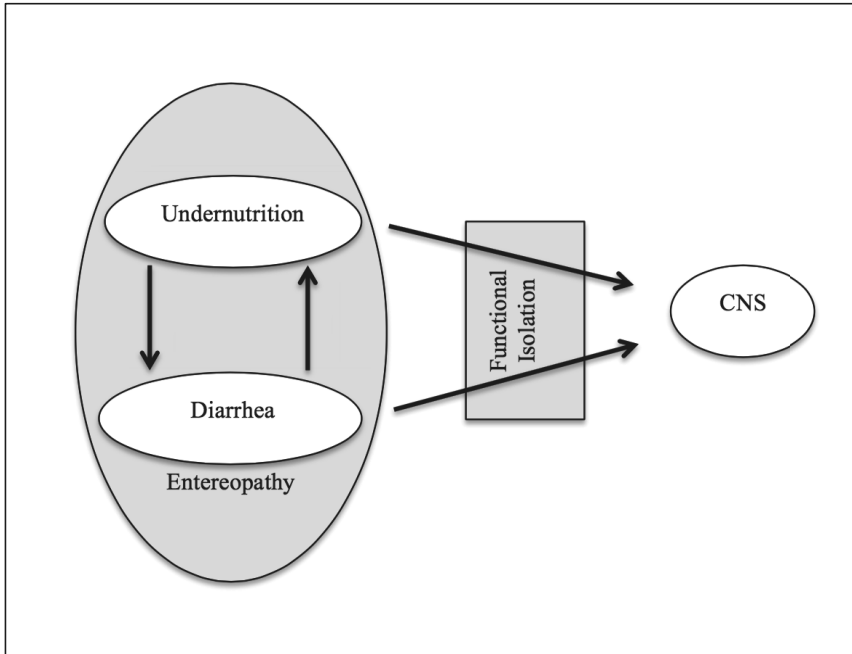


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illnesses leading to adverse neurodevelopment, the children with impaired neurodevelopment may be more susceptible to undernutrition and illnesses. However, these syndromes are rare and cannot account for all the differences in ASQ-3 scores in the study sample. Furthermore, children who were reported by their caregivers to have chronic illnesses ahead of enrollment were not included in the study.

In our results, when adjusting for HAZ and WHZ, diarrhea was still associated with the total ASQ-3 score. One way to understand this finding is that our growth and morbidity variables are reflections of different risk factors for neurodevelopment independent of each other. Our subscale analyses support the independent associations between growth and diarrhea and neurodevelopment. An increment in the HAZ score is followed by a reduced risk of being in the lower quartile of the gross motor domain alone, and increasing WHZ reduces the risk of being in the lower quartile of the communication subscale. For diarrhea, an increase in days with diarrhea is associated with an increased risk of being in the lower quartile of fine motor and problem solving subscales. Thus there might be specific pathways between stunting and diarrhea and neurodevelopment, leading to specific impairments in the developing brain.

It has been argued that the effect of diarrhea on the developing brain is through stunting, and that the association between diarrhea and neurodevelopment disappears when taking stunting into account (94, 95). The result from our study takes this discussion further. Diarrhea impairs growth both through reduced intake of nutrients and through inflammation. At the same time malnourished children have a higher burden of infections such as diarrhea (the nutrition – infection cycle) (160, 161). Thus, diarrhea might be in the causal pathway of stunting, and at the same time, stunting might be in the causal pathway of diarrhea. These relationships are illustrated in the model in Figure 5. For both conditions however, the effects on the developing brain is mediated by the psychosocial environment, the quality of stimulation and the responsive caregiving (4).



*Figure 5.* Model of the causal pathways between diarrhea and undernutrition leading to neurodevelopment.

#### **4.1.2 The significance of psychosocial factors**

While the study of biological factors and neurodevelopment is the main aim of this thesis, our results also demonstrate the importance of psychosocial factors for the neurodevelopmental outcomes. In the second paper, adjusting for relevant child characteristics (age, sex, breastfeeding status) and family income, variables grouped as stimulation and learning opportunities, such as number of toys and books in the home, hours of play with other children, and the caregivers promotion of child development, explained most of the variation in the ASQ-3 scores.

Two of the variables in the stimulation and learning opportunities are questions to the mothers concerning their understanding of the importance of promoting their child's development. The questions are selected from the stimulation and teaching dimension

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of the HOME inventory (101). Our results indicate that many caregivers lack knowledge of the importance of promoting their child's development. The lack of knowledge observed in the current study, has also been found by others in LMICs (99). In reviews of the HOME Inventory, there are reports of cultural variations in parental effort to teach children various skills. In general, however, variability of exposure within countries is associated with child competence (101). Our results support previous findings in suggesting that the lack of knowledge about child development is linked to adverse neurodevelopment.

In addition to the significance of caregivers' knowledge, our data describe the importance of having access to stimulating toys and books. Inadequate stimulation has been presented as one of the main risk factors for adverse neurodevelopment for children in LMICs (1-3). In the MICS study carried out in 28 LMICs, mother-child cognitive activities such as reading, storytelling and naming/counting/drawing are suggested as one of two domains of responsive parenting having impact on child development (97). This domain is closely linked to the social and economic status of the country as measured by the HDI (98). The results from the MICS study indicate that a low standard of living and low levels of support in a country are closely related to there being less cognitive activities for children. The investment perspective may be relevant to understand the mechanisms of poverty in LMICs in general, and our findings in this North Indian low to middle income study setting in particular (113). A previous study in the USA suggests that while family income was related to behavior problems through maternal emotional distress and parenting practices in the family stress pathway, the parental investment in a stimulating learning environment mediated the association between income and cognition (104). In the current study, we lack data to investigate the link between family stress and behavior ratings. However, the selected psychosocial variables such as toys, books and playtime, which we have labeled stimulation and learning opportunities, may capture parts of the parental investment. The caregivers' ability to provide sufficient time and resources may thus explain the variation in the ASQ-3 scores. The discussion of our

findings from an urban North Indian setting in light of findings from a US setting should be done with care. However, a consistent finding in the literature is that the relation between socio-economic status and HOME Inventory scores is independent of cultural values (162). Thus, the parental investment perspective may be feasible to understand the mechanisms between poverty and neurodevelopment also for children in this North Indian study setting.

Based on previous descriptions of the low to middle income study setting, the current study population may be described as a high-risk population in terms of high burdens on both biological and psychosocial risk factors. Functional isolation linking biological risks with the psychosocial environment may be a relevant mechanism to understand the impact biological risks have on the neurodevelopmental outcomes seen in our studies (33). For instance, due to behavioral consequences such as weakness and apathy, malnutrition and illness may have an impact on the level of activity and exploration in the child. Compared to healthier children, these children may independently take less advantage of their environment, gather less information from his or her surroundings and access less stimulation (2). Furthermore, sick and malnourished children may be inconsistent in their contact and give unclear signals due to fuzziness and irritability. Thus malnutrition and illnesses may have consequences for the quality of the mother-child interaction (96). By experiencing difficulties in regulating their interaction with others, these children may receive less sensitive attention than healthier children and/or be misconceived. As a result, they may be at risk for under stimulation and/or inadequate care. Thus, a child that may need more competent caregiving is at risk of receiving less (2). The mother-child interaction is at additional threat in cases of maternal distress and depression impairing the sensitivity and responsiveness of the caregiver even more (111). Malnutrition and illness may also have consequences for how the child is perceived by the environment and consequently for the quality of stimulation. For instance, children that are stunted may be perceived as younger than they are. As a result, these

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children may be treated as less mature and receive less sophisticated and age-appropriate stimulation from their surroundings (163).

In high-risk populations, caregivers struggle to provide even basic care for their children. Children burdened with malnutrition and illnesses are particularly vulnerable to the negative consequences of inadequate care from their caregivers. This illustrates how biological and psychosocial factors are interrelated in the course of child development. Interventions that promote child development through caregiver sensitivity and responsiveness in addition to nutrition and basic hygiene advice could optimize development for vulnerable children.

## 4.2 Methodological and ethical considerations

### 4.2.1 Strengths of the study

The current study is a carefully conducted RCT in a study population well known to the investigators. The randomization procedures were optimal including allocation concealment. Both participants and study personnel were blinded to the intervention group, and the rate of attrition was low. Furthermore, the adherence to supplementation was excellent as documented daily by field workers and reflected by the effect of supplementation on plasma levels of vitamin B12, folate and tHcy (138). Information on morbidity was collected biweekly during the six-month follow-up period and resulted in high quality data on childhood illnesses. Finally, we have detailed information on several potential cofounders.

We translated and adjusted the ASQ-3 to Hindi for this study. The presence of both personnel with knowledge and experience in child development and personnel with expertise from the local community at the 11-days workshop preparing for the assessment was important to ensure validity and reliability of data. Assessments were performed directly with the children in the presence of their caregivers. All children

that were included for testing completed their session and there were very few missing observations. Both during training and throughout the study, there was a high degree of inter-rater agreement in the measurements.

The ASQ-3 provides brief information about child performance in various domains, and gives both a total score and 5 subscale scores (124). In the current study, we have information on both the global capacity of the young children and on specific domains (43, 116, 164). In the RCT, for example, we see that although the effects on the total ASQ-3 scores are borderline significant, significant improvements are registered in the specific domains of gross motor and problem solving abilities.

#### **4.2.2 Limitations**

The ASQ-3 is a screening tool for child development that was originally constructed in the USA. In a study on early child development, a global and more comprehensive assessment tool such as the Bayley scales is often considered the gold standard. This scale provides detailed information on the child's capacity and allows for comparisons between groups with high precision (118). The Bayley scales are widely used in similar research in LMICs, but require thorough training of personnel. Due to various characteristics in the current study, we could not use the Bayley scales and had to rely on a tool providing less comprehensive information on child development. Since the ASQ-3 has not been formally validated in a North Indian population, we had no previous information about the feasibility of the screening tool ahead of study start. In our calculations following data collection, we see that there are limitations in some of the psychometric qualities of our translated and adjusted ASQ-3. A limitation of our study is that there was not sufficient time to pilot and adjust the questionnaires ahead of the start of the study. Such adjustments would have improved the quality of our data. Resulting in higher precisions to the measurements, and consequently lower sample size requirements and/or increased power.

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With no formally validated cut-offs, we used the scores on a continuous scale or as categorized on the lowest 25<sup>th</sup> percentile. Although our data may be used to indicate associations between risks and neurodevelopment, we cannot determine developmental delays in the current study setting, which may represent a limitation to our results. Furthermore, it must be noted that the ASQ-3 has not been validated for the use on a continuous scale. As a consequence there are uncertainties on the scaling of the scale, and the distribution is uneven due to floor and ceiling effects.

The ASQ-3 is the sole main outcome measure of neurodevelopment in the current study. It may be argued that the inclusion of a socio-emotional scale (i.e. the Ages and Stages Questionnaire – Socio-Emotional) would represent a significant improvement to the study, giving insights into other domains of these children's early development. Furthermore, our measurements are measures of the functional outcomes of the CNS observed in the children's behavior. Thus, through our data we gain little insight on mechanisms in the structural correlates of the CNS that would require different methods.

Another limitation to our study is that the assessment of the psychosocial environment only consists of eight questions to the caregivers. These are not from a standardized questionnaire, but were selected based on earlier studies in the current population (49) as well as two single questions from the HOME inventory (101). In the field of child development, there are a range of factors that may impact children and their development. Our questionnaire covers only a very limited part of this field. Although suggested as a major risk factor for impaired development, we did not collect data on caregiver's mental health and depressive symptoms in particular. Thus, in our study we cannot investigate how the caregiver's mental status impacts neurodevelopment, which could provide alternative perspectives and explanations to our findings. It should be noted however, in spite of various limitations to the questionnaire, we do capture factors that are important for child development in this setting.

Data on childhood illnesses were collected by trained field workers biweekly. The results indicate high burdens of diarrhea, ALRI and clinical pneumonia. It should not be ruled out that the high prevalence reported in the study might be due to overestimations by the caregivers due to our low threshold for clinic visits. As a result of the constant reminders of the conditions, caregivers may judge their children to be more burdened by diseases than they actually are. This may represent a possible bias in our data.

The main study in which we base our results was originally initiated to investigate the impact of vitamin B12 and/or folic acid on childhood infections and growth. The neurodevelopmental component was included after the start of the main study. The age range for the study is 24 months, which is appropriate in the investigation of infections in young children. However, it could be argued that this age range is too wide for the investigation of the impact of supplements on the developing brain.

The required sample size needed to detect an effect on neurodevelopment is lower than for detecting an effect on morbidity (57). Even though the sub study was initiated after the main study commenced, we managed to reach a reasonable sample size to detect meaningful differences between the groups. However, for the observed main differences in the total ASQ-3 scores, the study would have benefitted from a somewhat higher sample size.

Being included after the start of the study, we were not able to measure neurodevelopment at baseline. If we had measured at baseline, we could have estimated the effect of the intervention on change in ASQ-scores rather than on the end-study scores only. This measure would also have reduced the effect of baseline differences.

Despite an optimal randomization process and a relatively large sample size in the RCT, there are baseline differences among the four intervention groups in relevant variables. These baseline differences represent a limitation to our study as it could



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confound our results. Factors associated with poor development were more commonly observed in the placebo group, and accordingly, could lead to an overestimation of the effects of the interventions. We therefore repeated the analyses adjusting for these baseline variables including sex, age, breastfeeding status, HAZ and WAZ and family income (log transformed). The adjustments resulted in only modest changes to the effect estimates, and thus our main findings of benefit to gross motor and problem solving skills remain. However, we cannot rule out residual confounding, and that the adjustment did not remove the effects of the confounding.

#### **4.2.3 Reliability and validity of the translated version of the ASQ-3**

The main outcome measure in the current study was the ASQ-3, which was translated and adjusted for the current study setting. The process of ensuring validity and reliability of a translated and adjusted assessment tool is a continuous and ongoing process. A weakness in our study is the lack of piloting of the ASQ-3 ahead of the main data collection. During data analysis following end study, we discovered problematic items that could impair both the validity and reliability of the tool in this setting.

We used standardized alphas to evaluate the internal consistency of the translated and adjusted assessment tool. In the results, the alpha values varied from highly internally consistent to unsatisfactory and also took negative item covariance. The evaluations of the items that appeared to impair the alpha values gave new information on cultural specific factors of development in the current setting. For example, the calculations prevailed that on all items concerning walking and running children got a YES. This indicates that in the study setting, children walk and run earlier than what is expected in an American setting for this age group. In general, items on how the child relates (rock and cuddle) with stuffed animals seems to impair the alpha values, indicating that these items are not suitable in this setting. Additional adjustments in the forms following the alpha analysis would be appropriate to enhance its feasibility in the current setting.

The poor alpha values of our data may be due to both the number of constant items or to random errors. The Cronbach's alpha analysis is sensitive to the numbers of items in the analysis. The fewer items, the more challenging it is to achieve satisfactory alpha values (155). Constant items (lack of variability of the responses as with the ability to walk and run) are excluded from the alpha analysis. In our analysis, 18 items were constant and were excluded from the analysis. This made it even more difficult to achieve satisfactory alpha values. Random errors are likely to be similar between the study groups and should not result in biased effect estimates. Constant items and poor internal consistency may result in biased ASQ-scores. However, biased ASQ-scores will probably not cause biased effect estimates in the RCT design. Poor internal consistency due to constant items will shift the effect sizes towards the null. The consequences of poor internal consistency may be reduced effect sizes, a decline in the precision of the estimates and elevated p-values. Consequently, rather than false positives (type 1 errors), the suboptimal internal consistency of the ASQ-3 may have increased the risk of false negatives (type 2 errors). Thus, our findings of enhanced motor abilities and problem solving skills in the group of children that received vitamin B12 and folic acid compared to placebo were found despite an increased risk of false negatives.

Factor analyses are commonly used to evaluate the construct validity of a scale. In our study, factor analysis was not feasible due to the number of different ASQ-3 questionnaires (11 in total) to match the wide age range. We thus have to evaluate the construct validity by other methods. In LMICs, standardized tools are scarce, and to validate against a gold standard (concurrent validity) are often not possible (116). An alternative approach is to gather support for the validity through the assessment of the convergent validity against factors known to be related to early child development. In the current study, we had no gold standard measures taken at the same time as the ASQ-3, but we had information about several factors known from the literature to be related to neurodevelopment, e.g. linear growth, socio-economic status and stimulation and learning opportunities (3). The associations between the ASQ-3 score

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and factors such as growth, childhood illnesses, socio-economic status and stimulation and learning opportunities support the convergent validity of the translated and adjusted ASQ-3 in the current setting (114).

#### **4.2.4 Measurement factors**

There are several measurement factors that need to be discussed in relation to our findings. First of all, the use of personnel with no formal education and experience in the field of child assessment may have impacted our findings. Gross motor abilities may, for instance, be less challenging to assess for lay people than the other subscales. Thus, this subscale may have provided more precise estimates as discussed earlier. The lack of findings in the communication subscale may be surprising since both receptive and expressive language is thought to be sensitive domains in the current age group (18). However, the systematic assessment of language skills could be challenging for an inexperienced staff. At the same time, a large proportion of the items in the communication scale were answered based on caregivers report. Consequently the data on communication skills may have been biased and represent less precise information on the children's abilities, partly explaining the lack of findings in this domain.

The lack of findings in the personal social subscale may be due to challenges in the cross-cultural transference of the scale. The personal social subscale in the current study was the scale with the overall lowest alpha values. At the same time, it also featured a high level of items not observed by the testers. Difficulties in the personal social subscale of the ASQ-3 have been described by others in LMICs (165). Daily life skills is to a large extent defined by cultural expectations (4, 120), and thus the personal social subscale may be challenging to transfer across cultures. Thus, the lack of findings in personal social abilities may be due to the fact that the skills assessed by the ASQ-3 are not relevant to describe the children in the current study setting. The caregivers may have difficulties relating to or understand the questions, and thus give relevant answers to the questions on their child's personal social abilities. The

low alpha values and high degree of maternal report in this subscale may bias our data, and thus impair its ability to systematically describe the children and to detect differences among groups.

#### **4.2.5 Generalizability**

The data for this thesis is collected in a well-conducted RCT. Thus our results should provide a valuable addition to the existing evidence of the importance of vitamin B12 and folic acid for the developing brain. However, although our findings support findings from previous studies conducted in a variety of populations, our findings must be handled with care in terms of generalizability.

First, the current study setting is characterized by a range of factors known to compromise child well-being such as low socio-economic status, high prevalence of chronic undernutrition, widespread deficiency of micronutrients critical for development and high incidents of childhood illnesses. Due to the high prevalence of vitamin B12 and folate deficiency documented in previous studies (57), the study setting was ideal to investigate the efficacy of vitamin B12 and folic acid supplementation on neurodevelopment in a factorial design. However, the study sample represents a group of particularly vulnerable children due to multiple risks, which has consequences for the generalizability of the results to other populations. For instance, our results indicate that the effect of supplementation is most evident in susceptible subgroups, such as children that are stunted at baseline and children with biochemical evidence of vitamin B12 deficiency through elevated tHcy. It should be noted that these conditions are rare in children growing up in high-income countries, and thus our results may mainly apply for children growing up under poor conditions in LMICs. Consequently, before generalizing our findings linking vitamin B12 and folic acid to neurodevelopment, these should be confirmed in upcoming studies in various populations.

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The generalizability of the findings in paper II should also be considered closely. For these results, the low to middle-income study setting is of utmost importance in the interpretations of the findings. A wide range of risks that are linked to poverty settings must be considered. Although we report strong associations between several risk factors such as chronic undernutrition, diarrhea and inadequate stimulation and learning environment, we have little knowledge on how these associations will act in other populations where the socio-economic conditions are different. The independent linear association between diarrhea and neurodevelopment that we find in the current study have for instance not been found by others in other study settings (95). We cannot rule out that there are a range of specific causes for our particular findings that are not applicable in other populations.

ASQ-3 does not have a formal Hindi version, and in paper III we evaluated the ASQ-3 translated and adjusted specifically for the current North Indian study population. Although our evaluation shows that the ASQ-3 is promising for the current study, for further use our version has to be critically reviewed since additional adjustments may enhance the scale's psychometric qualities.

#### **4.2.6 Ethical considerations**

The declaration of Helsinki of ethical principles for medical research involving human subjects guides all medical research in human subjects worldwide (166). The basic principal for this declaration is the fundamental respect for the human beings, as well as the investigators sole duty to promote and safeguard health of the individual. While there is always need for new research, respect for the individual's well-being should precede the interest of science and society.

The interest and study questions for the current thesis is based on the fact that biological risks such as vitamin B12 deficiency, undernutrition and illnesses are widespread among children growing up in poverty in LMICs. Several previous studies indicate that there is a link between these factors and neurodevelopment in

young children. Data are collected within the framework of a RCT involving supplementation of vitamin B12 and/or folic acid to young North Indian children. Although ample evidence, there are today no official recommendations to secure sufficient vitamin B12 status in vulnerable children. In our opinion, there is a need for more evidence on the effect of vitamin B12 on neurodevelopment, preferable through RCTs with sufficient sample sizes and intervention periods. The results from our study could bring us closer to such clarification, although replications in other populations and follow-up studies on the long-term effects are called for.

The fifth principle of the Helsinki declaration states that: *In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.* Following this principal, how can we justify not providing supplement of vitamin B12 and folic acid to all children in the study, knowing that vitamin B12 and folate deficiency is prevalent and that supplement would possible secure their vitamin status and subsequent brain development? The arguments so far concern how the knowledge of vitamin B12 and neurodevelopment is important for children on a societal level. The well-being of the individual subject has not been addressed. In the present study, a field clinic was set up with medical doctors and nurses to attend to the participating children. In case of illness, the children received care from the field clinic, and were referred to more specialized health care units when this was called for. The provision of such health care is from the sponsor institution, SAS understood as an ethical obligation to the participants who commit their time for the research. After decades of performing research in the area, SAS is well known in the neighborhood and trusted for their ability to care for their participants. Based on this reputation SAS are capable of including children on a large scale for their studies. The organization has an international reputation of being a highly competent and serious research institution. A consequence of these high standards is of course that studies are costly, and demanding both in resources and competence.

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Principle number 19 in the Helsinki declaration states that: *Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.* With currently no official recommendations to spread vitamin B12 in the current area, children will not be offered vitamin B12 supplement unless more evidence is collected on its beneficial effects. Thus, one may argue that the current study is of the interest for the individual participating child, as well as for children on a societal level.

## 4.3 Implications

### 4.3.1 Clinical implications

Vitamin B12 deficiency is widespread, particularly in poor populations and in selected subgroups due to culture, religion and dietary convictions (52). Groups that suffer from conditions such as chronic intestinal illnesses or pernicious anemia are also at risk of vitamin B12 deficiency. In case of maternal deficiency, infants exclusively breastfed are at higher risk for deficiency than children that are on complementary feeding (55).

The results from the first paper of this thesis suggest that daily vitamin B12 and folic acid supplements to young North Indian children benefit neurodevelopment. Furthermore, our results indicate that children suffering from chronic undernutrition ( $HAZ < -2$ ) and with biochemical evidence of vitamin B12 deficiency (plasma  $tHcy > 10$ ) are particularly susceptible to the supplementation. The adjustment of official recommendations according to our findings would demand more evidence on the beneficial effect of these vitamins on neurodevelopment, the impact of timing, dose and concurrent risk factors. Seeing our results in conjunction with previous results, there is today not enough evidence to support a general recommendation of vitamin B12 or folic acid supplementation irrespective of current plasma levels. However, it is possible to assume that supplementation is of significance for the

developing brain in susceptible subgroups suffering from suboptimal levels of these vitamins. Consequently, a raised awareness of the importance of securing sufficient levels of these vitamins in periods of massive brain growth would be an important measure to optimize developmental potential in children at risk of deficiency.

As with vitamin deficiencies, stunting and childhood diarrhea are conditions that are widespread among children growing up in poverty (10, 83). Approximately 26% of children in LMICs are stunted, and diarrhea and pneumonia are the most prevalent causes of mortality and morbidity for children in poor populations (83). The negative association between stunting and the burden of childhood illnesses and neurodevelopment shown in the present study should be a powerful argument for improving growth and for reducing the incidence of these conditions beyond saving children`s lives.

Our results document that there are low levels of knowledge on child development in the caregivers in the current study setting, which is supported by others (99).

Furthermore, our results show associations between the children`s opportunity for stimulation and learning and their ASQ-3 scores. Interventions in LMICs demonstrate that the promotion of stimulating and responsive caregiving improves caregiving skills with beneficial effects on child development (2, 15, 19, 102). For long-term benefits on child development, recent evidence suggests that interventions should integrate knowledge of modifiable biological risks with the promotion of psychosocial factors such as responsive caregiving and stimulation and learning opportunities for the child (5, 19, 20, 103).

#### **4.3.2 Implications for further research**

Our results showing the beneficial effects of the vitamin B12 and folic acid on neurodevelopment in these North Indian young children needs to be replicated in RCT designs in similar populations with broader and more thorough assessment tools. In upcoming studies in the current age range, it would be beneficial to include a



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more comprehensive global measure such as the Bayley scales. The Bayley scales have been used in a previous study in the current study setting, and are a widely used tool in similar studies in LMICs (103, 115, 167). The Bayley scales also include a socio-emotional scale that may provide important insight to the effects of supplementation. The inclusion of specific measures of executive functioning would give more insight of specific effects of vitamin B12 on the developing brain as seen in similar studies (116). Furthermore, to assess the impact of fatigue and irritability on the quality of the mother-child interaction and the level of activity and exploration in the child may give important indicators on the effectiveness of supplementation (50, 79, 127). Finally, several neuroimaging techniques such as for instance the EEG, ERP and the vagal tone would be beneficial to investigate mechanisms in the developing brain, as shown in previous studies (168, 169). To optimize the precision of all measurements, considerable time and effort should be put into training, piloting and adjustments of the assessment tools ahead of study start. Finally, a narrower age-range and a larger sample size should be secured.

The findings of an association between diarrheal illness and neurodevelopment in these young North Indian children should also be replicated. Preferable in studies with close morbidity follow-up, information on a range of possible cofounders including growth, and with broad assessments of various developmental domains.

For the investigation of long-term effects of the biological risks addressed in this thesis, follow-ups of the current study sample would be beneficial. Such follow-ups would facilitate the investigation of possible trajectories from that of vitamin B12 and/or folic acid supplementation in early childhood and later neurodevelopment, and the long-term consequences of childhood stunting and heavy burdens of diarrhea. In later childhood, there are a range of available assessment tools both to assess global capacity and to assess specific skills and abilities. In an Indian setting, frequently the Kaufmann assessment battery has been used (66), however, a validated version of the WISC-IV was recently launched in India ([www.pearsson.in](http://www.pearsson.in)) and should be a

reasonable alternative for the assessment of general intelligence in older Indian children. Furthermore, in order to get a broader understanding of the condition of these children, it would be of interest to include specific measures of cognitive functioning as seen in previous studies (64, 70, 78), as well as measures on socio-emotional functioning and mental health, and school functioning and academic achievements (170).

Finally, following recent research evidence and recommendations, interventions that integrate modifiable biological factors such as vitamins, stunting and childhood illnesses with that of psychosocial factors following both the investment and the family stress pathway, would give new insights in the work to improve child development in poverty settings in LMICs (2, 5, 15, 19, 20). Such insights could lead to a range of positive immediate and long-term consequences for the vulnerable children worldwide and maximize their developmental potential.

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RESEARCH

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# The assessment of developmental status using the Ages and Stages questionnaire-3 in nutritional research in north Indian young children

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## Abstract

**Objective and background:** For large epidemiological studies in low and middle-income countries, inexpensive and easily administered developmental assessment tools are called for. This report evaluates the feasibility of the assessment tool Ages and Stages Questionnaire 3.edition (ASQ-3) "home procedure" in a field trial in 422 North Indian young children.

**Methods:** ASQ-3 was translated and adjusted for a North Indian Hindi setting. Three examiners were trained by a clinical psychologist to perform the assessments. During the main study, ten % of the assessments were done by two examiners to estimate inter-observer agreement. During all sessions, the examiners recorded whether the scoring was based on observation of the skill during the session, or on caregiver's report of the child's skill. Intra class correlation coefficient was calculated to estimate the agreement between the raters and between the raters and a gold standard. Pearson product moment correlation coefficient and standardized alphas were calculated to measure internal consistency.

**Principal findings:** Inter-observer agreement was strong both during training exercises and during the main study. In the Motor subscales and the Problem Solving subscale most items could be observed during the session. The standardized alphas for the total ASQ-3 scale across all ages were strong, while the alpha values for the different subscales and age levels varied. The correlations between the total score and the subscale scores were consistently strong, while the correlations between subscale scores were moderate.

**Conclusions/significance:** We found that the translated and adjusted ASQ-3 "home procedure" was a feasible procedure for the collection of reliable data on the developmental status in infants and young children. Examiners were effectively trained over a short period of time, and the total ASQ scores showed adequate variability. However, further adjustments are needed to obtain satisfying alpha values in all subscales, and to ensure variability in all items when transferred to a North Indian cultural context.

**Keywords:** Developmental assessment, Ages and Stages questionnaire, India, Global health, Health survey, Nutritional epidemiology

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## Background

Poverty, poor health, poor nutrition and deficient care are major risk factors for brain development and cognitive functioning in infants and young children in low and middle-income countries. Large epidemiological studies are needed to identify modifiable risk factors and to clarify their relative importance for development [1,2]. For this purpose, easily administered and inexpensive assessment tools ensuring reliable and valid measurements of developmental status are called for.

There is a range of quality criteria recommended for the assessment of child development in low and middle-income countries. In their toolkit prepared for the World Bank Human Development Group, Fernald, Kariger, Engle and Raikes [3] emphasize the significance of careful cultural adjustments of tools, and of assessing the psychometric qualities of the tools within the given cultural context [3]. Further, they recommend inexpensive items and materials that are easily obtainable, easy to use and enjoyable for the child. The assessment tool should meet the total range of the assessed children in order to discriminate between groups of children. Assessment tools that can be used in a wide age range and that are easily adaptable to a given cultural context are recommended.

There are official recommendations for standards in the translational and adaptation process when transferring an instrument to a given cultural setting [4]. Furthermore, unique cultural beliefs, expectations and norms in child-rearing practices may give rise to cultural specific variations in how children develop their skills. Therefore, when an instrument is transferred to a new cultural setting, its original standardized norms or population-based cut off scores are of little value. With no determined norms or cut offs, the utility of a tool should be limited to the comparison between groups, and not as a tool to diagnose or determine developmental delays [3].

Comprehensive tests of development such as the Bayley Scale of Infant and Toddler Development and the Mullen Scales of Early Learning are often referred to as the gold standard in the developmental assessment of infants and young children [5]. These tests offer comprehensive information on a child's current developmental status, are administered directly with the child, offer normative scores for the areas which are assessed, and psychometric qualities are shown to be highly satisfactory [6]. The comprehensive development tests require thorough training of personnel, preferable professionals with clinical experience within the age group, and administration is often time-consuming. Normative data are commonly limited to the country where the comprehensive tests are created and the process of obtaining normative data for other populations is time-consuming.

The briefer approaches to developmental assessments are often developed as screening instruments designed to

identify children with developmental delays who require further assessment [7]. Common factors of screening instruments are that they are efficient to administer and have standardized population-based cut off scores [8]. Screening instruments will differ however, in comprehensibility, i.e. the assessment of simple milestones or of wider aspects of children's development, in whether they are based on direct assessment with the child or on secondary reports from informants close to the child, and in terms of psychometric qualities [3,8].

The Ages and Stages Questionnaire 3 ed. (ASQ-3) is a widely used screening tool for infants and young children's development assessing development in five domains: Communication, Gross Motor, Fine Motor, Problem Solving and Personal Social [9]. The screening tool has been developed in the US, has a US standardized sample and its use has been widespread in assessing development in children aged 1 – 66 months at a low cost with cut off scores identifying developmental delays. The ASQ-system is originally a parent-completed questionnaire, but it may also be completed by a professional interacting with the child and caregiver referred to as the "home procedure" in the ASQ-3 manual. Psychometric parameters of the original ASQ-3 have been examined based on completion of a normative sample of approximately 18 000 respondents [9].

The ASQ-3 is available in English and Spanish versions. However, earlier versions of the ASQ have been translated and validated into several languages, including French, Turkish, Korean, Norwegian and Dutch [8,10-13]. There is one study evaluating the ASQ-2 for clinical purpose in four age groups in North India. Reports from the study showed correlations from 0.76 to 0.80 between scores on ASQ and DASII, a comprehensive developmental test widely used in India. In addition, the psychometric properties were reported to be good although with some variations across age groups. Based on their results, the report concludes that the ASQ had satisfactory usability for developmental screening in a North Indian setting [14]. There are also reports of the use of ASQ-2 for research purpose. For example in a multinational study ("Magpie Trial") involving several countries across different continents [15], a Norwegian randomized placebo-controlled intervention trial in very low birth weight infants [16], and a study in vulnerable children in the Andean region, Ecuador [17].

Reports from studies involving the ASQ-2 commonly describe that changes to items in the ASQ forms were necessary to ensure validity in the given cultural context [10,11,13,17]. In the Indian ASQ study for example, questions concerning mirrors and forks were mostly left unanswered, most likely because of its cultural inappropriateness. Furthermore, many developmental assessment tools, including ASQ-3, are dependent upon parent-completion. Hence, a challenge when collecting

data on the developmental status of children in low and middle-income countries is the caregiver's possible illiteracy and/or inexperience with questionnaires. To get past this challenge, it is widespread in research to use comprehensive tests assessing the child directly, although these tools may be both time and resource consuming. The ASQ-3 "home procedure" where lay-people can be trained to perform the assessment on the child directly may represent an efficient alternative approach in both time and resources for the collection of data on child development in larger studies. The ASQ-2 "home procedure" was used in the previously mentioned study in Ecuador due to caregiver's inability to complete the questionnaires [17].

Studies have shown that the ASQ-2 is promising as a developmental assessment in low and middle-income countries although cultural adjustments are necessary. However, to our knowledge, little has been done to evaluate the ASQ-3 "home procedure" in epidemiological studies. Hence, how to effectively train multiple examiners and conduct the assessment in large studies ensuring reliable data is not known. The overall aim of the current report is to assess the feasibility of the ASQ-3 "home procedure" for measuring developmental status in young children in a field trial in New Delhi, India. The report addresses necessary alterations and translations in the forms when transferring the ASQ-3 to a North Indian setting, as well as cultural adjustments in the administration of the home procedure. We assessed whether it was possible to, over a short time period, effectively train examiners to collect reliable data on infants and young children's developmental status by the ASQ-3 "home procedure", and we measured the internal consistency of the ASQ-3 when transferred to a North Indian Hindi version.

## Methods

### Ethic statement

The study was approved by the ethics committees of the Society for Essential Health Action and Training (India), Society for Applied Studies (India), Christian Medical College (India), and the Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST).

### Participants

The sample was part of a randomized, doubled blind, placebo controlled trial to measure the efficacy of routine administration of folic acid and vitamin B12 to prevent childhood infections ([clinicaltrials.gov](http://clinicaltrials.gov): NCT 00717730). One thousand children were enrolled and we included the last 440 enrolled children for developmental assessment. The study site was in the low and middle socioeconomic settings of Tigrī and Dakshinpuri in New Delhi. The areas

are typical urban neighbourhoods with a total population of about 300,000.

For the main study, a door-to-door survey was conducted to identify households with children aged six to 30 months. Children were then screened for eligibility by a physician and field supervisors and allocated randomly to one of the intervention groups. Prior to enrolment, an information sheet was read to the caregiver for signing and informed consent was obtained. After six months of supplementation and follow up, the children were assessed by the ASQ-3. There were no additional exclusion criteria for the ASQ-3 assessment. Written and verbal consent was gathered from the caregiver separately for the ASQ-3 component of the study. The Data collection period lasted from May to September 2011.

### Instrument

ASQ-3 is a comprehensive checklist of developmental status, standardized for children 1-66 months with age-appropriate questionnaires. For earlier ages there are questionnaires for every two month interval, the intervals increase with age. The ASQ-3 has five subscales: Communication, Gross Motor, Fine Motor, Problem-Solving and Personal-Social. Each form contains 30 items, six for each subscale, written in a simple language. Some questions are specific for certain age groups, while other items are used for a wider age range and are repeated in the different age-specific questionnaires. The questionnaires are designed to be completed by caregivers, but can also be administered by a professional in the "home procedure" described in the ASQ-3 manual [9]. In the "home-procedure", the professional plays an active part in the assessment of the child providing necessary material for the direct assessment of skills during the sessions. The "home procedure" is not necessarily performed in the home of the family, but in any given arena where the child, caregiver and professional meet.

### Procedure

#### Training

For 11 days immediately before initiation of the 5 months ASQ-data collection, three field supervisors (the examiners) were trained by the main author to perform the ASQ-3 "home procedure". The main author is a clinical psychologist with experience in giving training in the assessment of infants and young children in similar projects. The field supervisors had experience in working in the local community with the study population, two had degrees at master's level. None of the supervisors had formal training in developmental psychology. They were under the supervision of medical doctors, and responsible for the work of the field workers in the main study. During the 11 days training, administration, the understanding of the inherent ideas of items and scoring, as well as

approaches and techniques in terms of rapport building was discussed and practiced. Standardization exercises were performed in 30 children aged 12-36 months during the same training.

#### **Translation**

Eleven ASQ-3 forms, covering the ages 12-36 months, were formally translated to Hindi, the spoken language in the area. The translation process followed recommended procedures [4]. The field supervisors were responsible for the forward translations. The translations were thereafter back-translated to English by an employee of the Society for Applied Studies, otherwise not involved in the study. The original forms and the back translations were then reviewed and discussed by the team. No adjustments were made to the initial translation following these discussions. The translations for each age group were typed and laminated, and then used as a support to the original ASQ-3 forms during sessions.

#### **Cultural adjustments**

The assessment room was decorated with culturally appropriate decorations. The assessments were performed on a carpet on the floor as suitable for the children in the community. Materials for the sessions were purchased at local markets, and were considered to be correct for the local community. For some communication items, pictures were downloaded from the Internet in order to ensure appropriateness for the culture, (i.e. child that eats and child that plays with a ball).

Each item in the 11 relevant forms was discussed in the local research team (physician and field supervisors) in order to adapt the ASQ-3 to the study population. The feasibility of each item, possible difficulties in the translation process and possible challenges in the transition to the local community were discussed. Some small necessary adjustments were discussed and made to various items. These include slight changes of the examples in items to ensure the cultural appropriateness. I.e. in a communication item, examples of directions for the child were slightly adjusted from: "Find my coat" to: "Find my shoe", and from: "Get your book" to: "Get your (other relevant belonging during the session)".

Four items were identified not to be appropriate for the study population and were changed. These were an item involving a fork, an item involving a zipper, an item involving a mirror and one requiring the child's knowledge of both his/hers first and last name. Forks are not regular utensils in this area, and in the Personal Social subscale, the concept of fork was replaced with the concept of chapatti (flat bread), and the item was changed from: "Does your child eat with a fork?" to: "Does your child take chapatti with Dal (lenses)? Children's clothing in this area does not normally include zippers, and thus

children would be unfamiliar with the concept of zippers. The ASQ-3 item involving a zipper is an item in the communication subscale and the aim of the item is to assess the child's understanding of the concepts *up* and *down*, not the inherent capacities of a zipper. The zipper was therefore replaced with a magnet that the child could move up and down on a magnetic board on the wall. The concept of *zipper* was literally changed with the concept of *magnet* in the relatively long item text. Mirrors are not common in the study area, and many children would be unfamiliar in interacting with their mirror image and could not be expected to offer a toy to its image the first time interacting with a mirror. The item involving a mirror was changed from: *While looking at herself in the mirror, does your child offer a toy to her own image?* to: *While looking at herself in the mirror, does your child smile and interact with the reflection?* And finally, family names are seldom used in this population, and thus children in the study area are only expected to be familiar with their first name. In the discussions, the team found it difficult to find a replacement that would be reasonable, and the item was thus only slightly changed to involve only the first name. The item: "When you ask, "What is your name?" does your child say both her first and last name?" was changed to: "When you ask, "What is your name?" does your child say her first name?"

#### **Administration of the ASQ-3**

The ASQ-3 assessment was performed immediately following the end study procedures for the main study by one of the three examiners in a rotational order. Appointments with the families were made ahead, and a field worker would escort the child and caregiver to the clinic. Due to caregivers' possible inexperience with filling out questionnaires and/or illiteracy, a revised version of the "home procedure" outlined in the ASQ-3 manual was used. In the home procedure, the examiners try to elicit the relevant skills in the child during sessions, and necessary materials are natural parts of the assessments. Caregivers serve as important contributors in supporting their child as well as in providing help to elicit behaviours. The approach allowed for the assessment of skills with materials that the children often would not possess in their homes, i.e. beads and blocks. During the assessments the examiners gave the child time to play and practice (approximately five minutes) with the relevant material, and scored the items based on the child's accomplishment after the brief time of practice. The materials necessary for the screening were gathered in a standardized "Material kit", ensuring that the same materials were used in every session. Assessment would last for approximately 20-30 minutes. Each item was scored according to the ASQ-3 manual, Yes, Sometimes,



and Not Yet. The sessions started with “ice-breaking” sessions, where the children played with toys while the caregiver received relevant information for the ASQ-3 session from the caregiver and additional demographic information was gathered. Throughout the sessions the examiner held a friendly and non-threatening atmosphere in order to make both the child and caregiver comfortable in the new setting. When necessary, the examiners used toffees as motivators.

The examiners intention was to observe as much of the relevant skills and abilities as possible during the sessions, and not to rely solely on caregivers report on whether the child had developed the relevant skill. However, some items were not possible to assess during sessions, i.e. “Does the child feed him/herself with a spoon”, and the examiner would then have to rely on the mothers’ information. For every item, the examiners noted whether the scoring was based on examiners observation or the caregiver’s report.

Following the completion of the ASQ-3 assessment, caregivers received a brief feedback of the child’s performance during the session, including small advice on relevant developmental topics when this was considered necessary by the examiner.

#### **Inter-observer agreement**

Standardization exercises during the initial training, and the testing of ten % of sessions by two examiners during the main study were initiated to measure and ensure appropriate inter-observer agreement. The standardization exercises were performed in 30 children within the 11-day training period. The examiners alternated in performing the assessment, while the others observed and scored the session together with the first author who served as the gold standard. In addition, during the main study, ten % of the assessments, a total of 42 sessions, were randomly selected and scored by two examiners.

#### **Statistical analyzes**

Double data entry by two data encoders followed by validation was completed within 48 hours of the assessment sessions. Data was coded according to the ASQ-3 manual, 0 = Not yet, 5 = Sometimes and 10 = Yes. For missing items, we followed the instructions from the ASQ-3 manual and an adjusted total score was computed by dividing the total subscale score by the number of items answered in that scale, and then adding this number depending on the amount of items missing [9]. We calculated the Intra-class correlation coefficient (ICC), when comparing any of the examiners with the gold standard (first author) during the exercises, and when comparing two examiners during the data collection. Pearson product moment correlation coefficient was calculated between the five subscales and the total ASQ-3

score. Standardized alpha values, as appropriate when standard scores are summed to form scale scores [18], were calculated for the total score and for the five subscales for the 11 age intervals. Alpha values greater than 0.80 were considered to indicate high internal consistency, values from 0.60 to 0.80 were considered satisfactory, and alpha values from 0.40 to 0.60 were considered moderately internally consistent. Data was analyzed in Stata (StataCorp, College Station, TX), version 12.

#### **Results**

Of the 440 children included in the ASQ-3 assessment study, 18 children were not available at the scheduled visit; the total number of children assessed with the ASQ-3 was accordingly 422. All 422 available children completed their session. In the 422 completed questionnaires, 0.21% of the responses were missing.

#### **Background characteristics**

Table 1 shows the demographic information of the children assessed with the ASQ-3. Two hundred and fifty-nine were younger than 23 months. There was an even distribution of girls and boys. At baseline, most of the children were breastfed (86.3%), ten % of the children were wasted, 40.1% were stunted, and 31.0% were underweight. Average years of schooling for the fathers and mothers were 8.6 and 7.0 years, respectively. Of the fathers, 99.1% worked, while only 6.1% of the mothers reported to have work outside of the home. A total of 194 children lived in a joint family and the family size ranged from 3-25 with an average of 5.8 family members. The average number of children in the families was three.

#### **Inter-observer agreement**

Table 2 shows the ICCs between the gold standard (first author) and examiner one, two and three in the standardization exercises during training. All values show strong correlations between the different examiners and the gold standard across subscales and on the total scores. Table 3 shows the ICCs of the quality checks between examiner one and two during the supplementation trial, all values are consistently strong.

#### **Source of information for the scoring of items**

For different subscales, there were variations on the number of items observed during sessions and items scored based on caregivers report, see Figure 1 for details on the percentage of observed items during sessions. The Gross Motor, Fine Motor and the Problem Solving subscale were the subscales where most items were observed by the examiners. In the Communication and Personal Social subscales, more items are based on caregiver’s report of children’s relevant skills. The Personal

**Table 1 Demographic characteristics of children in the cohort**

	N	Mean/%	SD
<b>Child characteristics</b>			
Total	422		
Age in month			
12-23 months	259	61.3%	
24-36 months	163	38.7%	
Sex			
Girls	206	48.8%	
Boys	216	51.2%	
Breastfeeding status at baseline	364	86.3%	
Baseline Z score weight for length (wasted), < -2, n (%)	42	10%	
Baseline Z score length for age (stunted), < -2, n (%)	169	40.1%	
Baseline Z score weight for age (underweight), < -2, n (%)	131	31%	
<b>Family situation</b>			
<b>Economy</b>			
Annual income (median/range in 1000 rupees)	73000		12000-870000
Families who own colour TV, scooter or cooler, n (%)	377	89.3%	
<b>Maternal characteristics</b>			
Age		25.7	5.5
Years of schooling		7	6.3
Mothers who work, n (%)	27	6.1%	
<b>Paternal characteristics</b>			
Years of schooling		8.6	4
Fathers who work, n (%)	418	99.1%	
<b>Household characteristics</b>			
Type of family			
Nuclear, n (%)	228	54%	
Joint, n (%)	194	46%	
Number of children in the family		3	2.3
Family size		5.8	2.6

Social is the subscale where the least of the items were based on the examiners observations during sessions.

#### Variability

Means and standard deviation for the total score and the five subscales are summarized in Table 4. For the 422 assessed children, the mean total ASQ-3 score was 231.9 (SD = 50) with scores ranging from a minimum of 30 to

a maximum of 300. For the subscales the mean scores range from 44.8 to 47.8, all with a range from zero to 60. However, across all age levels, 18 of the total 330 (5.4%) items administered showed no variations in the scoring (constant items), because all participants had developed the relevant skill for these items. Ten of these items were in the Gross motor subscale, five in the Personal Social subscale and one in each of the remaining subscales. Five of the constant items in the Gross motor subscale are assessing the child's ability to walk and run on different age levels (i.e. Gross motor item 3, 18 months: *Does your child walk well and seldom fall?* and Gross motor item 4, 24 months: *Does your child run fairly well, stopping herself without bumping into things or falling?*). Two of the constant items in the Personal Social subscale are the same on different age categories and are assessing the ability to drink from a cup or glass (i.e. Personal Social item 4, 22 months: *Does your child drink from a cup or glass, putting it down again with little spilling?*). None of the constant items were items that were adjusted during the translation process.

#### Internal consistency

The Pearson product moment correlation coefficients across all age levels between the total ASQ-3 score and the subscales and between the different subscales are shown in Table 5. The correlations between the total ASQ-3 scores and the subscales are strong and the correlations between the five subscales are moderate.

Table 6 shows the standardized alphas for the total ASQ-3 score and the five subscales by age interval. The 66 alpha values span from negative values (no values in the table) to .92. In the Communication subscale, three values are within highly internal consistency and four values are within satisfactory. In the Gross Motor subscale, two values are highly internally consistent and three values are satisfactory. In Fine Motor and Problem Solving, six values are satisfactory. In Personal Social three values are satisfactory. In this last subscale the values for 24 and 36 months are marked as missing due to negative average covariance. For 24 months two items (item 4 and 5) were identified causing the negative average covariance. Item 4 is a question concerning the child's pretend play with a stuffed animal or doll and item 5 is on the child's ability to steer around objects and back out of corners when pushing a little wagon, stroller or other toy on wheels. For 36 months one item (item 6) was identified to cause negative average covariance, this item is on the child's ability to take turns by waiting while another child or adult takes a turn.

To assess the effect of the four adapted items on the internal consistency, further analysis were performed in the relevant subscales and age groups. Removing the

**Table 2 Intra class correlations between the gold standard and examiner 1, 2 and 3 during training exercises**

	N	Total score	Communication	Gross motor	Fine motor	Problem solving	Personal social
		ICC (95% CI)	ICC (95% CI)	ICC (95% CI)	ICC (95% CI)	ICC (95% CI)	ICC (95% CI)
<b>Examiner 1</b>	27	0.99 (0.98-1)	0.99 (0.99-1)	0.97 (0.95-0.99)	0.90 (0.83-0.97)	0.94 (0.90-0.99)	0.99 (0.98-1)
<b>Examiner 2</b>	30	0.98 (0.97-0.99)	0.99 (0.99-1)	0.97 (0.94-0.99)	0.86 (0.77-0.95)	0.96 (0.93-0.99)	0.97 (0.95-0.99)
<b>Examiner 3</b>	30	0.99 (0.98-1)	0.99 (0.99-1)	0.99 (0.99-1)	0.96 (0.92-0.99)	0.89 (0.82-0.96)	0.99 (0.98-1)

adapted item increased the alpha value of Personal Social, 27 month from 0.36 to 0.45, while in the communication subscale for 33 and 36 months, the values decreased from 0.81 to 0.64 and 0.92 to 0.87 respectively. For Personal Social 16, 18, 20 and 24 months the values remained unchanged.

### Discussion

The feasibility of the ASQ-3 “home procedure” was assessed in a field trial in a North Indian urban setting. Ahead of the five months data collection, there was an 11 days training, including translation, cultural adjustments and standardization of the examiners. In the translational process, four items were changed in order to be appropriate for the study population. The general feedback both during the training of the examiners and during the clinical trial, indicated that children, caregivers and examiners commonly found the ASQ-3 “home procedure” enjoyable to attend. The examiners experienced the ASQ-3 “home procedure” as a reasonable and feasible instrument to administer in the current clinical trial. During the study, all initiated sessions were completed and with very few missed observations. The ICC-values show a high degree of inter-observer agreement both during standardization and the main study, indicating feasibility of the ASQ-3 in terms of the collection of reliable data.

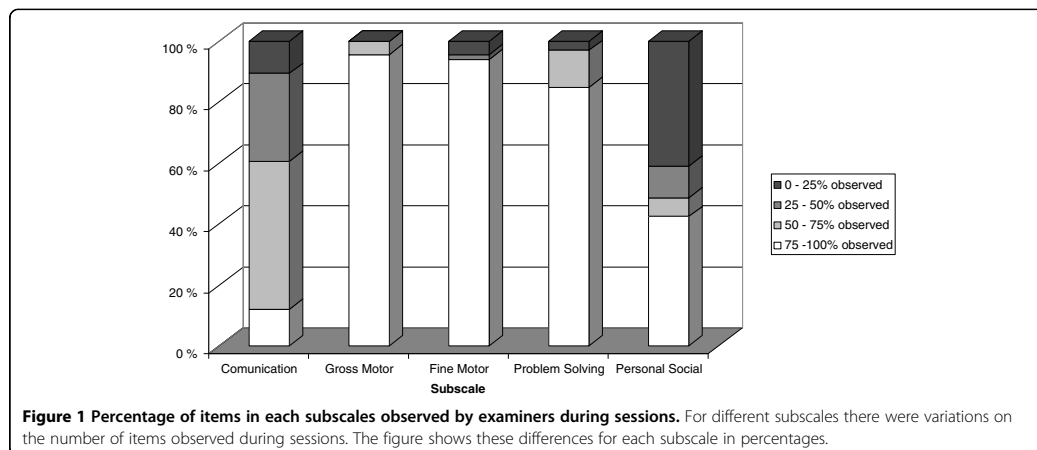
Furthermore, the total ASQ scores were in the entire range of possible values, however, some items did not show any variability. The correlation coefficients showed satisfactory concurrence between the five subscales and the total scale, but the standardized alpha values varied in the different subscales and age levels indicating some weakness of the internal consistency.

For the cultural adjustments of the ASQ-3 forms, four items in the 11 relevant forms were identified as improper in a North Indian context, and were changed. This is in accordance with other studies on the translation and adjustments of the ASQ to new cultural contexts reporting similar changes at item level [10,11,17]. It may seem that some items are more challenging to use in other cultures. For example, the item concerning a fork was changed in the present study, likewise a study in Ecuador reports that items involving using a fork were removed as they are not commonly used [17]. In the previously mentioned study from India, the items with forks were also mostly left unanswered indicating that the items were irrelevant for the children in the sample [14]. Furthermore, mirrors were found to be uncommon for the present study population, also demonstrated in previous adaptations [14]. In the ASQ-3 manual, the mirror-items are highlighted as possible problematic items for many cultures [9]. This could suggest that there are some items that are more cultural specific than others, and which should be considered with particular care while interpreting the results from studies, as well as when necessary adjustments are made in future studies.

In the present study eighteen items showed no variability since all children in the specific age categories had developed the relevant skill for the item, for example the skill of walking in the Gross Motor subscale. This might have been incidental since groups at each age levels were small (ranging from 16 to 52 participants in each age category). However, the number of constant items may also be an expression of cultural differences in child rearing practices and expectations to children’s development between North India and the US. This last assumption gives rise to the idea that the 18 constant items

**Table 3 Intra class correlation between examiner 1 and examiner 2 during the main study**

	Total score	Communication	Gross motor	Fine motor	Problem solving	Personal social
ICC	0.95	0.91	0.92	0.94	0.91	0.90
(95% CI)	(0.93-0.98)	(0.86-0.96)	(0.88-0.97)	(0.91-0.98)	(0.86-0.96)	(0.84-0.96)



are not developmentally appropriate for this North Indian sample of infants and young children, and should be adjusted and/or regrouped age appropriately prior to further use.

The internal consistency of the ASQ-3 when transferred to a North Indian setting was expressed by correlations between the total scores and the subscale scores, and by standardized alphas. The strong and consistent correlation coefficients between all of the five subscales and the total ASQ-3 scale indicate concurrency. The moderate correlation coefficients between the five subscales are expected, indicating a certain degree of concurrence between the subscales, but at the same time underlining that the subscales measure different developmental skills. These results are in accordance with the correlations between the different subscales and the total scores described in the ASQ-3 manual [9]. For the standardized alphas however, the picture is not as clear. The 66 alpha values range from highly internally consistent to unsatisfactory and in two instances, negative values. The standardized alphas for the total scale at the different age groups generally indicate that the scale is highly internally consistent and measuring the same thematic areas. For the subscales however, the values vary. The calculations of the standardized alphas therefore unfold additional problematic items causing unsatisfactory alpha values, and even, negative item covariance. These items are inconsistent with the other items in the subscale, and therefore might not assess the same developmental area in

this setting. Analysis on relevant subscales when removing adapted items does not consistently lead to improved internal consistency, and thus indicating that these are not the primary cause of the poor internal consistencies. The problematic items should be scrutinized further in order to get an understanding to why certain items in this cultural setting show inconsistency. With further adjustments to certain items there might be a possibility to improve the internal consistency of the scales, and then increase the level of reliability.

The calculations of the standardized alphas are sensitive to the number of items that are included in the analysis [18]. In the alpha calculations of the total scale, 30 items are included, while only six items are included in the calculations of the subscale alphas. Constant items are excluded from the analysis of standardized alphas, and therefore, the number of items may be even fewer than six on certain age levels in this study since a total of 18 items are constant. This may reduce the alpha values in the relevant subscales and age levels even further. Two alpha values are particular problematic in our calculations. These are in the Personal Social scale at 24 and 36 months where items cause negative average covariance, and therefore violate the assumptions of the calculations, resulting in no alpha values shown in the results.

In the technical report of the ASQ-3 manual, the standardized alpha values from their sample of 18 000 children are listed. It was concluded that the overall internal

**Table 4 Means, standard deviation and range of the total ASQ-scale score and the five subscale scores for all children**

	N	Total scale	Communication	Gross motor	Fine motor	Problem solving	Personal social
Mean (SD)	422	231.9 (50)	47.8 (15.4)	46.2 (14.1)	46.7 (13.5)	44.8 (13.9)	46.4 (12.4)
Range	422	30-300	0-60	0-60	0-60	0-60	0-60

**Table 5 Pearson product moment correlation coefficients between the five subscales across age levels and the total ASQ-score**

	Communication	Gross motor	Fine motor	Problem solving	Personal social
<b>Communication</b>					
<b>Gross Motor</b>	0.37				
<b>Fine Motor</b>	0.37	0.36			
<b>Problem Solving</b>	0.50	0.35	0.52		
<b>Personal Social</b>	0.31	0.39	0.43	0.41	
<b>Total</b>	0.73	0.69	0.73	0.77	0.68

0.5-1 = strong, 0.3-0.5 = moderate and 0-0.3 = weak correlation.  
 All correlations are significant at  $p < .000$ .

consistency of the subscales was good to acceptable. However, the table of the alphas for all the age intervals has values from 0.51 to 0.87. The Personal Social subscale is the scale with the poorest values. In a study on the cross cultural adaption of the ASQ-2 to a Korean setting, the standardized alpha values of all subscales ranged from 0.30 to 0.91, again with the poorest values in the Personal Social subscale [11]. In their discussion of the study, Heo, Squires and Yovanoff [11] argue that Personal Social items such as eating and dressing skills will give rise to differences between the Korean and the US sample. Gladestone et al. [19] argue similarly in their report on the modification of Western screening tools to a Malawian setting that cultural differences often appear in the area of social development. These assumptions are in accordance with the present study, where the Personal Social subscales offers the overall poorest alpha values. In the process of further adjusting the ASQ-3 to

a North Indian setting, the Personal Social subscale should be handled with particular care.

We administered the ASQ-3 as “home procedure”. Feedback and observations during the sessions indicate that the ASQ-3 “home procedure” in general was an enjoyable time both for children and caregivers. Examiners experienced the adjusted ASQ-3 as reasonable in assessing children from the area. This indicates that the face validity of the adjusted ASQ-3 was satisfactory. Sessions were brief and all 422 children completed their session once it was initiated. Children were given time during sessions to practice with possible unfamiliar material and were scored based on their accomplishments during sessions. Based on the possibility of collecting information both from observation and caregiver’s report missing data were scarce. These factors support the feasibility of the ASQ-3 “home procedure” in large population-based studies. Furthermore, the developmental assessment was conducted at a low cost. The examiners were not psychologists, the ASQ-3 kit was purchased online, and only one kit was required for the study site. Necessary materials and equipment for the “home procedure” were purchased at local markets, or downloaded from the Internet. Accessible tools at low cost, that are easy to use and which are enjoyable for the children in a given culture are in accordance with the recommendations of Fernald, Kariger, Engle and Raikes [3] in their toolkit for the assessment of child development in low and middle-income countries.

However, the “home procedure” approach does require some training of examiners, in addition to practice sessions after the initial training. In our study we conducted an 11 days training, which also included discussions of cultural adjustments. The ICCs both of the standardization exercises during training and the quality

**Table 6 Standardized alphas by total ASQ-3 scale and subscales**

Age interval	N	Total scale	Communication	Gross motor	Fine motor	Problem solving	Personal social
		Std. $\alpha$	Std. $\alpha$	Std. $\alpha$	Std. $\alpha$	Std. $\alpha$	Std. $\alpha$
12	51	0.77	0.33	0.69	0.62	0.62	0.53
14	43	0.88	0.46	0.82	0.54	0.78	0.57
16	37	0.90	0.67	0.90	0.76	0.69	0.67
18	34	0.75	0.58	0.47	0.62	0.46	0.26
20	42	0.90	0.81	0.73	0.66	0.71	0.76
22	34	0.72	0.70	0.16	0.49	0.17	0.43
24	39	0.82	0.76	0.58	0.29	0.49	.*
27	47	0.84	0.73	0.64	0.59	0.45	0.36
30	37	0.84	0.57	0.35	0.63	0.51	0.70
33	42	0.87	0.81	0.48	0.71	0.63	0.50
36	16	0.91	0.92	0.28	0.30	0.70	.*

$\alpha > 0.80$  - highly,  $\alpha = 0.60-0.80$  - satisfactory, and  $\alpha = 0.40-0.60$  - moderate internally consistent.  
 .\* negative average covariance.

check during the study period show that the examiners through intensive training and subsequent practice managed to obtain a high degree of concurrence in their scorings. The satisfactory ICCs serve as further support that the ASQ-3 "home procedure" may be a beneficial approach to efficiently obtain reliable data on child developmental status for research purpose.

A challenge of the ASQ-3 "home procedure" for research purpose is that, although examiners intention was to observe as much of the children's skills during sessions as possible, some ASQ items fail to provide this possibility due to its inherent structure. Analysis shows that the Motor scales and the Problem Solving scales include most items that may be observed by examiners during an assessment session. The two remaining scales, Communication and Personal Social include more items that require information from the caregiver to score. The scales may therefore be perceived to provide data of different quality, three of the scales provide objective information scored by trained examiners, and two of the scales are more reliant of the subjective report from caregivers.

Parental report do provide a risk of inaccuracy and/or overstatements in the report of the child's development due to factors such as social desirability, caregivers inexperience in interpreting their child's skills and/or their inability to accurately report the child's behaviour [3]. However, the ASQ-system is developed and based on the conviction that caregivers can provide information for proper assessments of their children. For instance, a study comparing the ASQ completion of low and middle-income parents in the US with subsequent assessment by the Bayley Scale of Infant and Toddler Development, shows no differences in the accuracy of scoring in the two groups of parents, giving support to the idea that parents-completion of child development questionnaires give reliable data also in high risk groups [20]. For now, when utilizing the ASQ-3 "home procedure" for research purpose in this cultural setting, data should be carefully interpreted with the difference in the quality of information in mind.

The total ASQ-3 scores range from zero (no scores) to 300 (full score), in our study the scores ranged from 30 to 300. The five subscales ranged from zero to 60 (full subscale score). Our results imply that although the data are not perfectly normally distributed, the ASQ-3 managed to identify children in both ends of the scale. The total ASQ-scores has a mean of 231.9 and SD of 50, while for the subscales the mean scores range from 44.8 to 47.8. A study by Kerstjens et al. [13] compares mean subscale values between Dutch, US, Norwegian and Korean samples. The mean values from our study are generally lower on all subscales, except for the Fine motor subscale were mean values from our studies are

slightly larger than in the Dutch and US sample, but still lower than in the Norwegian and Korean sample. The intention of this study has not been to formally validate the ASQ-3 for a North Indian setting and establishing cut off scores for developmental delay in the children. The differences of mean subscale values should therefore be interpreted with care. Fernald, Kariger, Engle and Raikes [3] emphasize that when cut off scores are not established for the given culture were the screening tool is used, its use should be limited to that of comparing groups. The differences between mean values in our study from other studies underline this statement. Until further validation has been conducted on the ASQ-3 for this particular population, there are no cut-off scores feasible for this North Indian sample, and data should be limited to the comparison of groups.

When evaluating the transference of an assessment tool to a new cultural context, test-retest reliability is of importance. Within the framework of this study, such evaluation was not possible. This is a definite weakness of the study. Furthermore, piloting of the translated questionnaire prior to the study would be preferable, and give room for further adjustments ahead of the study start based on preliminary calculations of internal consistencies, variability and constant items. These limitations of the study, together with other remarks in the Discussion section should set the groundwork for further attempts to transfer the ASQ-3 to new cultural settings.

## Conclusion

The present study has evaluated the feasibility of the ASQ-3 "home procedure" as an easily administered and inexpensive assessment tool for the collection of data on developmental status in infants and young children in an epidemiological study in a North Indian urban setting. Our results are promising in terms of the possibility to effectively train examiners to collect reliable data in a large study. However, for future utility in similar research setting, particular attention must be held to further adjustments of items, as well as the possibility of re-grouping items more age-appropriately, in order to enhance the internal consistency of the scales. The report underlines the significance of close awareness to cultural adjustments when transferring an assessment tool to a new cultural context, both in terms of translation and adaptation of items and in terms of cultural appropriate administration.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

NB, ST, TAS, TK, MH and IK designed research; TK, IK and Study group conducted research; NB, ST, TK and TAS were responsible for the data management; TAS and IK performed statistical analysis, MH and IK wrote the manuscript, MH, TAS and IK had the primary responsibility of the final

content. All authors contributed to the writing and approved the final version of the manuscript.

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RESEARCH ARTICLE

# Vitamin B12 and Folic Acid Improve Gross Motor and Problem-Solving Skills in Young North Indian Children: A Randomized Placebo-Controlled Trial

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## Abstract

### Objectives

Deficiencies of vitamin B12 and folate are associated with delayed development and neurological manifestations. The objective of this study was to measure the effect of daily supplementation of vitamin B12 and/or folic acid on development in young North Indian children.

### Methods

In a randomized, double blind trial, children aged six to 30 months, received supplement with placebo or vitamin B12 and/or folic acid for six months. Children were allocated in a 1:1:1:1 ratio in a factorial design and in blocks of 16. We measured development in 422 children by the Ages and Stages Questionnaire 3rd ed. at the end of the intervention.

### Results

Compared to placebo, children who received both vitamin B12 and folic acid had 0.45 (95% CI 0.19, 0.73) and 0.28 (95% CI 0.02, 0.54) higher SD-units in the domains of gross motor and problem solving functioning, respectively. The effect was highest in susceptible subgroups consisting of stunted children, those with high plasma homocysteine (> 10 μmol/L) or in those who were younger than 24 at end study. With the exception of a significant improvement on gross motor scores by vitamin B12 alone, supplementation of either vitamin alone had no effect on any of the outcomes.

design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

## Conclusion

Our findings suggest that supplementation of vitamin B12 and folic acid benefit development in North Indian Children.

## Trial Registration

ClinicalTrials.gov [NCT00717730](https://clinicaltrials.gov/ct2/show/study/NCT00717730)

## Introduction

Poor vitamin B12 status is common among young children in many low- to middle-income countries (LMIC) [1–5]. Deficiency in vitamin B12 has been associated with decreased cognitive performance among elderly [6], and case studies in infants show that severe vitamin B12 deficiency can dramatically affect the developing brain [3, 7]. Observational studies in children have reported associations between vitamin B12 deficiency and neurodevelopment [8–10]. For instance, in a cohort study in North Indian children, marginal vitamin B12 status was associated with lower scores on the mental development index of the Bayley Scales of Infant and Toddler Development (Bayley) 2nd ed. [9]. Moreover, six weeks old Norwegian infants with evidence of poor vitamin B12 status had substantially improved motor development one month after a vitamin B12 injection [11].

Poor folate status may also occur in some populations in LMIC, including India [1, 4]. The biochemical and metabolic effects of vitamin B12 and folate are closely related. Deficiency of either vitamin results in elevation of plasma total homocysteine (tHcy) [12, 13], and the consequences for neurodevelopment are similar [10]. In addition to the co-occurrence and possible synergistic effects of micronutrients on development, several relevant factors such as growth, infections and early life psychosocial factors are associated with developmental status in children [14]. Hence randomized placebo-controlled trials (RCT) are called for to clarify the effect of vitamin B12 and/or folate deficiency on early child development [10].

The objective of the current study was to measure the effect of two recommended daily allowances of vitamin B12 and/or folic acid for six months on neurodevelopment. In a RCT in young North Indian children, we compared scores of the different developmental domains of the Ages and Stages Questionnaire 3rd. ed. (ASQ-3) (communication, gross motor, fine motor, problem-solving and personal social) between a placebo group and three intervention groups receiving vitamin B12 and/or folic acid for six months.

## Materials and Methods

### Participants and study setting

The children (n = 422) included in this study participated in a RCT (n = 1000) on the effect of vitamin B12 and/or folic acid supplementation on childhood infections and growth in New Delhi, India [15]. The trial was first registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT00717730 in July, 2008, and at [www.ctri.nic.in](http://www.ctri.nic.in) as CTRI/2010/091/001090 in August, 2010. The authors confirm that all ongoing and related trials for this intervention are registered.

The study children aged six to 30 months were recruited from low to middle socioeconomic class families living in the Tigri and Dakshinpuri area in New Delhi with a total population of about 300,000, and randomized in blocks of 16. The last 440 enrollments were requested to participate in this developmental assessment sub study. Enrollment was from November 2010

to March 2011, and the developmental assessment was conducted from May through September 2011. The study was approved by the ethics committees of the Society for Essential Health Action and Training (India), Society for Applied Studies (India), Christian Medical College, Vellore (India), and the Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST), July 2008.

## Enrollment and randomization

A door-to-door survey was conducted to identify eligible children in all households in the area. A physician and field supervisors screened the children for ongoing illnesses and measured the hemoglobin levels. Cases of anemia were treated with oral iron as per national guidelines. Availability of informed consent and no plans to move away over the next six months were considered for enrollment. We excluded children with severe acute malnutrition (weight-for-height z-scores  $< -3$ ), and severe anemia (hemoglobin  $< 7$  g/dL), and they were referred for treatment according to national guidelines. Children already using folic acid and/or vitamin B12 supplements were not included in the study. We also excluded children that participated in other trials and children with illnesses requiring hospitalization. With no screening of developmental delays prior to enrollment, only children with known developmental disabilities were excluded. Only one child from each household was recruited for the study. Written informed consent was obtained prior to enrollment from the caregiver on behalf of the children. In case of non-literates, an impartial witness witnessed the consent. All witnesses were registered in a list. The ethics committees approved the consent procedure. Demographic information was collected at enrollment.

Using a factorial design, children were randomized in a 1:1:1:1 ratio in blocks of 16 to one of four treatment groups: placebo, vitamin B12 only, folic acid only, and vitamin B12 and folic acid (in the following, referred to as vitamin B12/folic acid). The randomization was stratified into infants ( $< 12$  months) and older children ( $\geq 12$  months) by assigning blocks to either of these two strata. The vehicle for the vitamins and the placebo, was a lipid-based paste provided in jars pre-labeled by the producer with a subject identification number and no indication on study group. A scientist at the University of Bergen, who was otherwise not involved in the study, provided the randomization list using Stata Version 10 (StataCorp, College Station, TX, USA) linking the unique child identification number with the intervention group. The subject identification number was the only indication that could link the paste to the study group. Ensuring double blinding, the placebo and the vitamin supplements were identical in appearance and taste and the allocation was masked to the participants as well as the study team throughout the data collection period.

Inclusion and exclusion criteria, as well as the other study procedures were the same for the main study and the current sub study.

## Interventions

We have previously demonstrated that gastrointestinal illnesses as well as folate and vitamin B12 deficiency is common in this population [4, 16, 17]. We therefore decided to provide folic acid and vitamin B12 at doses that were approximately twice the recommended daily allowances. The lipid based paste was chosen because of its acceptability in similar populations and because it is a feasible way to provide vitamins and minerals to child populations without degradation or contamination. The paste was prepared by NUTRISET, Ltd (Malaunay France). The interventions were given to the enrolled children daily by field workers. On Sundays and on public holidays the caregivers administered the supplementation to the children according to instructions. When families were travelling, field workers provided the supplement for the

planned travel period in smaller units. All children across study groups were supplemented with one spoon (5 g) if they were 6 to 11 months, and two spoons (10 g) if they were 12 months and above. Each 10 g of supplement contained 54.1 kcal total energy, 0.7 g proteins and 3.3 g fat. For the intervention groups folic acid only, vitamin B12 only or vitamin B12/folic acid, the 10 g supplement also contained 150 µg folic acid or 1.8 µg vitamin B12 (as cyanocobalamin), or the combination of both. For the younger children, the 5 g supplement contained half of the vitamin doses of the older children. All children received the intervention for six months.

## Developmental assessment

Development was assessed at the end of the study following six months supplementation. The timing of the assessments was identical for all children in the sub study. Development was assessed using the ASQ-3, a developmental screening tool constructed in the US [18]. The ASQ-2<sup>nd</sup> ed. has been validated against a developmental assessment tool in North India, and found to have good test characteristics for detecting developmental delay in this setting [19]. The ASQ-2 has also been used as an outcome measure in epidemiological studies worldwide [20–22], where both continuous and dichotomous outcomes (cut-offs) have been reported.

The ASQ-3 consists of age-appropriate questionnaires, all containing 30 items divided into five subscales: Communication, Gross motor, Fine motor, Problem-solving and Personal-social, summing up to five subscale scores (range 0 to 60) and a total score (range 0 to 300). Eleven forms (for age 12–36 months) were translated to Hindi following official recommendations [23], and items not suited for the cultural setting were identified and slightly adjusted [24]. In the translated ASQ-3 version, the standardized alphas for the total ASQ-3 scores were strong, indicating an overall acceptable internal consistency [24].

Three field supervisors were trained to administer the ASQ-3 directly with the child at the research clinic in presence of caregivers [25]. The examiners elicited the relevant skills from the child during sessions using standardized materials. The caregiver served as an important contributor in supporting the child, eliciting behaviors and gave relevant information of the child's development when necessary. The three field supervisors were trained by the first author, a clinical child psychologist with experience in training and the assessment of infants and young children. During the 11 days of training, the field supervisors were standardized in performing the procedure, and they reached a high inter-observer agreement both during training and in a separate quality control where 10% of the observations were done in duplicate throughout the study [24].

To assess the caregiver's promotion of child development we carefully selected two questions from the standardized assessment tool Home Observation for Measurement of the Environment (HOME) [26] that were asked the caregivers during the session. One question was on "Mother's belief that child's behavior can be modified" and one was on "Mother's encouragement of developmental advances".

## Growth and biochemical markers

Trained field supervisors measured weight and length at baseline and after six months of supplementation. Weight was measured to the nearest 50 g using Digitron scales. Length was measured using locally manufactured infantometers reading to the nearest 0.1 cm.

Venous blood samples were obtained at baseline for all children, and at end study in a subsample of randomly selected blocks (94 children for the subsample). Three mL of blood was collected into an evacuated tube containing EDTA (BD, Franklin Lakes, NJ, USA). Immediately following blood sampling, plasma was separated from the blood cells by centrifugation at room temperature (450 x g x 10 min), transferred into storage vials and stored at -20 °C until

analysis. Plasma tHcy was analyzed using commercial kits (Abbott Park, IL, USA) [27]. Plasma concentrations of vitamin B12 and folate were determined by microbiological assays using a chloramphenicol-resistant strain of *Lactobacillus casei* and colistin sulfate-resistant strain of *Lactobacillus leichmannii*, respectively [28].

### Power calculations

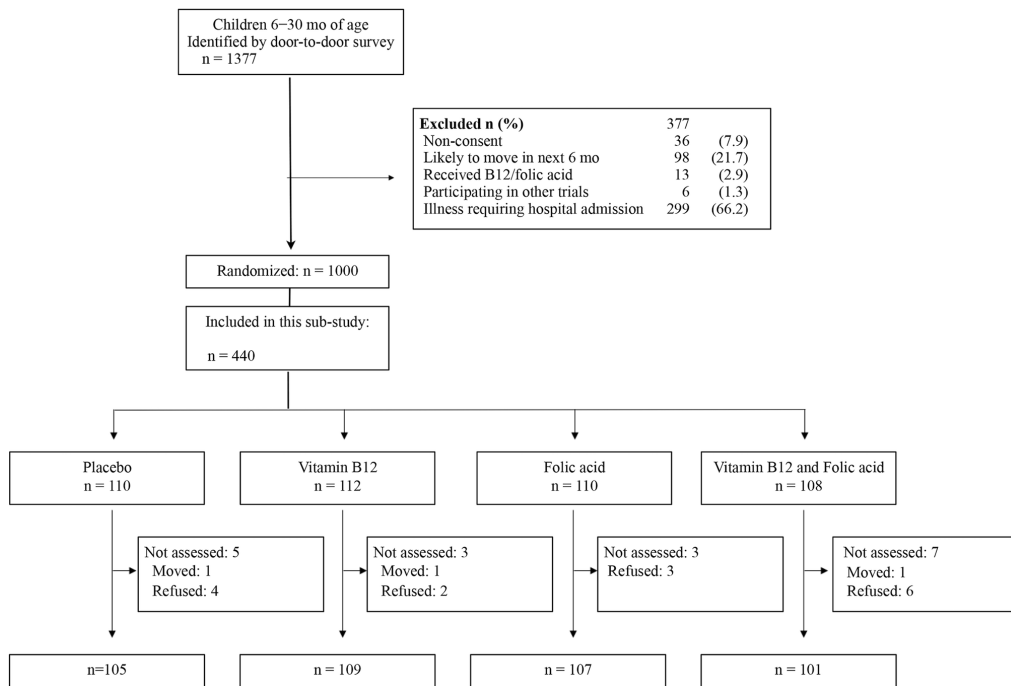
We included the last 422 enrollments in this sub-study. The power to detect a standardized mean difference (based on a t-test) of 0.4 (i.e. 20 points in total ASQ-3 scores) and 0.5 (25 points in total ASQ-3 scores) between the placebo and any of the treatment groups was 83 and 95 per cent, respectively. In these calculations, which were done by the “power” command in Stata, we used a two-sided alpha error of 0.05.

### Statistics and data management

The data was entered twice by two data entry clerks followed by validation by a computer manager. A total of 0.21% of the ASQ-3 responses were missing. For missing items an adjusted total score was computed by dividing the total subscale score by the number of completed items in the scale [29]. This number was then added depending on the amount of items missing. Height-for-age, weight-for-age and weight-for-height z scores were calculated using the most recent WHO growth charts [30]. The ASQ-3 scores for the total sample are presented as means (SD). We used linear regression to compare the intervention groups: vitamin B12, folic acid and vitamin B12/folic acid against the placebo on a continuous scale. We also used multiple logistic regression on the total and subscale ASQ-3 scores categorized on the 25th percentile. In these models, we also examined the effects in various predefined subgroups based on the following baseline characteristics (cut-offs in brackets): age (<12 months), stunting (< -2 z scores height/length-for-age), wasting (< -2 z scores weight-for-height/length), being underweight (< -2 z scores weight-for-age), low plasma vitamin B12 (<200 pmol/L), low plasma folate (<7.5 nmol/L), and high plasma tHcy (>10 μmol/L). This is the same approach for presenting main and subgroup effects as we used when presenting the effect of the interventions on the incidence of infections [15]. For the subgroup models, we adjusted for sex, age, breastfeeding status, height-for-age z-scores and mother’s encouragement of developmental advances. Post hoc, we examined the effect in an additional subgroup: ≤ 24 months vs. > 24 months at end study (corresponding to ≤18 months vs. >18 months at enrollment). We also performed the overall analyses adjusting for important baseline factors such as sex, gender, breastfeeding status, height-for-age, weight-for-age z-scores and log transformed family income. We included interaction terms in the models to measure whether the effects between the subgroups were significantly different. In these models, we also measured the interaction between folic acid and vitamin B12 supplementation. We calculated standardized mean differences by dividing the mean differences by the overall SDs of the different outcomes. Statistical analyses were performed in Stata, version 13 (Stata corporation, College Station, TX). All analyses were done following an intention-to-treat protocol.  $P < 0.05$  (two-tailed) was considered significant.

### Results

**Fig 1** shows the flow of the participants through the study. Among the 1000 children randomized into the main study, the last 440 enrollments were included for developmental assessment. Three children were not available for assessment and 15 did not wish to participate, hence the final number of participants was 422. Baseline characteristics for the children in the four intervention groups are presented in **Table 1**. As reported from the main study, adherence was



**Fig 1. Trial profile of a randomized, placebo controlled trial on the effect of vitamin B12 and/or folic acid administration on development in 6–30 months old North Indian children.**

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excellent and 96% of the scheduled doses were ingested [15]. Furthermore, compared to the placebo group, plasma vitamin B12 concentrations increased substantially in the group of children that received vitamin B12 alone or in combination with folic acid. Likewise, plasma folate levels increased significantly in children who received folic acid alone or in combination with vitamin B12 (Table 2).

### The effect of the intervention on development

Overall, the total ASQ-3 score was 12.6 (95% CI -1.1, 26.3) ( $P = 0.071$ ) points higher in the group of children who received six months of vitamin B12/folic acid supplementation compared to those who received placebo (Table 3). Higher scores in the vitamin B12/folic acid supplementation group were also observed for the Gross motor subscale ( $P = 0.001$ ) and the Problem-solving subscale ( $P = 0.048$ ), while no significant effect was observed for the Communication, Fine motor and Personal social subscales (Table 3). The mean standardized effect sizes for the Total, Gross Motor and the Problem-solving subscales were 0.25 (95% CI -0.02, 0.53), 0.46 (95% CI 0.19, 0.73) and 0.28 (95% CI 0.02, 0.54) SD units respectively.

In groups that either received folic acid or vitamin B12 alone, we did not find significant differences from placebo with the exception of children who received vitamin B12 without folic acid: The gross motor scale was 4.0 (95% CI 0.3, 7.8) points higher in the vitamin B12 group



than in the placebo group (Table 3). The corresponding effect size was 0.29 (95% CI 0.02, 0.55) SD units.

We repeated the analyses using logistic regression after dichotomizing the outcomes at the 25 percentiles. Similar results were observed as for the linear regression reported above (Table 4). We also undertook these comparisons adjusting for baseline differences; the adjusted analyses resulted in only modest changes to the estimates and the levels of significance (S1 and S2 Tables).

**Table 1. Baseline characteristics of the 422 children age 6–30 months.**

	Placebo (n = 105)	Vitamin B12 (n = 109)	Folic Acid (n = 107)	Vitamin B12 & Folic Acid (n = 101)
<b>Child characteristics</b>				
Age, months	16.2 ± 7.4 <sup>1</sup>	15.3 ± 6.6	15.8 ± 7.6	15.2 ± 6.6
6 – 11 months, n (%)	36 (36.2)	34 (31.2)	35 (32.7)	34 (33.7)
12 – 30 months, n (%)	69 (63.8)	75 (68.8)	72 (67.3)	67 (66.3)
Boys, n (%)	62 (59.0)	52 (47.7)	51 (47.7)	51 (50.5)
Still breastfed, n (%)	92 (87.6)	97 (89.0)	88 (82.2)	87 (87.0) <sup>2</sup>
<b>Z scores:</b>				
Weight-for-height (WHZ)	-1.0 ± 0.9	-0.8 ± 0.9	-0.7 ± 1.0	-0.8 ± 0.9
Height-for-age (HAZ)	-1.9 ± 1.2	-1.7 ± 1.2	-1.7 ± 1.2	-1.7 ± 1.0
Weight-for-age (WAZ)	-1.7 ± 1.0	-1.5 ± 1.0	-1.4 ± 1.1	-1.4 ± 0.9
Wasted (<-2 WHZ), n (%)	15 (14.3)	12 (11.0)	8 (7.5)	7 (6.9)
Stunted (<-2 HAZ), n (%)	47 (44.8)	45 (41.3)	43 (40.2)	34 (33.6)
Underweight (<-2 WAZ), n (%)	42 (40.0)	28 (25.7)	36 (33.6)	25 (24.8)
<b>Family characteristics</b>				
Annual family income (INRx1000) <sup>3</sup>	72 (48, 120)	82 (60, 120)	72 (60, 126)	84 (62, 140)
Has television, scooter or cooler, n (%)	90 (85.7)	98 (89.9)	98 (91.6)	91 (90.1)
Living in joint family, n (%)	45 (42.9)	44 (40.4)	54 (50.5)	51 (50.5)
Family size	5.8 ± 2.4	5.8 ± 2.9	5.8 ± 2.4	5.9 ± 2.4
Age of mother, years	25.8 ± 4.6	26.5 ± 8.2	25.0 ± 3.6	25.3 ± 3.9
<b>Mothers years of schooling n (%)</b>				
No schooling (>5)	22 (21)	30 (27.5)	24 (22.4)	22 (21.8)
Primary (5 years complete)	65 (61.9)	50 (45.9)	46 (43)	48 (47.5)
Middle (10 years complete)	6 (5.7)	13 (11.9)	9 (8.4)	11 (10.9)
Higher (>10 years)	12 (11.4)	16 (14.7)	28 (26.2)	20 (19.8)
<b>Fathers years of schooling</b>				
No schooling (>5)	16 (15.2)	13 (11.9)	13 (12.2)	11 (10.9)
Primary (5 years complete)	36 (34.3)	36 (33.1)	41 (38.3)	39 (38.6)
Middle (10 years complete)	26 (24.8)	37 (33.9)	23 (21.5)	27 (26.7)
Higher (>10 years)	27 (25.7)	23 (21.1)	30 (28)	24 (23.8)
Mothers who work, n (%)	6 (5.7)	9 (8.3)	8 (7.5)	3 (2.7)
Attending Anganwadi centre <sup>4</sup> , n (%)	6 (5.8)	13 (11.9)	12 (11.2)	9 (8.9)
<b>Folate and vitamin B12 status at baseline</b>				
Vitamin B12 < 200 pmol/L, n (%)	36 (34.3)	36 (33.0)	39 (36.5)	27 (26.7)
Folate < 7.5 nmol/L, n (%)	27 (25.7)	38 (34.8)	30 (28.0)	36 (35.6)
tHcy > 10 µmol/L, n (%)	67 (63.5)	60 (53.21)	62 (57.9)	58 (57.4)

<sup>1</sup> Mean±SD all such values.

<sup>2</sup> Missing information from 1 child.

<sup>3</sup> Indian Rupees in median (Interquartile range).

<sup>4</sup>Childcare centre.

**Table 2. Concentrations of markers of vitamin B12 and folate status and change compared to the placebo.**

	Placebo (n = 105)		B12 (n = 109)		Folic acid (n = 107)		B12 & Folic acid (n = 101)	
<b>Baseline</b>	Median	IQR <sup>1</sup>	Median	IQR	Median	IQR	Median	IQR
Plasma vitamin B12 (pmol/L)	260	(170–353)	269	(178–419)	286	(180–473)	307.0	(198–439)
Plasma folate (nmol/L)	10.4	(7.0–18.4)	9.1	(6.1–18.2)	11.8	(6.7–20.3)	9.7	(6.1–17.8)
Plasma tHcy (µmol/L)	12.7	(9.1–17.2)	10.6	(8.7–15.0)	11.8	(8.7–15.2)	10.4	(8.2–13.5)
<b>End of study</b>	(n = 24)		(n = 22)		(n = 24)		(n = 24)	
Plasma vitamin B12 (pmol/L)	285	(199–368)	362	(271–708)	297	(225–405)	446	(302–565)
Plasma folate (nmol/L)	16.6	(11.9–22.5)	11.8	(8.0–19.3)	49.0	(28.6–64.0)	50.5	(31.2–78.4)
Plasma tHcy (µmol/L)	12.4	(10.3–15.5)	8.0	(6.5–9.5)	8.1	(6.7–11.7)	7.6	(5.8–10.0)
<b>Change from baseline to end of study compared to placebo</b>			Mean diff. <sup>2</sup>	95% CI <sup>3</sup>	Mean diff.	95% CI	Mean diff.	95% CI
Vitamin B12 (pmol/L)	0		118	(17, 219)	6	(-93, 105)	102	(4, 202)
Folate (nmol/L)	0		-3.2	(-15.0, 8.6)	28.5	(17.0, 40.0)	34.3	(22.8, 45.7)
tHcy (µmol/L)	0		-1.7	(-5.0, 1.5)	-1.2	(-4.5, 2.1)	-5.3	(-8.6, -2.1)

<sup>1</sup> Interquartile range.

<sup>2</sup> Mean difference change in concentration from baseline.

<sup>3</sup> 95% Confidence interval.

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### Subgroup analyses

In the subgroup analyses we present the results from the logistic regression analyses. Stunted children that received six months of vitamin B12/folic acid supplementation had substantially and significantly reduced odds [Odds ratio (OR): 0.26 (95% CI 0.09, 0.78) (*P* = 0.016)] of being in the lowest quartile of the ASQ-3 score. This was also the case for children with elevated levels

**Table 3. The effect of vitamin B12 and/or Folic acid on ASQ-3 total and subscale scores.**

	Placebo (n = 105)	B12 (n = 109)		Folic acid (n = 107)			B12 & Folic acid (n = 101)			
	Mean (SD)	Mean (SD)	Mean Diff. <sup>1</sup>	95%CI <sup>2</sup>	Mean (SD)	Mean Diff.	95%CI	Mean (SD)	Mean Diff.	95%CI
<b>Total ASQ-3</b>	228.0 ± 47.2	230.6 ± 52.2	2.6	(-10.8, 16.0)	228.6 ± 55.8	0.6	(-12.7, 14.3)	240.6 ± 43.2	12.6	(-1.1, 26.3)
<b>Subscale</b>										
Communication	47.4 ± 14.8	47.8 ± 15.9	0.4	(-2.8, 4.6)	48.2 ± 15.7	0.8	(-3.4, 5.0)	47.9 ± 15.4	0.5	(-3.8, 4.7)
Gross motor	42.8 ± 15.2	46.8 ± 13.6	4.0	(0.3, 7.8)*	45.8 ± 15.3	3.0	(-0.8, 6.8)	49.3 ± 11.4	6.5	(2.7, 10.3)**
Fine motor	47.6 ± 13.2	47.1 ± 13.3	-0.5	(-4.1, 3.1)	44.5 ± 14–9	-3.1	(-6.8, 0.5)	47.6 ± 12.2	0	(-3.7, 3.7)
Problem-solving	44.3 ± 14.1	43.1 ± 15.6	-1.2	(-4.9, 2.5)	43.9 ± 13.3	-0.4	(-4.1, 3.3)	48.1 ± 11.5	3.8	(0.0, 7.6)*
Personal social	45.9 ± 12.4	45.7 ± 12.5	-0.2	(-3.5, 3.2)	46.4 ± 12.9	0.5	(-2.8, 3.9)	47.7 ± 12.0	-1.8	(-1.6, 5.2)

\**p* < 0.05.

\*\**p* < 0.01.

<sup>1</sup> Mean difference in total ASQ scores from Placebo.

<sup>2</sup> 95% Confidence interval.

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**Table 4. ORs<sup>1</sup> (95% CIs<sup>2</sup>) for being in the lower quartile of ASQ-3 total and subscale scores compared with placebo.**

	Placebo (n = 105)	B12 (n = 109)		Folic acid (n = 107)		B12 & Folic acid (n = 101)	
	OR	OR	95% CI	OR	95% CI	OR	95% CI
<b>Total ASQ-3</b>	1	0.66	(0.37, 1.19)	0.75	(0.42, 1.34)	0.53	(0.29, 0.99)*
<b>Subscale</b>							
Communication	1	0.95	(0.53, 1.68)	0.89	(0.50, 1.59)	0.88	(0.49, 1.59)
Gross motor	1	0.74	(0.43, 1.28)	0.74	(0.43, 1.28)	0.39	(0.22, 0.71)**
Fine motor	1	0.99	(0.56, 1.74)	1.57	(0.90, 2.73)	0.93	(0.52, 1.66)
Problem-solving	1	0.97	(0.57, 1.67)	0.83	(0.48, 1.43)	0.52	(0.29, 0.93)*
Personal social	1	0.98	(0.56, 1.71)	1.05	(0.60, 1.83)	0.72	(0.40, 1.28)

\*p<0.05.

\*\*p<0.01.

<sup>1</sup> Odds Ratio.

<sup>2</sup> 95% Confidence interval.

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of tHcy at baseline with significantly reduced odds [OR: 0.38 (95% CI 0.16, 0.92) ( $P = 0.032$ )] of being in the lowest quartile. Based on the assumption that the period up to 24 months is a critical time for brain development, we also measured the effect of the interventions separately for children who were  $\leq 24$  months at end study vs. the older children. In children  $\leq 24$  months at the end of the study ( $\leq 18$  months at enrollment), vitamin B12/folic acid supplementation led to significantly lower odds of being in the lower quartile of the ASQ-3 score (OR: 0.37, 95% CI 0.17–0.83,  $P = 0.015$ ). These effects were not seen in children aged 19 to 30 months at enrollment (Table 5). None of the subgrouping variables significantly modified the effect of the interventions on the ASQ-3 scores, and the interaction between folic acid and vitamin B12 supplementation was not significant.

## Discussion

In our study, we found a borderline significant positive effect of receiving vitamin B12 and folic acid for six months on the total ASQ-3 scores. We also found a significant and positive effect of vitamin B12 and folic acid in the developmental domains of gross motor functioning and problem-solving skills. The effects of supplementation were most evident in children who were stunted, and in children who had high plasma tHcy consistent with poor folate and/or vitamin B12 status, and in children who completed the study before their 2<sup>nd</sup> birthday [31]. Except for vitamin B12 in relation to gross motor functioning, there was no significant benefit of giving vitamin B12 or folic acid alone on early child development.

North Indians, including children, often have poor vitamin B12 status due to dietary practices (vegetarianism) and poverty [2]. Poor vitamin B12 status was also seen in the present study. In addition, a large proportion had low plasma folate, and nearly 60% of these young children had elevated tHcy at baseline. Children who received supplementation of vitamin B12 and folic acid for six months had substantial improvement in vitamin status and a reduction in plasma tHcy concentrations [15].

Through our assessment across developmental domains, we found that children who received supplementation of vitamin B12 and folic acid for six months had improved gross motor outcomes and problem-solving skills, while there were no significant changes in the domains of communication, fine motor and personal-social skills. The results are in line with previous observational studies that have documented an association between vitamin B12

**Table 5. ORs<sup>1</sup> (95% CIs)<sup>2</sup> for being in the lower quartile of ASQ-3 total in the intervention group compared with placebo in subgroups.<sup>3</sup>**

	N	Placebo	Vitamin B12		Folic acid		B12 & Folic acid	
		OR	OR	95% CI	OR	95% CI	OR	95% CI
<b>Subgroups</b>								
<b>Age in months at baseline</b>								
6 to 11 months	138	1	0.51	(0.17, 1.50)	0.79	(0.26, 2.39)	0.48	(0.16, 1.48)
12 to 30 months	283	1	0.74	(0.33, 1.63)	1.12	(0.50, 2.48)	0.59	(0.25, 1.36)
6 to 18 months	149	1	0.46	(0.22–0.99)*	0.73	(0.34–1.57)	0.37	(0.17–0.83)*
19 to 30 months	273	1	1.65	(0.49–5.60)	1.87	(0.55–6.33)	1.20	(0.33–4.43)
<b>Growth at baseline</b>								
Wasted	42	1	0.57	(0.06, 5.25)	4.29	(0.29, 62.61)	1.34	(0.11, 16.38)
Not wasted	379	1	0.67	(0.34, 1.32)	0.92	(0.47, 1.78)	0.53	(0.26, 1.08)
Stunted	169	1	0.48	(0.19, 1.22)	0.77	(0.29, 2.02)	0.26	(0.09, 0.78)*
Not stunted	252	1	0.95	(0.39, 2.34)	1.25	(0.51, 3.08)	1.11	(0.46, 2.71)
Underweight	131	1	0.54	(0.17, 1.64)	0.84	(0.29, 2.45)	0.64	(0.21, 1.97)
Not underweight	290	1	0.78	(0.36, 1.72)	1.05	(0.47, 2.35)	0.49	(0.21, 1.18)
<b>Biochemical markers at baseline</b>								
Vitamin B12 <200 pmol/L	137	1	0.48	(0.17, 1.38)	0.68	(0.24, 1.90)	0.61	(0.20, 1.85)
Vitamin B12 ≥200 pmol/L	284	1	0.87	(0.39, 1.95)	1.15	(0.51, 2.65)	0.59	(0.25, 1.38)
Folate <7.5 nmol/L	131	1	0.95	(0.28, 3.28)	1.44	(0.41, 5.13)	0.58	(0.16, 2.11)
Folate ≥7.5 nmol/L	290	1	0.65	(0.30, 1.38)	0.84	(0.40, 1.76)	0.60	(0.27, 1.32)
tHcy >10 μmol/L	247	1	0.66	(0.30, 1.46)	0.73	(0.33, 1.61)	0.38	(0.16, 0.92)*
tHcy ≤10 μmol/L	174	1	1.10	(0.37, 3.29)	1.86	(0.61, 5.67)	1.10	(0.37–3.27)

\*p<0.05.

<sup>1</sup>Odds Ratio.

<sup>2</sup>95% Confidence interval.

<sup>3</sup>Adjusted for sex, age, breastfeeding status, stunting and Mother’s encouragement of developmental advances (dichotomus).

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status and aspects of development [9, 32]. However, the specific developmental domains that are related to vitamin B12 differ between studies and are not directly comparable due to methodological differences. For example, in the study in North Indian children, marginal vitamin B12 status was related to lower scores on the mental development index of the Bayley 2nd ed, but not to the scores on the psychomotor scale [9]. These scales are not directly comparable to the ASQ domains. The association between gross motor functioning and vitamin B12 has been documented across several studies, i.e. in a Dutch vegan study group, where vitamin B12 deficient infants suffered from slower gross motor and language development compared to non-deficient infants [32]. Further, in a recently published RCT, Norwegian infants with poor vitamin B12 status and who were referred to a pediatrician due to feeding problems had substantially and significantly improved feeding and gross motor functioning following a vitamin B12 injection [11]. These findings are consistent with the findings in the present study. However, in the study of Norwegian children, they did not assess other developmental domains. Furthermore, while the Norwegian study relates to the rare situation of a child being submitted to hospital for feeding problems, our data relate to associations of the general Indian population of people belonging to middle to poor socioeconomic classes.

Given the nature of the developing brain with periods of regional brain growth spurts and rapid maturation, it is challenging to compare results on specific developmental areas across age groups. Functions under development are particularly sensitive to influences, which may

give rise to developmentally dependent outcomes [33, 34]. Consequently, we need to consider the timing of the exposure as well as the period of assessment [34, 35]. For instance, in the Dutch study of vegan families, findings indicate long-term consequences of early vitamin B12 deficiency on cognitive performance in adolescence [8]. In early childhood, these adolescents had significantly lower functioning in the areas of gross motor and language development compared to a control group [32]. In our study, we measured the short-term effects following six months supplementation. The time required for improvements to be detectable in the developing brain and possible to assess, may be domain specific [36]. Thus, although we did not find significant effects on communication skills, fine motor functioning and personal social abilities, an impact on these domains may require longer exposure time, or improvements may become apparent later in life. The interruption of myelination has been suggested as a possible mechanism linking vitamin B12 deficiency and adverse neurodevelopment [7]. Myelination facilitates communication in the brain and interruptions may influence the speed of conduction and thus the process of learning and acquisition of skills. Our result may indicate that the mechanisms of improved myelination give a more rapid effect in gross motor and problem-solving skills than for the other domains assessed. On the other hand, it has been argued that myelin repair is a slow process, and the improvements observed after short-term vitamin B12 supplement may be related to other effects such as increased energy-production in the central nervous system [37]. Thus, the improvements in the current study may be due to increased energy and attention, which are important factors for the performance both for gross motor abilities and problem-solving skills. Further research is required to achieve a deeper understanding of the specific consequences of vitamin B12 and folate deficiency on the developing brain.

Being a time of rapid growth, the period from gestation to 24 months of age is a sensitive period for brain development in which the brain is particularly susceptible to various exposures [33, 35, 38]. In support of this, there was a significant benefit of the vitamin B12 and folic acid supplementation in children who were  $\leq 18$  months at the start of supplementation and received all supplementation before they reach 24 months of age. Thus, our findings support that the timing of nutritional influence is of significance for the outcome of neurodevelopment.

The effect of vitamin B12 and folic acid supplementation on total ASQ-3 scores was most apparent in stunted children and in children with elevated tHcy. Childhood stunting is a proxy for several factors that are associated with poor neurodevelopment [39], consequently there might be a large potential for improvements among children who are stunted. The hypothesis of “functional isolation” where a malnourished child fails to elicit appropriate care and stimulation from the caregiver due to behavior symptoms such as irritability and apathy, may, in part, explain the effect in this subgroup [40]. Stunting could also reflect poor long-term vitamin B12 or folate intake, and the improved scores in children receiving vitamin B12 and folic acid may suggest that developmental delay in stunted children partly is related to low folate or vitamin B12 status. This possibility is supported by the finding that children with elevated levels of tHcy at baseline had a significant beneficial effect of vitamin B12 and folic acid supplementation on the total ASQ-3 score, while those with normal tHcy concentration did not. Stunting or elevated tHcy did not significantly modify the effect of the intervention on the total ASQ-3 score, but it should be noted that the trial was not powered to measure such interactions.

Our results give support to the hypothesis of the importance of vitamin B12 and folate for neurodevelopment and subsequent behavioral outcomes. Similarly, a recent report from the current study provides evidence for the importance of vitamin B12 for growth showing that poor vitamin B12 status in the children contributes to poor growth [41]. Clearly, there are several modifiable risk factors associated with adverse neurodevelopment other than vitamin B12 and folate deficiency [14]. There is sound evidence that adequate stimulation and responsive caregiving is crucial for healthy neurodevelopment as recently illustrated in a report from the

current study [42]. Furthermore, biological risk factors such as childhood infections and chronic undernutrition may lead to adverse neurodevelopment and unfulfilled developmental potential [14]. Further research is needed to understand the role of vitamin B12 and folate as one important factor for neurodevelopment in the complex interrelation between brain development and environmental influences.

### Strength and weaknesses

To our knowledge, this study is the first RCT that has measured the effects of vitamin B12 and/or folic acid on child development. This is a well-conducted RCT with few losses to follow-up and excellent adherence to the supplementation [15]. Despite an optimal randomization procedure involving more than 400 children, there were some baseline differences in family characteristics and physical growth between the groups. These represent a limitation to the study that may confound our effect estimates. We have repeated the analyses adjusting for these and other relevant baseline characteristics such as sex, age, breastfeeding status, height-for age and weight-for-height z-scores and log transformed family income. These adjustments resulted in minor alterations in the effect estimates and, hence, the level of significance. However, the main finding of a beneficial effect of vitamin B12/folic acid on gross motor and problem-solving skills remained significant (S1 and S2 Tables). If we had measured development at baseline in addition to at end study, we could have estimated the change in ASQ scores throughout the supplementation period and thereby adjusted for baseline differences in the ASQ scores. This was not possible due to the timing of the parent project and the time it took to prepare the instruments for the current sub-study. Despite the fact that each study group consisted of more than 100 children and that we carefully adjusted for baseline differences and other relevant variables, we cannot rule out residual confounding and our findings need to be confirmed before treatment recommendations can be made.

The ASQ-3 is a screening tool for the assessment of developmental delay constructed in the US with binary cut-offs, but has been used to measure developmental status on a continuous scale in several studies, as in the present study. It should be noted, that the questionnaires have not yet been validated for this purpose. We translated 11 ASQ-3 forms to Hindi particularly for this study, and the translated and adjusted ASQ-3 served as an easily administered and cost efficient assessment tool [24]. However, alpha values indicated questionable internal consistency in a few subscales and age categories. Poor internal consistency can be due to constant items (lack of variability of the responses) or random errors. In a randomized trial, random errors should be similar in the study groups and accordingly not result in biased effect estimates. More comprehensive assessment tools, such as the Bayley scales, or tools for social emotional functioning, could have added a broader picture of the children's skills and abilities. Finally, more advanced neuroimaging techniques might have identified unique changes to the developing brain in early childhood.

### Conclusion

In a RCT of supplementation with vitamin B12 and folic acid in six to 30 months old children we found beneficial effects on neurodevelopment as assessed by a screening tool in the domains of gross motor functioning and problem solving skills. These results need to be confirmed in other populations with more comprehensive assessment tools, and further research is recommended on the long-term effects of marginal vitamin B12 and folate status in the developing brain.

### Supporting Information

**S1 CONSORT Checklist.**  
(PDF)

**S1 Table. The effect of vitamin B12 and/or Folic acid on ASQ-3 total and subscale scores, adjusted for baseline characteristics.**

(DOCX)

**S2 Table. ORs (95% CIs) for being in the lower quartile of ASQ-3 total and subscale scores compared with placebo, adjusted for baseline characteristics.**

(DOCX)

**S1 Protocol. Study Protocol.**

(PDF)

**S2 Protocol. [ClinicalTrials.gov](#) Protocol registration receipt.**

(PDF)

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## Author Contributions

Conceived and designed the experiments: IK ST TK HR CSY NB TAS. Performed the experiments: ST TK. Analyzed the data: IK HR TAS. Contributed reagents/materials/analysis tools: CSY. Wrote the paper: IK MH HR TAS.

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RESEARCH ARTICLE

# Diarrhea, Stimulation and Growth Predict Neurodevelopment in Young North Indian Children

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## Abstract

### Background and Objective

Infants and young children in low to middle-income countries are at risk for adverse neurodevelopment due to multiple risk factors. In this study, we sought to identify stimulation and learning opportunities, growth, and burden of respiratory infections and diarrhea as predictors for neurodevelopment.

### Methods

We visited 422 North Indian children 6 to 30 months old weekly for six months. Childhood illnesses were assessed biweekly. At end study, we assessed neurodevelopment using the Ages and Stages Questionnaire 3<sup>rd</sup> ed. (ASQ-3) and gathered information on stimulation and learning opportunities. We identified predictors for ASQ-3 scores in multiple linear and logistic regression models.

### Results

We were able to explain 30.5% of the variation in the total ASQ-3 score by the identified predictors. When adjusting for child characteristics and annual family income, stimulation and learning opportunities explained most of the variation by 25.1%. Height for age (standardized beta: 0.12,  $p < .05$ ) and weight for height z-scores (std. beta: 0.09,  $p < .05$ ) were positively associated with the total ASQ-3 score, while number of days with diarrhea was negatively associated with these scores (std. beta: -0.13,  $p < 0.01$ ).

### Conclusion

Our results support the importance of early child stimulation and general nutrition for child development. Our study also suggests that diarrhea is an additional risk factor for adverse neurodevelopment in vulnerable children.



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## Introduction

There is sound evidence that deficient care and inadequate stimulation are key risk factors for adverse neurodevelopment in children [1–3]. Likewise, the evidence for poor growth and stunting as significant risk factors is convincing [4,5]. Pneumonia and diarrhea are important causes of morbidity and mortality in children in low to middle-income countries (LMIC) [6]. In some studies, diarrhea prevalence has predicted neurodevelopment [4,7–9], but a recent meta-analysis including these studies concluded that number of days with diarrhea did not predict neurodevelopment when taking stunting into account [10]. However, the very few studies included in this meta-analysis varied substantially in sample size, age of the participants, choice of cognitive measures and the quality of data on diarrhea.

In poor populations, risk factors co-occur giving rise to cumulative effects on neurodevelopment [2,5,11,12]. Complex relationships among these risk factors make it challenging to determine their independent contribution. In the present study, we have assessed developmental status and collected information on various risk and protective factors for adverse development such as socioeconomic status, child characteristics and stimulation and learning opportunities in a sample of 422 young North Indian children. The children participated in a six months study of folic acid and vitamin B12 supplementation on growth, diarrhea and other infections in New Delhi, India [13], and unique to this study is the thorough biweekly assessment of childhood illnesses such as acute lower respiratory infections (ALRI), pneumonia and diarrhea. The main aim of our study is to identify predictors for neurodevelopment in multiple regression models, and specifically to measure the extent to which diarrheal illness is associated with early child development.

## Materials and Methods

### Participants and study setting

The children ( $n = 422$ ) included in this study were part of a randomized, doubled blind, placebo controlled trial (RCT) ( $n = 1000$ ) on the effect of vitamin B12 and/or folic acid supplementation on childhood infections and growth in New Delhi, India (clinicaltrials.gov: NCT 00717730) [13]. Children aged 6 to 30 months were enrolled and randomized in blocks of 16, the last 440 randomized enrollments were requested to participate in the developmental assessment sub study. Of these, three children were not available for assessment and 15 did not wish to participate, hence the final number of participants was 422. The enrollment for this sample was from November 2010 through March 2011, and the developmental assessments were performed from May through September 2011. The study site was in the low and middle socioeconomic settings of Tigri and Dakshinpuri in New Delhi. These are typical urban neighborhoods with a total population of about 300,000. The ethics committees of the Society for Essential Health Action and Training (India), Society for Applied Studies (India), Christian Medical College (India), and the Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST) approved the study.

### Procedure

For enrollment, a door-to-door survey was conducted to identify households with eligible children. A physician and field supervisors screened the children and written informed consent was obtained from caregivers prior to enrollment. Availability of informed consent and no plans to move away over the next 6 months were considered for enrollment. We excluded children with severe acute malnutrition (weight for height z-scores less than -3), with severe anemia (hemoglobin <7 g/dL), and those who were using folic acid and/or vitamin B12

supplements. Information on child characteristics and socioeconomic status was collected at baseline. A team of field workers visited the children's household twice weekly for six months for close morbidity follow up. Weight and height was measured at baseline and at end study at the study clinic. At the end of six months follow up, developmental assessment was conducted and information on the child's stimulation and learning opportunities was collected at the study clinic. There were no additional exclusion criteria for the developmental assessment.

## Measurements

**Developmental assessment.** Neurodevelopmental status was measured by the Ages and Stages Questionnaire 3. ed. (ASQ-3), a comprehensive checklist, standardized for children 1–66 months with age-appropriate questionnaires [14]. The questionnaires contain 30 items that sums up to five subscales: Communication, Gross motor, Fine motor, Problem-solving and Personal-social (possible score range from 0 to 60), and a total score (possible score range from 0 to 300). Three field supervisors were trained to administer the ASQ-3 directly with the child at the research clinic in the presence of caregivers. The examiners elicited the relevant skills from the child during sessions using standardized materials. The caregiver served as an important contributor in supporting the child, eliciting behaviors and gave relevant information of the child's development when necessary. The three field supervisors were trained by the main author, a clinical child psychologist with experience in the assessment of infants and young children and in training of personnel.

All forms were translated to Hindi following official recommendations [15], and items not appropriate for the cultural setting were identified and slightly adjusted (for extensive information see [16]). During the 11 days of training, the field supervisors were standardized in performing the procedure, and they reached a high inter-observer agreement both during training and in the 10% quality control throughout the study. In the translated ASQ-3 version, the standardized alphas for the total ASQ-3 scores were strong, indicating an overall acceptable internal consistency [16].

**Stimulation and learning opportunities.** To assess the caregiver's promotion of child development we carefully selected two questions from the standardized assessment tool the Home Observation for Measurement of the Environment (HOME) [17]. One question was on "Mother's belief that child's behavior can be modified" and one was on "Mother's encouragement of developmental advances". These questions and other questions on the child's stimulation and learning environment, such as number of toys and books in the home, hours of play with other children and attendance to anganwadi centre (childcare) were asked the caregivers during the sessions.

**Childhood illnesses and growth.** At the biweekly field worker visits, mothers were asked about diarrheal illness, symptoms of respiratory infections and fever on any day since the last visit, and whether treatment had been sought for any illness. Respiratory rates were counted twice at each visit, temperature was measured and the child was examined for signs of dehydration if diarrhea or vomiting were present. Diarrhea was defined as the passage of  $\geq 3$  loose or watery stools in a 24-h period. ALRI was defined as cough or difficult breathing with elevated respiratory rate above the age-specific cut-off values ( $\geq 50$  breaths per min in infants and  $\geq 40$  breaths per min in older children) according to WHO-criteria, or cough or difficult breathing and lower chest indrawings. Clinical pneumonia was defined either by a combination of cough with crepitations or bronchial breathing by auscultations or as an episode of acute lower respiratory tract infection associated with at least one of the following features; lower chest indrawings, convulsions, not able to drink or feed, extreme lethargy, restlessness or irritability, nasal flaring or child is abnormally sleepy and difficult to wake up.

Anthropometry was assessed through weight and length measurements at baseline and end study at the study clinic. Weight was measured to the nearest 50 g using Digitron scales. Height was measured using locally manufactured infantometers reading to the nearest 0.1 cm.

## Data management and statistical analyses

The data was double entered by two data entry operators followed by validation. A total of 0.21% of the ASQ-3 responses were missing. For missing items an adjusted total score was computed by dividing the total subscale score by the number of completed items [18]. This number was then added depending on the amount of items missing. For each child, we summed up the item scores to five total subscale scores, and a total ASQ-3 score. We measured the association of relevant independent variables with the total ASQ-3 in multiple linear regression models. We selected the variables for the regression models as described elsewhere [19]. The variables that were included in the initial crude models were: number of family members, mother's age, mother's year of schooling, father's year of schooling, if family owns television or scooter or cooler, annual family income, joint versus nuclear family, attendance in anganwadi, number of toys in the family, family owns books, number of children in the family, hours of play with other children during the week, mothers belief that child's behavior can be modified, mothers encouragement of developmental advances, height for age z-scores (HAZ), weight for height z-scores (WHZ), number of days with diarrhea, incidents of clinical pneumonia and incidents of ALRI. Due to collinearity, weight for age z-scores were not included in the adjusted models. We confirmed this manual model by selecting variables in an automatic stepwise linear regression procedure. For the regression models the log-transformed values of annual family income and the log(base2) transformed values of days of diarrhea were used.

For the total ASQ-3 score, we present the selected variables in groups using a hierarchical (nested) regression approach [20]. The variable groups are: stimulation and learning opportunities (number of toys in the family, family owns books, hours of play with children during the week, mothers belief that child's behavior can be modified, mothers encouragement of developmental advances), growth (HAZ and WHZ) and childhood illnesses (number of days with diarrhea and incidence of clinical pneumonia). The variable groups were entered in the analysis in different steps constituting different models. For instance, stimulation and learning opportunities was entered in step 1 constituting model 1. In model 4 growth variables were added, and in model 7 the childhood illnesses variables were added to a full model. The remaining models (2, 3, 5 and 6) constitute different constellations of the variable groups alone and together. All regression models were adjusted for child characteristics (sex, age and breastfeeding status) and annual family income. The child characteristics variables were included regardless of their significance or influence on the other variables in the initial crude models.

The scores of the five subscales were highly skewed and categorized on the 25<sup>th</sup> percentile in the multiple logistic regression analysis. The selection of variables followed the same procedure as for main regression analysis. Only variables with  $P > 0.05$  are presented in the table. Data was analyzed in Stata version 12.

## Results

Of the 440 children three children were not available for assessment and 15 refused to participate. The final number of participants was 422.

### Demographic Characteristics

Demographic information of the children in the cohort is shown in [Table 1](#). There was an even distribution of girls and boys. Most of the children were breastfed (86.3% at baseline), 40.1%



**Table 1. Demographic and clinical characteristics of children in the cohort.**

	Baseline	N	Mean/%	SD
<b>Child characteristics</b>				
Total		422		
Age in month				
	12–23 months	259	61.3%	
	24–36 months	163	38.7%	
Sex				
	Girls	206	48.8%	
Breastfed		364	86.3%	
<b>Family situation</b>				
<i>Economy</i>				
	Annual income in INR <sup>1</sup> (median/range)		73000	12000–87000
	Families who own color TV or scooter or cooler, n (%)	377	89.3%	
<i>Maternal characteristics</i>				
	Age		25.7	5.5
	Years of schooling		7	6.3
	Mother's occupation, n (%) <sup>2</sup>			
	Governmental employee	1	0.2%	
	Non-governmental employee	8	1.9%	
	Self employed	7	1.7%	
	Daily wager, maid or un-employed	405	96%	
<i>Paternal characteristics</i>				
	Years of schooling		8.6	4
	Father's occupation, n (%)			
	Governmental employee	9	2.1%	
	Non-governmental employee	237	56.2%	
	Self employed	89	21.1%	
	Daily wager or un-employed	87	20.6%	
<i>Household characteristics</i>				
	Type of family			
	Nuclear, n (%)	228	54%	
	Joint, n (%)	194	46%	
	Number of children in the family		3	2.3
	Family size		5.8	2.6
<b>Stimulation and learning opportunities</b>				
	Hours of weekly play with other children		19	16.6
	Number of toys in the family			
	No toys	16	3.8%	
	Less than 5 toys	120	28.4%	
	5–10 toys	147	34.8%	
	More than 10 toys	139	32.9%	
	Families who owns books	253	60%	
	Attending Anganwadi center <sup>3</sup> , n (%)	40	9.5%	
<b>Anthropometry</b>				
	Z score height for age (stunted), < -2, n (%)	169	40.1%	
	Z score weight for length (wasted), < -2, n (%)	42	10%	
	Z score weight for age (underweight), < -2, n (%)	131	31%	
<b>Childhood illnesses from the biweekly home visits throughout the study period</b>				
	Number of days with diarrhea		6.6 days	7.1
	Incidents of Acute lower respiratory infection	159	37.7%	
	Incidents of Clinical Pneumonia	115	27.2%	

<sup>1</sup>Indian Rupees,

<sup>2</sup> One mother is deceased,

<sup>3</sup>Childcare center

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**Table 2. Hierarchical Regression Analysis for variables predicting total ASQ-3 scores in North Indian children 12–36 months<sup>1</sup>.**

VARIABLES	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
	Adjusted for Child Characteristics and annual family income <sup>2</sup>						
	B <sup>3</sup> (SE)	B (SE)	B (SE)	B (SE)	B (SE)	B (SE)	β <sup>4</sup>
<b>Stimulation and learning opportunities</b>							
Number of toys							
More than 10 toys	ref.			ref.	ref.		ref.
6–10 toys in the home	-4.4 (5.4)			-2.9 (5.3)	-2.2 (5.4)		-2.4 (5.2)
1–5 toys in the home	-5.8 (5.8)			-4.0 (5.8)	-2.8 (5.9)		-3.4 (5.7)
No toys in the home	-68.5*** (12.0)			-59.9*** (12.0)	-61.6*** (12.1)		-57.9*** (11.9)
Family own books (ref. No books)	4.9 (4.5)			5.7 (4.4)	5.7 (4.5)		7.0 (4.4)
Hours of weekly play with other children	0.6*** (0.1)			0.6*** (0.1)	0.6*** (0.1)		0.6*** (0.1)
Mother's belief that child's behavior can be modified (ref. No modification)	14.2** (5.0)			13.2** (4.9)	12.4* (4.9)		11.7* (4.9)
Mother's encouragement of developmental advances (ref. No encouragement)	18.0** (5.4)			17.7** (5.3)	17.0** (5.4)		17.2** (5.3)
<b>Growth</b>							
Height for age z-scores		7.8*** (2.2)		5.6* (2.0)		7.5** (2.1)	5.4* (2.0)
Weight for height z-scores		7.4** (2.6)		5.4* (2.4)		6.9** (2.6)	5.0* (2.4)
<b>Childhood Illnesses</b>							
Number of days with Diarrhea			-5.7** (1.8)		-5.1** (1.6)	-5.2** (1.8)	-5.0** (1.6)
Incidents of Clinical Pneumonia			-12.6* (5.3)		-8.9 (4.9)	-12.1* (5.2)	-9.4 (4.8)
Observations	421	421	421	421	421	421	421
<b>R-squared</b>	<b>0.251</b>	<b>0.104</b>	<b>0.086</b>	<b>0.279</b>	<b>0.284</b>	<b>0.137</b>	<b>0.305</b>

\*\*\* p<.001,

\*\* p<.01,

\* p<.05,

<sup>1</sup> For the 422 assessed children, the mean total ASQ-3 score was 231.9 (SD = 50) with scores ranging from a minimum of 30 to a maximum of 300.

<sup>2</sup> All models are adjusted for child characteristics (age, sex and breastfeeding status), and annual family income,

<sup>3</sup> unstandardized Beta coefficient,

<sup>4</sup> standardized regression coefficient, Beta values for model 7 only.

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were stunted (<-2 HAZ), 10% were wasted (<-2 WHZ) and 31% were underweight (<-2 WAZ). The average days of diarrhea during the study period, were 6.6 days (SD: 7.1), 14% of the children had no episodes of diarrhea, 53.3% had between 1–7 days and 32.7% had between 8–49 days with diarrhea during the 6 months period. At least one episode of ALRI was reported in 37.7%, and clinical pneumonia in 27.2% throughout the observation period.

### Predictors for developmental status

The predictors for the total ASQ-3 scores are shown in [Table 2](#). All models were adjusted for child characteristics and annual family income. These variables explained 4.7% of the variation in the total ASQ-3 score alone. In the full model (model 7), all variables together explained 30.5% of the variation.

**Stimulation and learning opportunities.** Stimulation and learning opportunities adjusted for child characteristics and annual family income, explained most of the variation in the total ASQ-3 scores alone by 25.1% (Table 2, model 1). When growth was added (Table 2, model 4), 27.9% of the variation was explained, while 28.4% was explained in the model including stimulation and learning opportunities and childhood illnesses (Table 2, model 5). Four variables of the stimulation and learning opportunities were significantly associated with the ASQ-3 score. Compared to those who had more than ten toys, those who had no toys in the home had substantially lower ASQ-3 scores ( $p < 0.001$  in all models). Number of hours of weekly play with other children ( $p < 0.001$  in all models), mother's belief that child's behavior can be modified ( $p < 0.01$  and  $p < 0.05$ ) and mother's encouragement of developmental advances ( $p < 0.01$  in all models) were all positively and significantly associated with the total ASQ-3 score.

**Growth.** Growth alone explained 10.4% of the variation in the total ASQ-3 score (Table 2, model 2), and when including childhood illnesses these explained 13.7% together (Table 2, model 6). HAZ and WHZ were positively and significantly associated with the total ASQ-3 score in all models ( $p < 0.001$ ,  $p < 0.01$ ,  $p < 0.05$ ), however the coefficients were attenuated when stimulation and learning opportunities was included. Fig. 1 shows the relationship between HAZ and the total ASQ-3 score in generalized additive models (GAM).

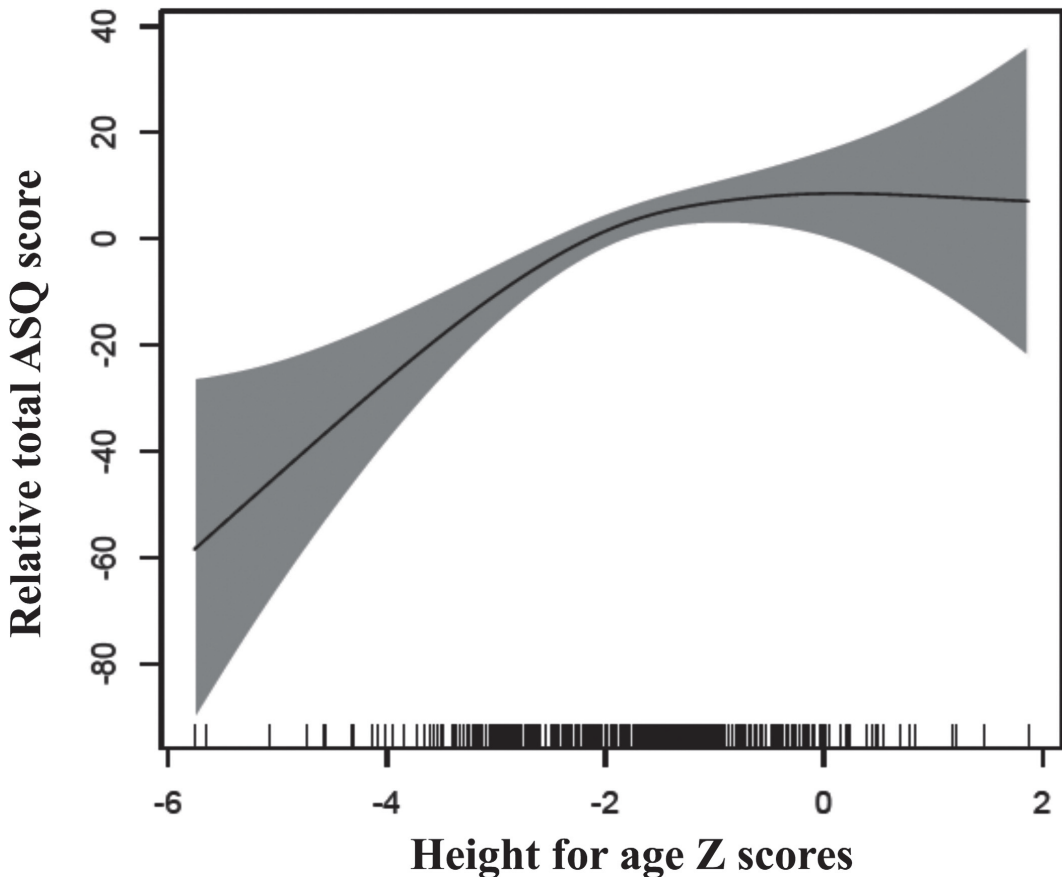
**Childhood illnesses.** The adjusted analysis of the childhood illnesses variables explained 8.6% of the variation in the total ASQ-3 score alone (Table 2, model 3). Number of days with diarrhea was negatively and significantly associated with the total ASQ-3 score ( $p < 0.01$  in all models). Fig. 2 shows the relationship between number of days with diarrhea and the total ASQ-3 score. Clinical pneumonia was significantly associated with the total ASQ-3 score in models where stimulation and learning opportunities was not present ( $p < 0.05$  in both models) (Table 2, model 3 and 6).

**Variables predicting the ASQ-3 subscales.** Table 3 shows the predictors for the ASQ-3 subscale scores from logistic regression models. Number of days with diarrhea was significantly associated with the Fine motor and Problem-solving subscales, and incidents of pneumonia with Communication and Fine motor subscales. HAZ was significantly associated with the Gross motor subscale only, while WHZ was significantly associated with the Communication subscale.

## Discussion

We were able to explain 30.6% of the variation in the total ASQ-3 score by the included predictors for neurodevelopment. Stimulation and learning opportunities was the variable group that explained most of the variation. Growth was also independently associated with developmental status. Furthermore, the variable days of diarrhea was an independent and consistent predictor for the ASQ-3 scores.

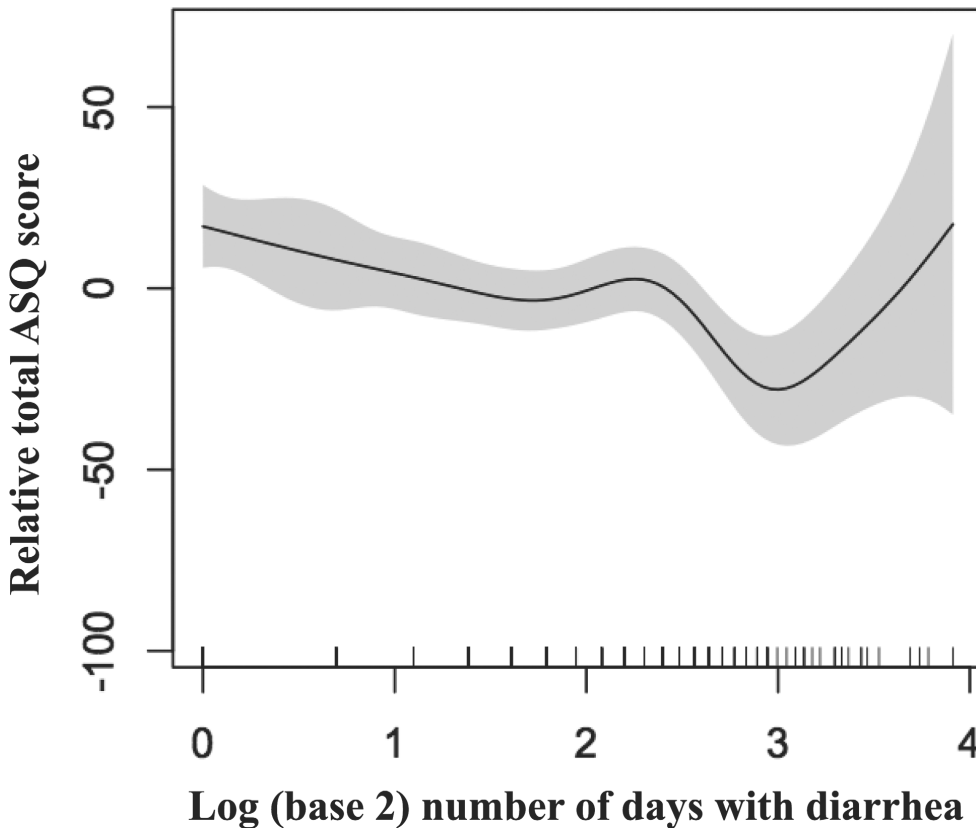
Factors in children's home environment, such as responsive caregiving and early learning opportunities are of indisputable importance for child development [2,21]. In our results this is clearly demonstrated by the variables on stimulation and learning opportunities explaining most of the variability of the neurodevelopmental scores alone. Stunting is another well-established risk factor for adverse neurodevelopmental outcomes [12]. This is supported in our study by the linear relationship between the ASQ-3 scores and HAZ-scores below -2, where the total ASQ-3 scores increase with increasing HAZ scores (Fig. 1). Furthermore, HAZ was associated with the total ASQ-3 scores with effect sizes ranging from 5.1 to 6.7 ASQ-3 points in all models. The effects of growth were seemingly stronger in models where stimulation and learning opportunities were not included.



**Fig 1. Associations between height for age z-scores and changes in ASQ-scores.** The graphs were constructed using generalized additive models in R, the solid line depicts the association of the ASQ-score and HAZ. The Y-axis is centered on the mean total ASQ-score. The shaded area spans the 95% confidence interval of this association.

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Each doubling of the number of days with diarrhea was associated with an average decrement of approximately five ASQ-3 points. The plots from the GAM revealed that this relation was linear (Fig. 2). Our results support previous findings, for example from a prospective cohort study in Brazil, reporting of associations between early childhood diarrhea and various developmental domains in later childhood [7–9]. These reports have been criticized, however, for not adequately adjusting for environmental and health related factors, as well as for their low sample size. Furthermore, it has been argued that stunting is a relevant cofounder in the association between diarrhea and cognitive development and that diarrhea morbidity only has an effect on the developing brain through stunting [4,6,10]. By demonstrating the significant association between diarrhea and neurodevelopment independent of growth, the present study improved upon previous findings. The assessment of illnesses was conducted biweekly for six



**Fig 2. Associations between log (base2) days of diarrhea and changes in ASQ-scores.** The graphs were constructed using generalized additive models in R, the solid line depicts the association of the total ASQ-score and log (base2) days of diarrhea. The Y-axis is centered on the mean total ASQ-score. The shaded area spans the 95% confidence interval of this association.

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months and we have information on several potential confounders, the results are thus based on a more extensive assessment than previous studies.

Analysis on the separate ASQ-3 subscales show that when adjusting for the other variables in the model, an increase in days of diarrhea was associated with an increased risk of being in the lower quartile in skills of fine motor and problem-solving abilities. Increasing HAZ was associated with a reduced risk of being in the lower quartile of the gross motor domain alone, while being wasted was associated with an increased risk of being in the lower quartile of communication skills. These differences may show that there are different pathways between those of diarrhea and growth and brain development, underscoring that the effect of diarrhea not only works through stunting. The independent association of diarrhea revealed in our results suggests that reducing diarrhea prevalence in children may be an important measure to enhance neurodevelopment.

**Table 3. Variables predicting ASQ-3 subscale score in North Indian children 12–36 months<sup>1</sup>.**

VARIABLES	Communication Odds Ratio (CI)	Gross Motor Odds Ratio (CI)	Fine Motor Odds Ratio (CI)	Problem Solving Odds Ratio (CI)	Personal Social Odds Ratio (CI)
<b>Child Characteristics and Socioeconomics status</b>					
Age in months	0.95** (0.92–1.00)				1.04** (1.01–1.08)
Mothers years of schooling		0.92** (0.88–0.97)			
Annual family income				0.63* (0.44–0.90)	0.69* (0.50–0.96)
<b>Stimulation and learning opportunities</b>					
Number of toys in the home					
More than 10 toys in the home	ref.	ref.	ref.	ref.	ref.
6–10 toys in the home					
1–5 toys in the home	1.91* (1.04–3.50)				
No toys in the home	11.04** (2.69–45.29)	3.88* (1.08–13.91)		4.31* (1.34–21.79)	
Family own books (ref. No books)					
Hours of weekly play with other children	0.98* (0.97–1.00)		0.98*** (0.96–0.99)	0.97*** (1.90–4.86)	0.98** (0.97–0.99)
Mother's belief that child's behavior can be modified (ref. No modification)			0.34*** (0.52–0.22)		
Mother's encouragement of developmental advances (ref. No encouragement)	0.58* (0.53–0.21)			0.33*** (0.53–0.21)	0.47** (0.99–0.93)
<b>Growth</b>					
Height for age z-scores		0.69 *** (0.56–0.84)			
Weight for height z-scores	0.67** (0.51–0.86)				
<b>Childhood Illnesses</b>					
Number of days with diarrhea			1.26** (1.07–1.49)	1.19* (1.01–1.40)	
Incidents of Clinical Pneumonia	1.85* (1.13–3.03)		1.63* (1.00–2.62)		

\*\*\* p<.001,

\*\* p<.01,

\* p<.05, logistic regression P-value,

<sup>1</sup> For the 422 assessed children, the mean subscales scores vary from 44.8 to 47.8, all with a range from 0 to 60.

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Various mechanisms may be involved in the impact of diarrhea diseases on brain development, such as for instance inflammation and/or reduced nutrient intake [22]. A possible indirect effect of childhood illnesses is the process of “functional isolation” where the child due to behavioral consequences of its condition face difficulties in eliciting appropriate caregiving behavior, and consequently fails to develop according to potential [23,24]. The hypothesis of “functional isolation” may in part explain why children burdened with pneumonia and/or diarrhea in our study have lower scores. Infected children may be weak, apathetic and irritable, and thus represent a challenge for the caregiver to provide proper responsive care.

Findings from a previous Peruvian study indicate that the various etiology of the diarrhea illness affect brain development differently, which also could explain why some studies find an association between diarrhea and neurodevelopment while other do not [4,22,25]. A limitation of our study is that the enrollment lasted for less than a year, and since it does not encompass all seasons, does not include all the variations of diarrhea illnesses. Thus, due to the lack of information on etiology in our study, our ability to demonstrate variations is limited. Developmental assessments were conducted immediately following the six months intensive follow-up,

and thus a second limitation of our study, is the lack of information on long-term effects of the risk- and protective factors.

The ASQ-3 has not been formally validated for a North Indian population. To our knowledge, there are currently no up to-date-tests for this age group formally validated for this setting. However, particular for this study, we translated and adjusted the relevant ASQ-3 forms for our age groups following official recommendations. This process and its evaluation have been described elsewhere [16]. The ASQ-3 has been used previously in a clinical setting in North India [26], as well as in research in LMIC [27], and its sensitivity and specificity have proven to be satisfying [28]. In the present study the ASQ-3 served as a feasible tool for the purpose of collecting reliable data on developmental status in our population. Both the total and subscale scores differentiated between variables, and several variables that predicted the total ASQ-3 score confirm previous findings in this field of research [2,3]. However, since the ASQ-3 is constructed as a screening test and not a diagnostic test, it is important to underscore that diagnosis of developmental delays requires a more sophisticated confirmatory test that was not performed here.

For vulnerable children in LMIC targeted interventions to improve neurodevelopment are called for [29]. Studies have demonstrated that interventions should include both factors of responsive caregiving and learning opportunities, and nutrition for the greatest impact on early child development [21,30]. Our study provides support for these results, and furthermore, that the continuing work to reduce the burden of diarrhea illness among vulnerable children may be an important step towards enabling children to fulfill their potential. In other words, the importance of reducing the burden of illnesses may not only be important for the reduction of childhood mortality, but also to enhance quality of life through improved brain development.

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## Author Contributions

Conceived and designed the experiments: IK ST TK NB TAS. Performed the experiments: ST TK NB. Analyzed the data: IK MH TAS. Contributed reagents/materials/analysis tools: ST TK NB TAS. Wrote the paper: IK ST TK MH NB TAS.

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**Errata for  
Biological risks and neurodevelopment in young  
North Indian children.**

*Results from a randomized controlled trial on vitamin B12 and  
folic acid*

**Ingrid Kvestad**



Avhandling for graden philosophiae doctor (ph.d.)  
ved Universitetet i Bergen

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september 15, 2015

## Errata

Paper II:

Table 2 in Paper II contains two mistakes in the  $\beta$  column. The  $\beta$ -values for *The Mothers belief that child`s behavior can be modified (ref. No modification)*, and *Mothers encouragement of developmental advances (ref. No encouragement)* should be positive and not negative as shown in the paper, and thus changed from -0.11 to 0.11 and -0.16 to 0.16 respectively.

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	Hølgersen, Helge, PhD	Mellom oss - Essay i relasjonell psykoanalyse.
<b>2005</b>		
V	Hetland, Hilde, Dr. psychol.	Leading to the extraordinary? Antecedents and outcomes of transformational leadership.
	Iversen, Anette Christine, Dr. philos.	Social differences in health behaviour: the motivational role of perceived control and coping.
<b>2005</b>	Mathisen, Gro Ellen, PhD	Climates for creativity and innovation: Definitions, measurement, predictors and consequences.
H		
	Sævi, Tone, Dr. philos.	Seeing disability pedagogically – The lived experience of disability in the pedagogical encounter.
	Wiium, Nora, PhD	Intrapersonal factors, family and school norms: combined and interactive influence on adolescent smoking behaviour.
	Kanagaratnam, Pushpa, PhD	Subjective and objective correlates of Posttraumatic Stress in immigrants/refugees exposed to political violence.
	Larsen, Torill M. B. , PhD	Evaluating principals` and teachers` implementation of Second Step. A case study of four Norwegian primary schools.

	Bancila, Delia, PhD	Psychosocial stress and distress among Romanian adolescents and adults.
<b>2006</b>		
V	Hillestad, Torgeir Martin, Dr. philos.	Normalitet og avvik. Forutsetninger for et objektivt psykopatologisk avviksbegrep. En psykologisk, sosial, erkjennelsesteoretisk og teorihistorisk framstilling.
	Nordanger, Dag Øystein, Dr. psychol.	Psychosocial discourses and responses to political violence in post-war Tigray, Ethiopia.
	Rimol, Lars Morten, PhD	Behavioral and fMRI studies of auditory laterality and speech sound processing.
	Krumsvik, Rune Johan, Dr. philos.	ICT in the school. ICT-initiated school development in lower secondary school.
	Norman, Elisabeth, Dr. psychol.	Gut feelings and unconscious thought: An exploration of fringe consciousness in implicit cognition.
	Israel, K Pravin, Dr. psychol.	Parent involvement in the mental health care of children and adolescents. Empirical studies from clinical care setting.
	Glasø, Lars, PhD	Affects and emotional regulation in leader-subordinate relationships.
	Knutsen, Ketil, Dr. philos.	HISTORIER UNGDOM LEVER – En studie av hvordan ungdommer bruker historie for å gjøre livet meningsfullt.
	Matthiesen, Stig Berge, PhD	Bullying at work. Antecedents and outcomes.
<b>2006</b>		
H	Gramstad, Arne, PhD	Neuropsychological assessment of cognitive and emotional functioning in patients with epilepsy.
	Bendixen, Mons, PhD	Antisocial behaviour in early adolescence: Methodological and substantive issues.
	Mrumbi, Khalifa Maulid, PhD	Parental illness and loss to HIV/AIDS as experienced by AIDS orphans aged between 12-17 years from Temeke District, Dar es Salaam, Tanzania: A study of the children's psychosocial health and coping responses.
	Hetland, Jørn, Dr. psychol.	The nature of subjective health complaints in adolescence: Dimensionality, stability, and psychosocial predictors
	Kakoko, Deodatus Conatus Vitalis, PhD	Voluntary HIV counselling and testing service uptake among primary school teachers in Mwanza, Tanzania: assessment of socio-demographic, psychosocial and socio-cognitive aspects
	Mykletun, Arnstein, Dr. psychol.	Mortality and work-related disability as long-term consequences of anxiety and depression: Historical cohort designs based on the HUNT-2 study
	Sivertsen, Børge, PhD	Insomnia in older adults. Consequences, assessment and treatment.
<b>2007</b>		
V	Singhammer, John, Dr. philos.	Social conditions from before birth to early adulthood – the influence on health and health behaviour

	Janvin, Carmen Ani Cristea, PhD	Cognitive impairment in patients with Parkinson's disease: profiles and implications for prognosis
	Braarud, Hanne Cecilie, Dr.psychol.	Infant regulation of distress: A longitudinal study of transactions between mothers and infants
	Tveito, Torill Helene, PhD	Sick Leave and Subjective Health Complaints
	Magnussen, Liv Heide, PhD	Returning disability pensioners with back pain to work
	Thuen, Elin Marie, Dr.philos.	Learning environment, students' coping styles and emotional and behavioural problems. A study of Norwegian secondary school students.
	Solberg, Ole Asbjørn, PhD	Peacekeeping warriors – A longitudinal study of Norwegian peacekeepers in Kosovo
<b>2007</b>		
<b>H</b>	Søreide, Gunn Elisabeth, Dr.philos.	Narrative construction of teacher identity
	Svensen, Erling, PhD	WORK & HEALTH. Cognitive Activation Theory of Stress applied in an organisational setting.
	Øverland, Simon Nygaard, PhD	Mental health and impairment in disability benefits. Studies applying linkages between health surveys and administrative registries.
	Eichele, Tom, PhD	Electrophysiological and Hemodynamic Correlates of Expectancy in Target Processing
	Børhaug, Kjetil, Dr.philos.	Oppseding til demokrati. Ein studie av politisk oppseding i norsk skule.
	Eikeland, Thorleif, Dr.philos.	Om å vokse opp på barnehjem og på sykehus. En undersøkelse av barnehjemsbarns opplevelser på barnehjem sammenholdt med sanatoriebarne beskrivelse av langvarige sykehusopphold – og et forsøk på forklaring.
	Wadel, Carl Cato, Dr.philos.	Medarbeidersamhandling og medarbeiderledelse i en lagbasert organisasjon
	Vinje, Hege Forbech, PhD	Thriving despite adversity: Job engagement and self-care among community nurses
	Noort, Maurits van den, PhD	Working memory capacity and foreign language acquisition
<b>2008</b>		
<b>V</b>	Brevik, Kyrre, Dr.psychol.	The Adjustment of Children and Adolescents in Different Post-Divorce Family Structures. A Norwegian Study of Risks and Mechanisms.
	Johnsen, Grethe E., PhD	Memory impairment in patients with posttraumatic stress disorder
	Sætrevik, Bjørn, PhD	Cognitive Control in Auditory Processing
	Carvalho, Susana Fonseca, PhD	Prevention of bullying in schools: an ecological model

**2008****H**

- Brønnick, Kolbjørn Selvåg  
Attentional dysfunction in dementia associated with Parkinson's disease.
- Posserud, Maj-Britt Rocio  
Epidemiology of autism spectrum disorders
- Haug, Ellen  
Multilevel correlates of physical activity in the school setting
- Skjerve, Arvid  
Assessing mild dementia – a study of brief cognitive tests.
- Kjønniksen, Lise  
The association between adolescent experiences in physical activity and leisure time physical activity in adulthood: a ten year longitudinal study
- Gundersen, Hilde  
The effects of alcohol and expectancy on brain function
- Omvik, Siri  
Insomnia – a night and day problem

**2009****V**

- Molde, Helge  
Pathological gambling: prevalence, mechanisms and treatment outcome.
- Foss, Else  
Den omsorgsfulle væremåte. En studie av voksnes væremåte i forhold til barn i barnehagen.
- Westrheim, Kariane  
Education in a Political Context: A study of Knowledge Processes and Learning Sites in the PKK.
- Wehling, Eike  
Cognitive and olfactory changes in aging
- Wangberg, Silje C.  
Internet based interventions to support health behaviours: The role of self-efficacy.
- Nielsen, Morten B.  
Methodological issues in research on workplace bullying. Operationalisations, measurements and samples.
- Sandu, Anca Larisa  
MRI measures of brain volume and cortical complexity in clinical groups and during development.
- Guribye, Eugene  
Refugees and mental health interventions
- Sørensen, Lin  
Emotional problems in inattentive children – effects on cognitive control functions.
- Tjomsland, Hege E.  
Health promotion with teachers. Evaluation of the Norwegian Network of Health Promoting Schools: Quantitative and qualitative analyses of predisposing, reinforcing and enabling conditions related to teacher participation and program sustainability.
- Helleve, Ingrid  
Productive interactions in ICT supported communities of learners

**2009****H**

- Skorpen, Aina  
Øye, Christine  
Dagliglivet i en psykiatrisk institusjon: En analyse av miljøterapeutiske praksiser
- Andreassen, Cecilie Schou  
WORKAHOLISM – Antecedents and Outcomes
- Stang, Ingun  
Being in the same boat: An empowerment intervention in breast cancer self-help groups

	Sequeira, Sarah Dorothee Dos Santos	The effects of background noise on asymmetrical speech perception
	Kleiven, Jo, dr.philos.	The Lillehammer scales: Measuring common motives for vacation and leisure behavior
	Jónsdóttir, Guðrún	Dubito ergo sum? Ni jenter møter naturfaglig kunnskap.
	Hove, Oddbjørn	Mental health disorders in adults with intellectual disabilities - Methods of assessment and prevalence of mental health disorders and problem behaviour
	Wageningen, Heidi Karin van	The role of glutamate on brain function
	Bjørkvik, Jofrid	God nok? Selvaktelse og interpersonlig fungering hos pasienter innen psykisk helsevern: Forholdet til diagnoser, symptomer og behandlingsutbytte
	Andersson, Martin	A study of attention control in children and elderly using a forced-attention dichotic listening paradigm
	Almás, Aslaug Grov	Teachers in the Digital Network Society: Visions and Realities. A study of teachers' experiences with the use of ICT in teaching and learning.
	Ulvik, Marit	Lærerutdanning som danning? Tre stemmer i diskusjonen
<b>2010</b>		
<b>V</b>	Skår, Randi	Læringsprosesser i sykepleieres profesjonsutøvelse. En studie av sykepleieres læringserfaringer.
	Roald, Knut	Kvalitetsvurdering som organisasjonslæring mellom skole og skoleeigar
	Lunde, Linn-Heidi	Chronic pain in older adults. Consequences, assessment and treatment.
	Danielsen, Anne Grete	Perceived psychosocial support, students' self-reported academic initiative and perceived life satisfaction
	Hysing, Mari	Mental health in children with chronic illness
	Olsen, Olav Kjellevoid	Are good leaders moral leaders? The relationship between effective military operational leadership and morals
	Riese, Hanne	Friendship and learning. Entrepreneurship education through mini-enterprises.
	Holthe, Asle	Evaluating the implementation of the Norwegian guidelines for healthy school meals: A case study involving three secondary schools
<b>H</b>	Hauge, Lars Johan	Environmental antecedents of workplace bullying: A multi-design approach
	Bjørkelo, Brita	Whistleblowing at work: Antecedents and consequences
	Reme, Silje Endresen	Common Complaints – Common Cure? Psychiatric comorbidity and predictors of treatment outcome in low back pain and irritable bowel syndrome

Helland, Wenche Andersen	Communication difficulties in children identified with psychiatric problems
Beneventi, Harald	Neuronal correlates of working memory in dyslexia
Thygesen, Elin	Subjective health and coping in care-dependent old persons living at home
Aanes, Mette Marthinussen	Poor social relationships as a threat to belongingness needs. Interpersonal stress and subjective health complaints: Mediating and moderating factors.
Anker, Morten Gustav	Client directed outcome informed couple therapy
Bull, Torill	Combining employment and child care: The subjective well-being of single women in Scandinavia and in Southern Europe
Viig, Nina Grieg	Tilrettelegging for læreres deltakelse i helsefremmende arbeid. En kvalitativ og kvantitativ analyse av sammenhengen mellom organisatoriske forhold og læreres deltakelse i utvikling og implementering av Europeisk Nettverk av Helsefremmende Skoler i Norge
Wolff, Katharina	To know or not to know? Attitudes towards receiving genetic information among patients and the general public.
Ogden, Terje, dr.philos.	Familiebasert behandling av alvorlige atferdsproblemer blant barn og ungdom. Evaluering og implementering av evidensbaserte behandlingsprogrammer i Norge.
Solberg, Mona Elin	Self-reported bullying and victimisation at school: Prevalence, overlap and psychosocial adjustment.
<b>2011</b>	
<b>V</b>	
Bye, Hege Høivik	Self-presentation in job interviews. Individual and cultural differences in applicant self-presentation during job interviews and hiring managers' evaluation
Notelaers, Guy	Workplace bullying. A risk control perspective.
Moltu, Christian	Being a therapist in difficult therapeutic impasses. A hermeneutic phenomenological analysis of skilled psychotherapists' experiences, needs, and strategies in difficult therapies ending well.
Myrseth, Helga	Pathological Gambling - Treatment and Personality Factors
Schanche, Elisabeth	From self-criticism to self-compassion. An empirical investigation of hypothesized change processes in the Affect Phobia Treatment Model of short-term dynamic psychotherapy for patients with Cluster C personality disorders.
Våpenstad, Eystein Victor, dr.philos.	Det tempererte nærvær. En teoretisk undersøkelse av psykoterautens subjektivitet i psykoanalyse og psykoanalytisk psykoterapi.
Haukebø, Kristin	Cognitive, behavioral and neural correlates of dental and intra-oral injection phobia. Results from one treatment and one fMRI study of randomized, controlled design.

	Harris, Anette	Adaptation and health in extreme and isolated environments. From 78°N to 75°S.
	Bjørknes, Ragnhild	Parent Management Training-Oregon Model: intervention effects on maternal practice and child behavior in ethnic minority families
	Mamen, Asgeir	Aspects of using physical training in patients with substance dependence and additional mental distress
	Espevik, Roar	Expert teams: Do shared mental models of team members make a difference
	Haara, Frode Olav	Unveiling teachers' reasons for choosing practical activities in mathematics teaching
<b>2011</b>		
<b>H</b>	Hauge, Hans Abraham	How can employee empowerment be made conducive to both employee health and organisation performance? An empirical investigation of a tailor-made approach to organisation learning in a municipal public service organisation.
	Melkevik, Ole Rogstad	Screen-based sedentary behaviours: pastimes for the poor, inactive and overweight? A cross-national survey of children and adolescents in 39 countries.
	Vøllestad, Jon	Mindfulness-based treatment for anxiety disorders. A quantitative review of the evidence, results from a randomized controlled trial, and a qualitative exploration of patient experiences.
	Tolo, Astrid	Hvordan blir lærerkompetanse konstruert? En kvalitativ studie av PPU-studenters kunnskapsutvikling.
	Saus, Evelyn-Rose	Training effectiveness: Situation awareness training in simulators
	Nordgreen, Tine	Internet-based self-help for social anxiety disorder and panic disorder. Factors associated with effect and use of self-help.
	Munkvold, Linda Helen	Oppositional Defiant Disorder: Informant discrepancies, gender differences, co-occurring mental health problems and neurocognitive function.
	Christiansen, Øivin	Når barn plasseres utenfor hjemmet: beslutninger, forløp og relasjoner. Under barnevernets (ved)tak.
	Brunborg, Geir Scott	Conditionability and Reinforcement Sensitivity in Gambling Behaviour
	Hystad, Sigurd William	Measuring Psychological Resiliency: Validation of an Adapted Norwegian Hardiness Scale
<b>2012</b>		
<b>V</b>	Roness, Dag	Hvorfor bli lærer? Motivasjon for utdanning og utøving.
	Fjermestad, Krister Westlye	The therapeutic alliance in cognitive behavioural therapy for youth anxiety disorders
	Jenssen, Eirik Sørnes	Tilpasset opplæring i norsk skole: politikeres, skolelederes og læreres handlingsvalg



	Saksvik-Lehouillier, Ingvild	Shift work tolerance and adaptation to shift work among offshore workers and nurses
	Johansen, Venke Frederike	Når det intime blir offentlig. Om kvinners åpenhet om brystkreft og om markedsføring av brystkreftsaken.
	Herheim, Rune	Pupils collaborating in pairs at a computer in mathematics learning: investigating verbal communication patterns and qualities
	Vie, Tina Løkke	Cognitive appraisal, emotions and subjective health complaints among victims of workplace bullying: A stress-theoretical approach
	Jones, Lise Øen	Effects of reading skills, spelling skills and accompanying efficacy beliefs on participation in education. A study in Norwegian prisons.
<b>2012</b>		
<b>H</b>	Danielsen, Yngvild Sørebo	Childhood obesity – characteristics and treatment. Psychological perspectives.
	Horverak, Jøri Gytre	Sense or sensibility in hiring processes. Interviewee and interviewer characteristics as antecedents of immigrant applicants' employment probabilities. An experimental approach.
	Jøsendal, Ola	Development and evaluation of BE smokeFREE, a school-based smoking prevention program
	Osnes, Berge	Temporal and Posterior Frontal Involvement in Auditory Speech Perception
	Drageset, Sigrunn	Psychological distress, coping and social support in the diagnostic and preoperative phase of breast cancer
	Aasland, Merethe Schanke	Destructive leadership: Conceptualization, measurement, prevalence and outcomes
	Bakibinga, Pauline	The experience of job engagement and self-care among Ugandan nurses and midwives
	Skogen, Jens Christoffer	Foetal and early origins of old age health. Linkage between birth records and the old age cohort of the Hordaland Health Study (HUSK)
	Leveresen, Ingrid	Adolescents' leisure activity participation and their life satisfaction: The role of demographic characteristics and psychological processes
	Hanss, Daniel	Explaining sustainable consumption: Findings from cross-sectional and intervention approaches
	Rød, Per Arne	Barn i klem mellom foreldrekonflikter og samfunnsmessig beskyttelse
<b>2013</b>		
<b>V</b>	Mentzoni, Rune Aune	Structural Characteristics in Gambling
	Knudsen, Ann Kristin	Long-term sickness absence and disability pension award as consequences of common mental disorders. Epidemiological studies using a population-based health survey and official ill health benefit registries.

	Strand, Mari	Emotional information processing in recurrent MDD
	Veseth, Marius	Recovery in bipolar disorder. A reflexive-collaborative exploration of the lived experiences of healing and growth when battling a severe mental illness
	Mæland, Silje	Sick leave for patients with severe subjective health complaints. Challenges in general practice.
	Mjaaland, Thera	At the frontiers of change? Women and girls' pursuit of education in north-western Tigray, Ethiopia
	Odéen, Magnus	Coping at work. The role of knowledge and coping expectancies in health and sick leave.
	Hynninen, Kia Minna Johanna	Anxiety, depression and sleep disturbance in chronic obstructive pulmonary disease (COPD). Associations, prevalence and effect of psychological treatment.
	Flo, Elisabeth	Sleep and health in shift working nurses
	Aasen, Elin Margrethe	From paternalism to patient participation? The older patients undergoing hemodialysis, their next of kin and the nurses: a discursive perspective on perception of patient participation in dialysis units
	Ekorås, Belinda	Emotional and Behavioural Problems in Children: Self-perception, peer relationships, and motor abilities
	Corbin, J. Hope	North-South Partnerships for Health: Key Factors for Partnership Success from the Perspective of the KIWAKKUKI
	Birkeland, Marianne Skogbrott	Development of global self-esteem: The transition from adolescence to adulthood
<b>2013</b>	Gianella-Malca, Camila	Challenges in Implementing the Colombian Constitutional Court's Health-Care System Ruling of 2008
<b>H</b>	Hovland, Anders	Panic disorder – Treatment outcomes and psychophysiological concomitants
	Mortensen, Øystein	The transition to parenthood – Couple relationships put to the test
	Årdal, Guro	Major Depressive Disorder – a Ten Year Follow-up Study. Inhibition, Information Processing and Health Related Quality of Life
	Johansen, Rino Bandlitz	The impact of military identity on performance in the Norwegian armed forces
	Bøe, Tormod	Socioeconomic Status and Mental Health in Children and Adolescents
<b>2014</b>	Nordmo, Ivar	Gjennom nåløyet – studenters læringserfaringer i psykologutdanningen
<b>V</b>	Dovran, Anders	Childhood Trauma and Mental Health Problems in Adult Life
	Hegelstad, Wenche ten Velden	Early Detection and Intervention in Psychosis: A Long-Term Perspective

	Urheim, Ragnar	Forståelse av pasientaggresjon og forklaringer på nedgang i voldsrate ved Regional sikkerhetsavdeling, Sandviken sykehus
	Kinn, Liv Grethe	Round-Trips to Work. Qualitative studies of how persons with severe mental illness experience work integration.
	Rød, Anne Marie Kinn	Consequences of social defeat stress for behaviour and sleep. Short-term and long-term assessments in rats.
	Nygård, Merethe	Schizophrenia – Cognitive Function, Brain Abnormalities, and Cannabis Use
	Tjora, Tore	Smoking from adolescence through adulthood: the role of family, friends, depression and socioeconomic status. Predictors of smoking from age 13 to 30 in the "The Norwegian Longitudinal Health Behaviour Study" (NLHB)
	Vangsnes, Vigdis	The Dramaturgy and Didactics of Computer Gaming. A Study of a Medium in the Educational Context of Kindergartens.
	Nordahl, Kristin Berg	Early Father-Child Interaction in a Father-Friendly Context: Gender Differences, Child Outcomes, and Protective Factors related to Fathers' Parenting Behaviors with One-year-olds
<b>2014</b>	Sandvik, Asle Makoto	Psychopathy – the heterogeneity of the construct
<b>H</b>	Skotheim, Siv	Maternal emotional distress and early mother-infant interaction: Psychological, social and nutritional contributions
	Halleland, Helene Barone	Executive Functioning in adult Attention Deficit Hyperactivity Disorder (ADHD). From basic mechanisms to functional outcome.
	Halvorsen, Kirsti Vindal	Partnerskap i lærerutdanning, sett fra et økologisk perspektiv
	Solbue, Vibeke	Dialogen som visker ut kategorier. En studie av hvilke erfaringer innvandrerdømmer og norskfødte med innvandrereforeldre har med videregående skole. Hva forteller ungdommenes erfaringer om videregående skoles håndtering av etniske ulikheter?
	Kvalevaag, Anne Lise	Fathers' mental health and child development. The predictive value of fathers' psychological distress during pregnancy for the social, emotional and behavioural development of their children
	Sandal, Ann Karin	Ungdom og utdanningsval. Om elever sine opplevingar av val og overgangsprosessar.
	Haug, Thomas	Predictors and moderators of treatment outcome from high- and low-intensity cognitive behavioral therapy for anxiety disorders. Association between patient and process factors, and the outcome from guided self-help, stepped care, and face-to-face cognitive behavioral therapy.
	Sjølie, Hege	Experiences of Members of a Crisis Resolution Home Treatment Team. Personal history, professional role and emotional support in a CRHT team.

	Falkenberg, Liv Eggset	Neuronal underpinnings of healthy and dysfunctional cognitive control
	Mrdalj, Jelena	The early life condition. Importance for sleep, circadian rhythmicity, behaviour and response to later life challenges
	Hesjedal, Elisabeth	Tverrprofesjonelt samarbeid mellom skule og barnevern: Kva kan støtte utsette barn og unge?
<b>2015 V</b>	Hauken, May Aasebø	« <i>The cancer treatment was only half the work!</i> » A Mixed-Method Study of Rehabilitation among Young Adult Cancer Survivors
	Ryland, Hilde Katrin	Social functioning and mental health in children: the influence of chronic illness and intellectual function
	Rønsen, Anne Kristin	Vurdering som profesjonskompetanse. Refleksjonsbasert utvikling av læreres kompetanse i formativ vurdering
	Hoff, Helge Andreas	Thinking about Symptoms of Psychopathy in Norway: Content Validation of the Comprehensive Assessment of Psychopathic Personality (CAPP) Model in a Norwegian Setting
	Schmid, Marit Therese	Executive Functioning in recurrent- and first episode Major Depressive Disorder. Longitudinal studies
	Sand, Liv	Body Image Distortion and Eating Disturbances in Children and Adolescents
	Matanda, Dennis Juma	Child physical growth and care practices in Kenya: Evidence from Demographic and Health Surveys
	Amugsi, Dickson Abanimi	Child care practices, resources for care, and nutritional outcomes in Ghana: Findings from Demographic and Health Surveys
	Jakobsen, Hilde	The good beating: Social norms supporting men's partner violence in Tanzania
	Sagoe, Dominic	Nonmedical anabolic-androgenic steroid use: Prevalence, attitudes, and social perception
	Eide, Helene Marie Kjærgård	Narrating the relationship between leadership and learning outcomes. A study of public narratives in the Norwegian educational sector.
<b>2015 H</b>	Wubs, Annegreet Gera	Intimate partner violence among adolescents in South Africa and Tanzania
	Hjelmervik, Helene Susanne	Sex and sex-hormonal effects on brain organization of fronto-parietal networks
	Dahl, Berit Misund	The meaning of professional identity in public health nursing
	Røykenes, Kari	Testangst hos sykepleierstudenter: «Alternativ behandling»
	Bless, Josef Johann	The smartphone as a research tool in psychology. Assessment of language lateralization and training of auditory attention.

Løvvik, Camilla Margrethe  
Sigvaldsen

Common mental disorders and work participation – the  
role of return-to-work expectations

Lehmann, Stine

Mental Disorders in Foster Children: A Study of  
Prevalence, Comorbidity, and Risk Factors

Knapstad, Marit

Psychological factors in long-term sickness absence: the  
role of shame and social support. Epidemiological  
studies based on the Health Assets Project.

