# Micronutrients, omega-3 fatty acids and cognitive performance in Indian schoolchildren

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**Thesis**

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## Abstract

In developing countries, approximately 30-40% of school-age children suffer from iodine and iron deficiencies. Poverty and consumption of monotonous diets are underlying causes of inadequate intakes of micronutrients and omega-3 fatty acids and may have severe consequences for children's cognitive development. Multiple micronutrient interventions have shown to benefit mental performance of children, but a systematic evaluation of the evidence is currently lacking. The omega-3 fatty acid, $\alpha$-linolenic acid (ALA) is converted into docosahexaenoic acid (DHA), a major structural component of the brain, which is important for normal development and maintenance of brain function. At present, it is unclear whether additional intake of omega-3 fatty acids improves cognitive performance in children.

The aim of this thesis was to investigate the role of multiple micronutrients and omega-3 fatty acids on cognitive performance in school-age children living in deprived environments, thereby addressing three main research questions.

The first query concerned the investigation of the size of effects of multiple micronutrient interventions on different cognitive domains. Findings of our meta-analysis comprising 17 studies in children 5-16 years of age, suggested that multiple micronutrients were beneficial for fluid intelligence (i.e. reasoning abilities) (0.14 SD; 95% CI: -0.02, 0.29) and academic performance (0.30 SD; 95% CI: 0.01, 0.58). Crystallized intelligence (i.e. acquired knowledge) seemed not affected (-0.03 SD; 95% CI: -0.21, 0.15) and for the other cognitive domains data were too limited to draw firm conclusions.

Secondly, we examined the role of omega-3 fatty acids on children's cognitive development, for which a literature review was conducted. Associations between omega-3 fatty acid status or dietary intake and cognitive performance were investigated by cross-sectional analysis using baseline data of a randomized controlled trial in 598 Indian schoolchildren (see below for details). We found no evidence for a beneficial effect of additional intake of omega-3 fatty acids, and of DHA in particular, on cognitive development in school-age children. Neither there was a significant relationship between omega-3 fatty acid status and cognitive performance.

Lastly, we studied the effect of different doses of micronutrients and omega-3 fatty acids, and their interaction, on cognitive performance. For that purpose, a randomized controlled trial in 598 Indian schoolchildren aged 6-10 years was conducted from November 2005 until March 2007. Children received either 15% or 100% of the Recommended Dietary Allowance of micronutrients in combination with either a low (140 mg ALA) or high dose (900 mg ALA plus 100 mg DHA) of omega-3 fatty acids for 12 months. Cognitive function was measured at baseline, 6 and 12 months. Our results showed that with some small differential effects on short term memory at 6 months (0.11 SD; 95% CI: 0.01-0.20) and fluid intelligence at 6 months (-0.10 SD; 95% CI: -0.17, -0.03) and 12 months (-0.12 SD; 95%

CI: -0.20, -0.04), the high and low dose of micronutrients were as effective for improving retrieval ability, cognitive speediness and overall cognitive performance. Neither there were differences between the omega-3 fatty acid treatments, nor an interaction between micronutrients and omega-3 fatty acids on cognitive outcomes.

In conclusion, although multiple micronutrients may benefit intellectual performance of schoolchildren, development of public health guidelines is currently premature. Further investigation on doses and composition of micronutrients would be needed to identify a cost-effective micronutrient supplement to optimize cognitive performance in children. Presently, no evidence exists for a positive effect of omega-3 fatty acids on cognitive performance in healthy children. A final trial using a higher dose and sufficiently long duration would be needed to conclude whether omega-3 fatty acid supplementation improves mental development at school age.

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# Chapter 1

## General Introduction

Today, more than 2 billion people world wide suffer from micronutrient deficiencies, of which deficiencies of iron, iodine, vitamin A and folate are most common (1). In school-age children living in developing countries, the prevalence rates of iron deficiency and inadequate iodine nutrition are estimated to be 40% (1) and 32% (2), respectively. Poverty and monotonous diets low in energy, protein, fat and micronutrients are the underlying causes of nutritional deficiencies, which may have severe consequences for physical growth, immune function and cognitive development of children (2).

During the International Conference on Nutrition (ICN) organized by the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) of the United Nations in 1992, 159 countries declared to make all efforts to improve the nutritional situation of their populations and eliminate micronutrient deficiencies. This message was reinforced in one of the Millennium Development Goals in 2000. Despite all efforts, the problem of undernutrition and related effects on development and health of children still exists. Recently, a series of three papers, have been published in The Lancet to raise awareness that more than 200 million of children under five years of age are not reaching their full developmental potential because of poverty, malnutrition, poor health and unstimulating home environments (3-5). Improving the intake of iron and iodine has been shown to be an effective strategy to enhance cognitive performance in children. However, the effectiveness of multiple micronutrient and other interventions needed further investigation (4). The research described in this thesis focuses on the role of multiple micronutrients and omega-3 fatty acids on cognitive development in children.

## Cognitive performance

### Brain development

The development of the human central nervous system starts in the first weeks after conception with the formation of the neural tube, which subsequently differentiates into the brain and spinal cord. Brain development involves processes of neurogenesis, neuronal migration, synaptic formation and myelination (4;5). Most rapid brain growth takes place from the third trimester of pregnancy until the first two years of life (6). By the age of 6 years the brain reaches 95% of its full size (5). During childhood, the brain progresses to maturity by generation of neurons, formation of new connections (synaptogenesis) and myelination and these processes continue into adulthood (**Figure 1**) (4;5;7). The various areas in the brain develop and mature at different rates. In the first 2 years of life, the most basic functions, such as the senses (taste, audition, smell, vision, touch) and the mobility apparatus (movement, walking) are developed. Areas involved in advanced spatial orientation and language follow later. The frontal lobes mature last. This area of the brain harbors more complex functions, such as planning ahead, complex problem solving and memory (4). These high-level psychological

processes of memory, learning, reasoning, attention, language and coordination of motor outputs are classified under the broad term "cognition" (8).

Cognitive development refers to the changes of the cognitive processes observed over longer periods of time (months or years) and is usually assessed in children by batteries of intelligence tests assessing specific cognitive abilities (8). The term cognitive performance is used when the intellectual ability of an individual is assessed at a certain time point. Cognitive development is determined by a number of factors, including socio-cultural, biological and psycho-social variables, and by genetic variation (4).

**How nutrition affects cognitive development**

Nutrition can have an impact on cognitive functioning directly and indirectly. Directly, nutrients are required as building blocks of the brain structures, and for the synthesis and function of neurotransmitters (6;7). Indirectly, nutrition may enhance cognitive development via general improvement of health and energy metabolism (8). Overall improvements in health status of a young child will result in more explorative behavior as the child will become more active. As a result, the caretaker will more frequently interact with the child and indirectly stimulate the child's cognitive development (9). In older children, improved health status has a positive impact on school attendance, and also increases activity and social interactions with other children, leading to enhanced cognitive development and performance (10). Thus, when general aspects of psychological development, such as well-being, motivation and concentration improve, it is likely that intelligence will also be positively affected.

The development of infants and children is particularly vulnerable to nutrient deficiencies due to rapid physical growth, and especially growth of the brain (11). The timing of the nutrition intervention is important, because some brain functions develop early in life and nutritional deficits during this period may irreversibly impair brain development and later cognitive functioning. This has been indicated by several studies showing that effects of severe or chronic iron deficiency during infancy were associated with poor performance in late childhood, even after treatment with iron when children were young (12). Furthermore, children's nutritional status at baseline may influence the efficacy of nutrition interventions on improving mental development. A meta-analysis on the effect of iron supplementation in children, demonstrated that effects on cognition were more pronounced in children who had iron deficiency (anemia) at the start of the intervention compared to non-deficient children (13). Thus, children with nutritional deficiencies may benefit more from nutrition interventions compared to children without deficiencies. Therefore, it may be hypothesized that micronutrient interventions may be more effective in improving cognition in malnourished populations in developing countries than in well-nourished children in developed nations.

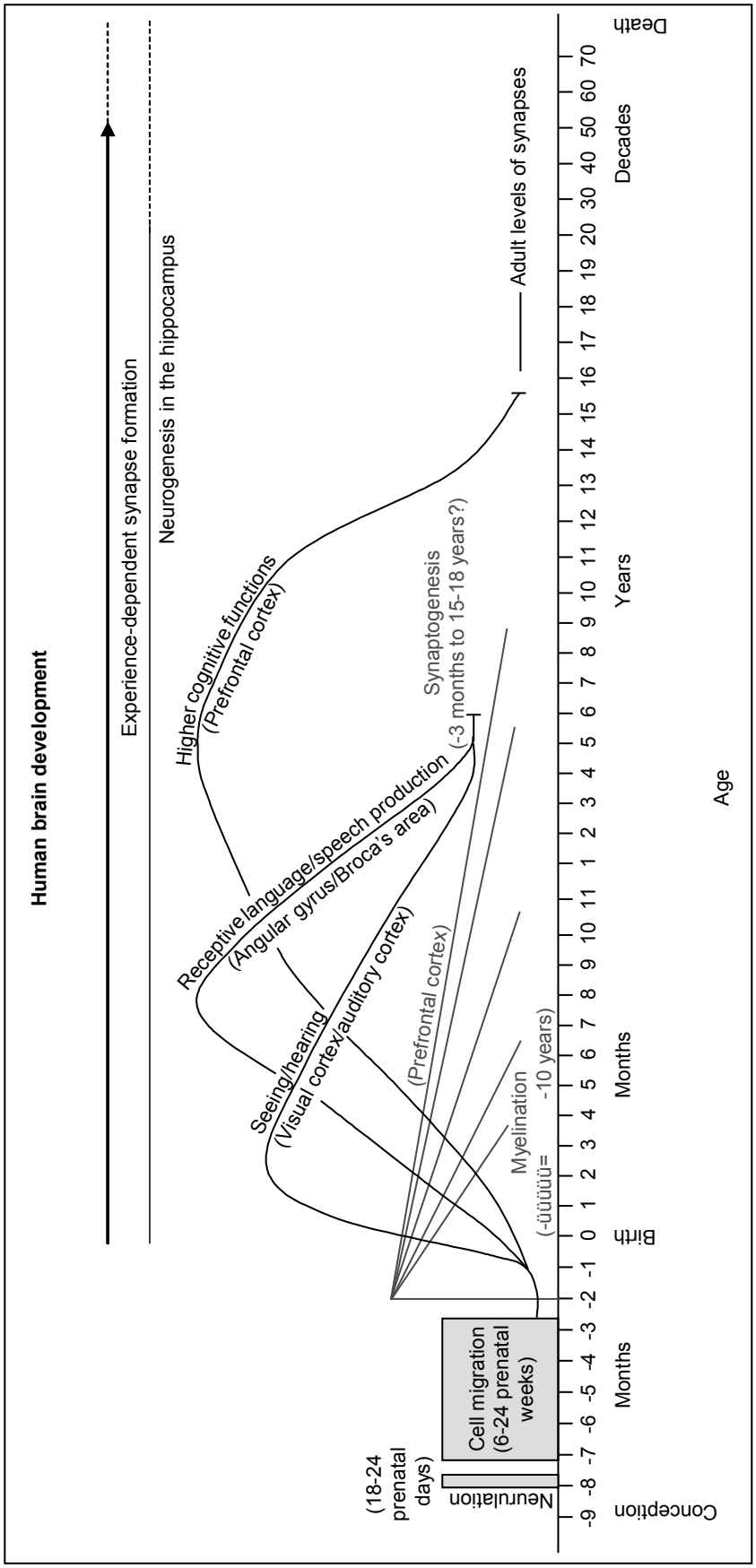


**Figure 1**. Development of the human brain.  Figure has been reproduced with permission (11).

## Measuring cognition in nutrition intervention studies

The effect of nutritional interventions on cognitive performance in children is usually measured through a set of psychological assessments. In infants and young children, these assessments mostly comprise measurements of general mental and psychomotor development, for example by the Bayley Scales of Infant Development. In children older than 2-3 years of age, the cognitive abilities start to differentiate and therefore testing becomes more specific. However, until 5 years of age some cognitive abilities such as reasoning and speed of processing are still very immature and difficult to assess. Intelligence is usually assessed by sets of short tests measuring various cognitive abilities that are collated and generate a general score of intelligence (14). The Kaufman Assessment Battery for Children and Wechsler Intelligence Scales for Children are examples of commonly used tools for assessment of intelligence.

The quality of a cognitive test depends on its validity and reliability. The validity reflects the extent to which the test measures the underlying construct or cognitive ability (e.g. short-term memory). Validity of a test can be established by correlating the test outcomes to outcomes of other tests that are known to measure the same construct (15). Reliability refers to the degree to which the test outcome yields the same score when individuals with the same attributes are repeatedly assessed. Reliability can be measured by methods of test-retest (i.e. the level to which the same individual obtains the same score when given the same test), parallel forms (i.e. the extent to which the same individual obtains the same score when given a test with different but equivalent items) and internal consistency (i.e. the degree to which the different items are consistent with the score within a scale) (15).

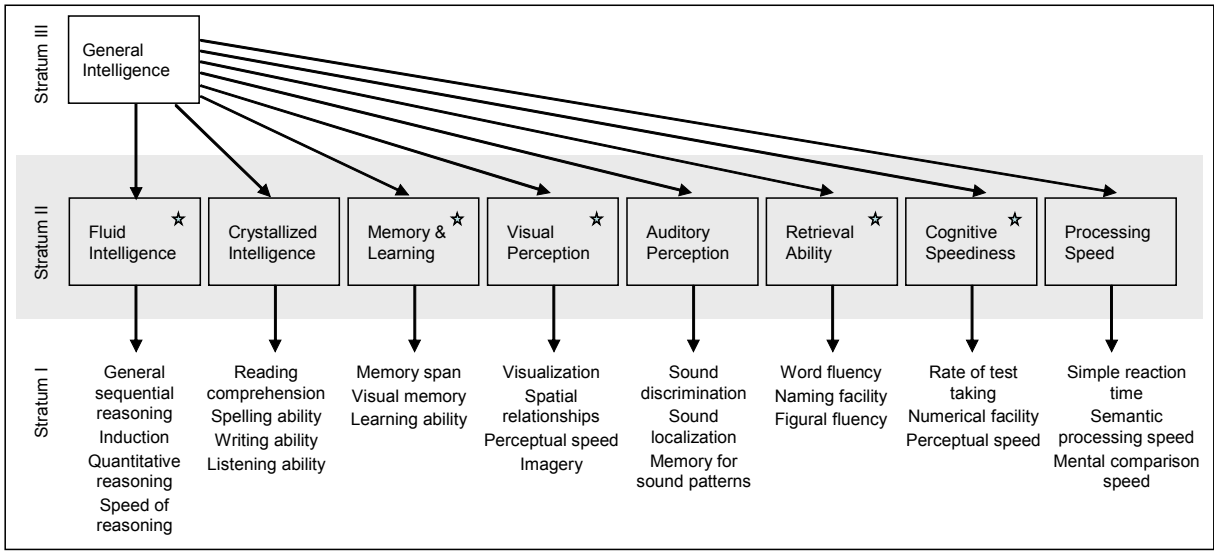


**Figure 2**. The three stratum structure of cognitive abilities adapted from Carroll (18).
Stratum I contains some examples of the 69 narrow cognitive functions that can be assessed by individual cognitive tests. Stratum II consists of eight broad cognitive domains which are represented in order of strength of correlations with general intelligence. Domains marked with an asterisk were postulated to be influenced by micronutrient interventions (19).

For use in nutrition intervention trials it is essential that tests are sensitive (i.e. have a high resolution) in order to pick up small effects (16). For research in developing countries, it is important that cognitive tests that originate from western countries, will be thoroughly adapted to local language and culture to ensure their validity and reliability (16;17). Use of non-validated test batteries may lead to problems in the interpretation of findings as subjects may not have understood the tests in a correct manner or as test administrators may not be familiar with the scoring system. When tests are too difficult (resulting in floor effects) or too easy (leading to ceiling effects) the distribution of test scores will be highly skewed and it will therefore not be possible to distinguish differential performance in children and to detect effects of nutrition interventions.

In this thesis, a hierarchical model of intelligence, named the three stratum structure of cognitive abilities by Carroll (18) will be used to classify cognitive tests into cognitive domains (**Figure 2**). The model is based on a factor-analytic survey of over 460 datasets reported in psychometric literature, covering about six decades of intelligence testing in various populations of different age, gender and culture. To construct the model, an exploratory factor analysis was employed on each individual dataset to identify the factors representing basic cognitive functions, which can be found in stratum I of Carroll's model. To each factor, a name was assigned based on the representation of cognitive functions loading on that factor. Subsequently, higher-order factor analyses were conducted to identify broad cognitive abilities and general intelligence factor. Synthesis of outcomes of the individual datasets resulted in a framework in which general intelligence (stratum III) is composed of eight cognitive abilities (stratum II) that correlate with general intelligence; and of 69 more basic and specific cognitive processes (stratum I) that are underlying the eight cognitive domains. These narrow cognitive functions, such as general sequential reasoning, reading comprehension or memory span, can be measured by individual cognitive tests. The broad cognitive abilities in Figure 2 are represented in order of strength of association of the ability with general intelligence. Thus, the factor fluid intelligence comprising reasoning abilities is most strongly related to general intelligence.

Based on previous literature, it is expected that nutrition interventions will mainly affect the domains of fluid intelligence (or reasoning ability), memory and learning (which covers mainly short-term memory), visual perception, retrieval ability, and cognitive speediness. In addition, sustained attention and academic performance which are not part of the model may be enhanced by nutrition interventions (19).

## Role of micronutrients and omega-3 fatty acids on cognition

In this thesis, we will focus on possible benefits of multiple micronutrients and omega-3 fatty acids on cognitive development. First, a brief overview on the role of single micronutrients on brain and cognitive function in school-age children

will be given, followed by a rationale for additional cognitive effects of multiple micronutrients compared to single micronutrients. Subsequently, the involvement of omega-3 fatty acids in mental development will be summarized and a justification for possible interactions between multiple micronutrients and omega-3 fatty acids on cognition will be presented.

***Iron***

Brain iron is required for proper myelination of the spinal cord and white matter of the cerebellar folds. In addition, iron is a cofactor for a number of enzymes involved in neurotransmitter synthesis and plays a role in neurotransmitter metabolism (8;20). Furthermore, iron deficiency may inhibit the synthesis of thyroid hormones and may indirectly hamper the development of the brain and central nervous system (21).

Longitudinal studies consistently indicate that children with early childhood anemia continue to have poor cognitive and motor development and school achievement into middle childhood (22). Recently, a meta-analysis of four trials showed a positive effect of iron supplementation of 30-100 mg/d (~100-300% of the Recommended Dietary Allowance (RDA)) on intellectual development in children ≥8 years of age (13). Effects were stronger in children who were iron deficient or anemic at baseline.

To summarize, there is substantial evidence for a beneficial effect of iron on cognitive development in school-age children.

***Iodine***

Iodine is an essential micronutrient that forms a vital component of the thyroid hormones, thyroxine and triiodothyronine, which are crucial regulators of the metabolic processes and physical and mental development in humans. Thyroid hormones play a major role in the growth and development, function and maintenance of the central and peripheral nervous system (21;23).

A meta-analysis of 18 studies in humans aged 2 months to 45 years, demonstrated a general loss of 13.5 IQ points in chronically iodine deficient populations, compared to non-iodine deficient groups (24). Eight randomized controlled intervention trials have addressed the question of reversibility of the consequences of iodine deficiency in school-age children using doses of 200-540 mg/d (~200-500% of the RDA). Of these studies, three demonstrated that iodine supplementation improved mental performance (25-27) and two showed that an improvement in iodine status following iodine supplementation was associated with better cognitive performance (28;29). Three studies did not find an improvement on cognition which may have been due to the fact that iodine supplementation was not sufficient to increase iodine status or to other underlying factors in the study designs (30-32). Nevertheless, the majority of these studies suggest that iodine

supplementation improves mental development of children.

***Zinc***

Besides its important role in the immune system (33), zinc is a co-enzyme required for neurogenesis, neuronal migration, and synaptogenesis (34). Zinc deficiency may affect cognitive performance by alterations in attention, activity, neuro-psychological behavior and motor development (35), but the exact mechanisms are unclear. Relatively few studies have examined the effect of zinc supplementation on cognitive performance in school-age children. Three randomized controlled trials in Chinese and Mexican-American children showed that zinc supplementation of 20 mg/d (~200% of the RDA) improved reasoning abilities (36-38), whereas two studies using doses of 10 mg/d (~100% of the RDA) in stunted Guatemalan and Canadian children did not (39;40). Therefore, evidence for a beneficial effect of zinc on cognitive performance is currently inconclusive.

***B-vitamins***

B-vitamins contribute to optimal functioning of the central nervous system through their role as cofactors in numerous catalytic reactions in the human body. Through these processes thiamine (vitamin $B_1$), riboflavin (vitamin $B_2$), pyridoxine (vitamin $B_6$), folate and cobalamin (vitamin $B_{12}$) are required for neurotransmitter synthesis and functioning (41-45), brain energy metabolism (46) and myelination of the spinal cord and brain (45).

Evidence for beneficial effects of most B-vitamins on cognitive performance in children is limited, and for vitamin $B_6$ and folate there are no studies reported in literature. One double-blind study in children aged 9-19 years showed that thiamine supplementation of 2 mg/d (~200% of the RDA) was beneficial for memory, intelligence and reaction time (47). Also, one human case study linked riboflavin to cognitive functioning by finding a reversible effect on neurological and visual abnormalities in a 2.5-year-old riboflavin deficient, anemic girl after a high dose of riboflavin (48). For vitamin $B_{12}$, evidence is limited to two observational studies. The first study, conducted in the Netherlands, showed that children aged 10-16 years, who consumed a macrobiotic diet early in life, performed less well on cognitive tests measuring fluid intelligence, spatial ability and short-term memory than omnivore children (49). Similarly, the second study, conducted in Guatemala, demonstrated that 8-12 year-old schoolchildren with vitamin $B_{12}$ deficiency had poorer scores on cognitive tests than children with an adequate vitamin $B_{12}$ status. However, these findings were not controlled for socio-economic status, hemoglobin, iron status, and blood lead levels (50).

Thus, although there are plausible mechanisms for the role of B-vitamins in functioning of the central nervous system, there is insufficient evidence for a causal effect of B-vitamins on cognitive performance during childhood.

**Multiple micronutrients**

Consumption of monotonous diets lacking important micronutrients will result in co-existing micronutrient deficiencies and therefore, multiple micronutrient interventions may be more effective to improving cognitive functioning than single micronutrient interventions (51). In addition, there may be synergistic effects of micronutrients on physical functions, such as involvement of iron and vitamin A in addition to iodine in thyroid metabolism (52;53) and requirements of vitamin A, B-vitamins and iron for erythropoeisis (54;55). On the other hand, micronutrients may interact negatively, by impairing their bioavailability and effectiveness in physiological processes which may hamper cognitive functioning. For instance, iron and zinc compete for intestinal uptake and high doses may inhibit mutual absorption and that of other minerals such as copper and manganese (56).

In 2001, a review of 13 studies in school-age children from developed countries showed that in 10 of these studies multiple micronutrient supplementation was effective in improving non-verbal intelligence (57). Since then, seven additional studies conducted in developing countries in malnourished children assessed the effect of multiple micronutrient fortified foods on cognitive performance (58-63), of which five demonstrated significantly beneficial effects on at least one of the cognitive performance indicators measured in children (58-61). The doses of micronutrients used in these studies varied considerably (i.e. for iron from 1.2-24 mg/d (~4-130% of the RDA)), but most trials provided micronutrients at doses between 30-100% of the RDA. Thus, doses given in multiple micronutrient studies are usually lower than those used in the trials investigating iron or iodine alone (>100% of the RDA), and it is currently unknown whether lower doses are as effective as higher doses.

In conclusion, a relatively large number of studies indicate that multiple micronutrients may enhance on cognitive performance in school-age children. However, a systematic approach to evaluate the totality of evidence and to quantify the effect of multiple micronutrients is currently lacking. Furthermore, it remains unclear which of the cognitive domains are most sensitive to micronutrient supplementation, whether effects depend on the nutritional status of subjects and what dose of micronutrients would be most effective to improve cognitive function.

**Omega-3 fatty acids**

Fatty acids are important for child growth and development. Approximately 25-30% of the fatty acids in the human brain consist of polyunsaturated fatty acids (PUFA) of which the omega-3 fatty acid docosahexaenoic acid (DHA) and the omega-6 fatty acid arachidonic acid (AA) are major components. DHA and AA are rapidly incorporated in the nervous tissue of retina and brain during the brain's growth spurt, which mainly takes place from the last trimester of pregnancy up

to 2 years of age (64-67). Beyond development of the central nervous system, omega-3 and omega-6 fatty acids may influence brain function throughout life by involvement in aspects of neuron function and of neurotransmitter synthesis (7). Although the human body can synthesize DHA from α-linolenic acid (ALA), the conversion of ALA to EPA and DHA is marginal (68-71), with an overall conversion rate of 4% at most (72). It is presently unclear whether conversion of dietary ALA is under all circumstances sufficient to meet optimal DHA requirements.

At the time of the onset of our studies, research in healthy school-age children without developmental disorders, such as attention-deficit hyperactivity disorder, was limited to one observational study in the Netherlands, which did not find a relationship between DHA status measured and cognitive performance in children aged 7 years (73). Recently, three randomized controlled trials have been reported in literature. Two of these studies did not find any effects of supplementation with either a low dose of 110 mg EPA plus DHA (58) for one year or with higher dosages of 400 and 1000 mg DHA for 8 weeks (74) on cognitive performance in healthy children aged 7-12 years. In contrast, a third study conducted in South-African children 7-9 years of age showed that children receiving an omega-3 rich fish-flour spread had better scores on verbal learning and memory tests compared to those receiving a control spread (75). However, it is possible that other nutrients in addition to the omega-3 fatty acids in the fish-flour have contributed to the cognitive effects.

In summary, evidence is currently too limited to conclude whether supplementation with DHA in addition to ALA may enhance intellectual development of children.

**Interactions between micronutrients and fatty acids**

Since diets of poor populations may lack both micronutrients as well as essential fatty acids, we hypothesized that supplementation of micronutrients with omega-3 fatty acids could have synergistic effects on improving cognitive function. There are two hypotheses for additional effects of multiple micronutrient supplementation and omega-3 fatty acids on cognition. Firstly, micronutrients may increase the conversion of ALA to DHA and subsequently enhance the brain's DHA status and improve cognitive function. Animal studies have indicated that iron, zinc, vitamin $B_6$ and vitamin E may enhance the conversion of ALA to DHA, through their role in the δ6-desaturation enzyme (76). In addition, a study in 6-11 year-old South African children showed that iron deficient children had lower concentrations of omega-3 fatty acids in erythrocytes compared to controls. After 15 weeks of supplementation with iron and vitamin C, the erythrocyte omega-3 fatty acid concentrations had been restored to levels comparable to those of control children (77). Thus, this study suggests that micronutrient supplementation might improve omega-3 fatty acid status of undernourished children.

Secondly, as multiple micronutrients have been shown to influence cognitive

functioning and because DHA is an important structural component of the brain, supplementation with both may synergistically improve cognitive outcomes. However, after the onset of our studies, no interactions between multiple micronutrients and EPA plus DHA supplementation were found on plasma fatty acid status and cognitive function in children aged 7-10 years in Australia and Indonesia (58).

Taken together, there are some indications that micronutrients may enhance omega-3 fatty acid status but the findings should be confirmed in a randomized controlled trial. Hypothetically, multiple micronutrients and omega-3 fatty acids may interact synergistically on cognitive performance, but this needs further investigation.

## Aim and outline of this thesis

Although nutrition may have the largest impact on neurodevelopment from conception to the age of two years when the central nervous system is rapidly developing; diet may also influence cognitive performance during childhood as the brain continues to develop into adulthood. Whereas single micronutrient interventions showed that iron and iodine improve intellectual development in school-age children, the evidence for multiple micronutrients from a relatively large number of studies has yet not been evaluated in a systematic manner. Although an earlier review indicated that micronutrients would most benefit non-verbal or reasoning abilities (57), it is currently unclear which of the cognitive domains are most sensitive to micronutrient interventions. Also, no clarity exists whether the effects of micronutrients are dependent on nutritional status of children, and what dose of micronutrients would be required to optimize intellectual development. Furthermore, while much research has focused on the role of DHA in particular on mental development during infancy, little is known about effects of omega-3 fatty acids, including DHA, on cognition in school-age children. Lastly, it is of interest to explore whether micronutrients and omega-3 fatty acids would interact synergistically on improving children's intellectual development.

The objective of this thesis is to study the role of multiple micronutrients and omega-3 fatty acids on cognitive performance in school-age children living in deprived environments. More specifically, the following research questions are addressed:

*1. To what extent do multiple micronutrient interventions influence different cognitive domains in children?*

This research question will mainly be answered through a meta-analysis of randomized controlled trials assessing the effect of multiple micronutrients on the different cognitive domains in children. Furthermore, associations between

micronutrient status and different cognitive domains have been explored using cross-sectional data and the effects of a high versus low dose of micronutrients on different cognitive abilities is investigated in a randomized controlled trial. We hypothesize based on observations from earlier micronutrient interventions that the size of effects of multiple micronutrients on cognitive outcomes will be small to moderate and that the domains of fluid intelligence, memory and learning, visual perception, retrieval ability, and cognitive speediness can be positively influenced. We expect effects to be larger in malnourished subjects with a higher risk of micronutrient deficiencies compared to well-nourished children.

*2. What is the role of omega-3 fatty acids on children's cognitive development?*

A literature review on the role and effect of omega-3 fatty acids on visual and cognitive development in infants and children has been conducted to address this question. In addition, the relationships between omega-3 fatty acid status and intake and cognitive function are explored using cross-sectional data and a randomized controlled trial has been conducted to investigate the effects of a high versus low dose of omega-3 fatty acids. As DHA is a major structural component of the brain and involved in neurotransmitter functioning, we postulate that supplementation with omega-3 fatty acids benefits cognitive performance of children.

*3. What is the effect of an intervention with different doses of multiple micronutrients and omega-3 fatty acids on cognitive performance in schoolchildren in India? Is there an interaction between multiple micronutrients and omega-3 fatty acids?*

A randomized controlled trial with a 2x2 factorial design investigating different doses of micronutrients and omega-3 fatty acids on cognition in children has been used to investigate these research questions. We hypothesize that higher doses of multiple micronutrients (close to 100% of the RDA) and omega-3 fatty acids would be more effective in improving intellectual development in children than lower doses below 50% of the RDA of these nutrients and that there are additional effects of omega-3 fatty acids plus micronutrients on cognitive outcomes.

The outline of this thesis is as follow:

- Chapter 2. Systematic review and meta-analysis on the effect of multiple micronutrients on cognitive performance in schoolchildren.
- Chapter 3. Literature review on the role and effect of omega-3 fatty acids on visual and cognitive development in infants and children.
- Chapter 4. Cross-sectional study to investigate the associations of indicators of body size, micronutrient and fatty acid status on cognitive performance of Indian schoolchildren.
- Chapter 5. Randomized controlled trial to investigate the effect of foods fortified with either a low or high dose of micronutrients and omega-3 fatty

acids on cognitive performance and growth in schoolchildren in India.
- Chapter 6. Cross-sectional study to assess the role of diet in relation to anthropometric measurements and cognitive performance of the Indian schoolchildren.
- Chapter 7. General discussion which comprises a reflection on the main outcomes of the work presented in chapters 2-6, followed by implications for public health and recommendations for future research.

**The study location in India**

The field work described in this thesis has been conducted in Bangalore city, Karnataka state, South India (**Figure 3**). Bangalore (re-named Bengaluru since 2006) is located at 920 m above sea level and has a relatively mild climate with three seasons, including hot (April-May) wet (June-September) and cool (October-March) seasons. With approximately 6 million inhabitants, Bangalore is the fifth largest city of India after Delhi, Kolkata, Mumbai and Chennai. As a consequence of rapid growth of the Information Technology industry sector and a rising economy, the vehicular traffic in Bangalore has increased enormously over the past ten years and has caused severe pollution to the environment. Besides the improvements in welfare, approximately 30% of the inhabitants live below the poverty line of US$2 per day (less than 27,000 Indian rupees per year) (78).

The study was conducted in collaboration with nutrition scientists and psychologists of St. Johns Research Institute, Bangalore who had access to the communities in the urban slums. Two primary schools located in the North-East of Bangalore catering to children living in the surrounding slum areas were selected for participation in the study. In order to maximize the effect of our intervention, we chose a study population of children from a low socio-economic background. These children consume diets that are low in micronutrients and omega-3 fatty acids and have therefore a high risk of micronutrient deficiencies.

**Figure 3**. Map of study location Bangalore, India.

Micronutrient deficiencies are thought to be widely prevalent among Indian schoolchildren. Approximately 40% of Indian schoolchildren suffer from iron deficiency (1), 23% from vitamin A deficiency (79), and 4% of 6-12 year-old children have goitre (80). The prevalence of both zinc and vitamin $B_{12}$ deficiencies are estimated to be high

based on national stunting rates (81) and low consumption of animal foods (82).

The two schools were selected out of eight primary schools that were screened for prevalence of anemia in children and feasibility to conduct the intervention study. No other nutrition or health interventions had taken place at the schools in the previous three months before the study start. Children were taught in the local Kannada language, which was important for the cognitive assessment.

## References


1. Allen L, de Benoist B, Hurrell RF, Dary O. Guidelines on food fortification with micronutrients. Geneva: World Health Organization and Food and Agriculture Organization of the United Nations, 2006.
2. de Benoist B, McLean E, Andersson M, Rogers L. Iodine deficiency in 2007: global progress since 2003. Food Nutr Bull 2008;29:195-202.
3. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B. Child development in developing countries 1 - Developmental potential in the first 5 years for children in developing countries. Lancet 2007;369:60-70.
4. Walker SP, Wachs TD, Gardner JM et al. Child development in developing countries 2 - Child development: risk factors for adverse outcomes in developing countries. Lancet 2007;369:145-57.
5. Engle PL, Black MM, Behrman JR et al. Child development in developing countries 3 - Strategies to avoid the loss of developmental potential in more than 200 million children in the developing world. Lancet 2007;369:229-42.
6. Fernstrom JD. Can nutrient supplements modify brain function? Am J Clin Nutr 2000;71:1669S-75S.
7. Yehuda S. Omega-6/omega-3 ratio and brain-related functions. World Rev Nutr Diet 2003;92:37-56.
8. Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. J Nutr 2001;131:568S-79S.
9. Walker SP, Chang SM, Powell CA, Grantham-McGregor SM. Effects of early childhood psychosocial stimulation and nutritional supplementation on cognition and education in growth-stunted Jamaican children: prospective cohort study. Lancet 2005;366:1804-7.
10. Sigman M, Whaley SE, Neumann CG et al. Diet quality affects the playground activities of Kenyan children. Food Nutr Bull 2005;26:S202-S212.
11. Thompson RA, Nelson CA. Developmental science and the media. Early brain development. Am Psychol 2001;56:5-15.
12. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev 2006;64:S34-S43.
13. Sachdev HPS, Gera T, Nestel P. Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. Public Health Nutr 2005;8:117-32.
14. Flanagan DP, Harrison PL. Contemporary Intellectual Assessment, Theories, Tests and Issues. New York: The Guilford Press, 2005.
15. McCartney K, Burchinal MR, Bub KL. Best practices in quantitative methods for developmentalists. Monographs of the Society for Research in Child Development. Oxford: Blackwell Publishing, 2006.
16. Isaacs E, Oates J. Nutrition and cognition: assessing cognitive abilities in children and young people. Eur J Nutr 2008;47 Suppl 3:4-24.
17. van de Vijver FJR, Leung K. Personality in cultural context: methodological issues. J Pers 2001;69:1007-31.
18. Carroll JB. Human cognitive abilities: A survey of factor-analytic studies. New York, NY, US: Cambridge University Press, 1993.
19. Hughes D, Bryan J. The assessment of cognitive performance in children: Considerations for detecting nutritional influences. Nutrition Reviews 2003;61:413-22.
20. Beard JL, Connor JR. Iron status and neural functioning. Ann Rev Nutr 2003;23:41-58.
21. Delange F. The role of iodine in brain development. Proc Nutr Soc 2000;59:75-9.
22. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 2001;131:649S-66S.
23. Hetzel BS. Iodine deficiency disorders (IDD) and their eradication. Lancet 1983;2:1126-9.
24. Bleichrodt N, Born MP. A meta-analysis of research on iodine and its relationship to cognitive development. In: Stanbury JB, ed. The damaged brain of iodine deficiency. New York, NY: Cognizant Communication Corporation 1994:195-200.
25. Dodge PR, Palkes H, Fierro-Benitez R, Ramirez I. Effect on intelligence of iodine in oil administered to young Andean children - a preliminary report. In: Stanbury JB, ed. Endemic Goiter. PAHO 1969:378-80.
26. Shrestha SR, West CE, Bleichrodt N, van de Vijver F, Hautvast JGAJ. Supplementation with iodine and iron improves mental development in Malawian children. Effect of iodine and iron supplementation on physical, psychomotor and mental development in primary schoolchildren in Malawi. Thesis Wageningen. Wageningen: Grafisch Service Centrum 1994:41-58.
27. Zimmermann MB, Connolly K, Bozo M, Bridson J, Rohner F, Grimci L. Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: a randomized, controlled, double-blind study. Am J Clin

Nutr 2006;83:108-14.
28. Bautista A, Barker PA, Dunn JT, Sanchez M, Kaiser DL. The effects of oral iodized oil on intelligence, thyroid status, and somatic growth in school-age children from an area of endemic goiter. Am J Clin Nutr 1982;35:127-34.
29. van den Briel T, West CE, Bleichrodt N, van de Vijver FJR, Ategbo EA, Hautvast JGAJ. Improved iodine status is associated with improved mental performance of schoolchildren in Benin. Am J Clin Nutr 2000;72:1179-85.
30. Bleichrodt N, Escobar del Rey F, Morreale de Escobar G, Garcia I, Rubio C. Iodine deficiency, implications for mental and psychomotor development in children. In: Delong GR, Robbins J, Connolly K, eds. Iodine and the brain. New York: Plenum Press 1989:269-88.
31. Huda SN, Grantham-McGregor SM, Tomkins A. Cognitive and motor functions of iodine-deficient but euthyroid children in Bangladesh do not benefit from iodized poppy seed oil (Lipiodol). Journal of Nutrition 2001;131:72-7.
32. Isa ZM, Alias IZ, Kadir KA, Ali O. Effect of iodized oil supplementation on thyroid hormone levels and mental performance among Orang Asli schoolchildren and pregnant mothers in an endemic goitre area in Peninsular Malaysia. Asia Pacific Journal of Clinical Nutrition 2000;9:274-81.
33. Hotz C, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Food and Nutrition Bulletin 2004;25:S94-S204.
34. Bhatnagar S, Taneja S. Zinc and cognitive development. British Journal of Nutrition 2001;85:S139-S145.
35. Golub MS, Keen CL, Gershwin ME, Hendrickx AG. Developmental zinc deficiency and behavior. J Nutr 1995;125:2263S-71S.
36. Penland JG, Sandstead HH, Alcock NW et al. A preliminary report: effects of zinc and micronutrient repletion on growth and neuropsychological function of urban Chinese children. J Am Coll Nutr 1997;16:268-72.
37. Sandstead HH, Penland JG, Alcock NW et al. Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children. Am J Clin Nutr 1998;68:470S-5S.
38. Penland JG, Sandstead HH, Egger N et al. Zinc, iron and micronutrient supplementation effects on cognitive and psychomotor function of Mexican-American schoolchildren. FASEB J 1999;13:A921.
39. Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW. Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. Am J Clin Nutr 1993;57:344-52.
40. Gibson RS, Vanderkooy PD, Macdonald AC, Goldman A, Ryan BA, Berry M. A growth-limiting, mild zinc-deficiency syndrome in some southern Ontario boys with low height percentiles. Am J Clin Nutr 1989;49:1266-73.
41. Bettendorff L. Thiamine in excitable tissues - Reflections on a non-cofactor role. Metabolic Brain Disease 1994;9:183-209.
42. Guilarte TR. Vitamin B6 and cognitive development: recent research findings from human and animal studies. Nutr Rev 1993;51:193-8.
43. Hutto BR. Folate and cobalamin in psychiatric illness. Compr Psychiatry 1997;38:305-14.
44. Sugden C. One-carbon metabolism in psychiatric illness. Nutr Res Rev 2006;19:117-36.
45. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev 2008;66:250-5.
46. Westermarck T, Antila E. Diet in relation to the nervous system. In: Garrow JS, James WPT, Ralph A, eds. Human nutrition and dietetics. London: Churchill Livingstone 2000:715-30.
47. Harrell RF. Mental response to added thiamine. Journal of Nutrition 1946;31:283-98.
48. Leshner RT. Riboflavin deficiency - a reversible neurodegenerative disease. Ann Neurol 1981;10:294-5.
49. Louwman MWJ, van Dusseldorp M, van de Vijver FJR et al. Signs of impaired cognitive function in adolescents with marginal cobalamin status. Am J Clin Nutr 2000;72:762-9.
50. Allen LH, Penland JG, Boy E, DeBaessa Y, Rogers LM. Cognitive and neuromotor performance of Guatemalan schoolers with deficient, marginal, and normal plasma vitamin B-12. FASEB J 1999;13:A544.
51. Mason JB, Lotfi M, Dalmiya N, Sethuraman K, and Deitchler M. Current progress and trend in the control of vitamin A, iodine, and iron deficiencies. The Micronutrient Report . 2001. Ottawa, The Micronutrient Initiative. Ref Type: Report
52. Zimmermann M, Adou P, Torresani T, Zeder C, Hurrell R. Persistence of goiter despite oval iodine supplementation in goitrous children with iron deficiency anemia in Cote d'Ivoire. Am J Clin Nutr 2000;71:88-93.
53. Zimmermann MB, Jooste PL, Mabapa NS et al. Vitamin A supplementation in iodine-deficient African children decreases thyrotropin stimulation of the thyroid and reduces the goiter rate. Am J Clin Nutr 2007;86:1040-4.
54. Zimmermann MB, Biebinger R, Rohner F et al. Vitamin A supplementation in children with poor vitamin A and iron status increases erythropoietin and hemoglobin concentrations without changing total body iron. Am J Clin Nutr 2006;84:580-6.
55. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr 2004;24:105-31.
56. Sandstrom B. Micronutrient interactions: effects on absorption and bioavailability. Br J Nutr 2001;85 Suppl 2:S181-S185.
57. Benton D. Micro-nutrient supplementation and the intelligence of children. Neurosci Biobehav Rev 2001;25:297-309.
58. Osendarp SJ, Baghurst KI, Bryan J et al. Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia. Am J Clin Nutr 2007;86:1082-93.

59. Vazir S, Nagalla B, Thangiah V, Kamasamudram V, Bhattiprolu S. Effect of micronutrient supplement on health and nutritional status of schoolchildren: mental function. Nutrition 2006;22:S26-S32.
60. van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benade AJS. Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary schoolchildren: a randomized controlled trial. (vol 69, pg 497, 1999). Am J Clin Nutr 1999;69:1294.
61. Manger MS, McKenzie JE, Winichagoon P et al. A micronutrient-fortified seasoning powder reduces morbidity and improves short-term cognitive function, but has no effect on anthropometric measures in primary schoolchildren in northeast Thailand: a randomized controlled trial. Am J Clin Nutr 2008;87:1715-22.
62. Solon FS, Sarol JN, Bernardo AB, I et al. Effect of a multiple-micronutrient-fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of schoolchildren in the Philippines. Food and Nutrition Bulletin 2003;24:S129-S140.
63. Jinabhai CC, Taylor M, Coutsoudis A, Coovadia HM, Tomkins AM, Sullivan KR. A randomized controlled trial of the effect of antihelminthic treatment and micronutrient fortification on health status and school performance of rural primary schoolchildren. Ann Trop Paediatr 2001;21:319-33.
64. Dobbing J, Sands J. Quantitative growth and development of human brain. Arch Dis Child 1973;48:757-67.
65. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. Early Hum Dev 1980;4:131-8.
66. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Hum Dev 1980;4:121-9.
67. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129-S138.
68. Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta 1994;1213:277-88.
69. Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N, Jr. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. J Lipid Res 2001;42:1257-65.
70. Salem N, Jr., Pawlosky R, Wegher B, Hibbeln J. In vivo conversion of linoleic acid to arachidonic acid in human adults. Prostaglandins Leukot Essent Fatty Acids 1999;60:407-10.
71. Vermunt SH, Mensink RP, Simonis AM, Hornstra G. Effects of age and dietary n-3 fatty acids on the metabolism of [13C]-alpha-linolenic acid. Lipids 1999;34 Suppl:S127.
72. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. Curr Opin Clin Nutr Metab Care 2002;5:127-32.
73. Bakker EC, Ghys AJA, Kester ADM et al. Long-chain polyunsaturated fatty acids at birth and cognitive function at 7y of age. Eur J Clin Nutr 2003;57:89-95.
74. Kennedy DO, Jackson PA, Elliott JM et al. Cognitive and mood effects of 8 weeks' supplementation with 400 mg or 1000 mg of the omega-3 essential fatty acid docosahexaenoic acid (DHA) in healthy children aged 10-12 years. Nutr Neurosci 2009;12:48-56.
75. Dalton A, Wolmarans P, Witthuhn RC, van Stuijvenberg ME, Swanevelder SA, Smuts CM. A randomised control trial in schoolchildren showed improvement in cognitive function after consuming a bread spread, containing fish flour from a marine source. Prostaglandins Leukot Essent Fatty Acids 2009;80:143-9.
76. Smit EN, Muskiet FA, Boersma ER. The possible role of essential fatty acids in the pathophysiology of malnutrition: a review. Prostaglandins Leukot Essent Fatty Acids 2004;71:241-50.
77. Smuts CM, Tichelaar HY, Vanjaarsveld PJ et al. The Effect of Iron Fortification on the Fatty-Acid Composition of Plasma and Erythrocyte-Membranes in Primary-Schoolchildren with and Without Iron-Deficiency. Prostaglandins Leukot Essent Fatty Acids 1994;51:277-85.
78. Dittrich C. Bangalore: globalization and fragmentation in India's high-tech capital. Asien 2007;103:S45-S58.
79. Singh V, West KP, Jr. Vitamin A deficiency and xerophthalmia among school-aged children in Southeastern Asia. Eur J Clin Nutr 2004;58:1342-9.
80. National Nutrition Monitoring Bureau. Prevalence of micronutrient deficiencies. NNMB Technical Report No. 22. Hyderabad: 2003.
81. Brown KH, Rivera JA, Bhutta Z et al. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 2004;25:S99-203.
82. Stabler SP, Allen LH. Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr 2004;24:299-326.

# Chapter 2

## Multiple micronutrient supplementation for improving cognitive performance in children: systematic review of randomized controlled trials

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## Abstract

**Background**: Although multiple micronutrient interventions have been shown to benefit children's intellectual development, a thorough evaluation of the totality of evidence is currently lacking to direct public health policy.

**Objective**: This study aimed to systematically review the present literature and to quantify the effect of multiple micronutrients on cognitive performance in schoolchildren.

**Methods**: The Institute for Scientific Information Web of Knowledge and local medical databases were searched for trials published from 1970 to 2008. Randomized controlled trials investigating the effect of ≥3 micronutrients compared with placebo on cognition in healthy children aged 0-18 years were included following protocol. Data were extracted by two independent researchers. The cognitive tests used in the trials were grouped into several cognitive domains (e.g. fluid and crystallized intelligence) and pooled effect size estimates were calculated per domain. Heterogeneity was explored through sensitivity and meta-regression techniques.

**Results**: Three trials were retrieved in children <5 years, and 17 trials in children aged 5-16 years. For the older children, pooled random effect estimates for intervention were 0.14 SD (95% CI: -0.02, 0.29; *P*=0.083) for fluid intelligence and -0.03 SD (95% CI: -0.21, 0.15; *P*=0.74) for crystallized intelligence, both of which were based on 12 trials. Four trials yielded an overall effect of 0.30 SD (95% CI: 0.01, 0.58; *P*=0.044) for academic performance. For other cognitive domains no significant effects were found.

**Conclusions**: Multiple micronutrient supplementation may be associated with a marginal increase in fluid intelligence and academic performance in healthy schoolchildren, but not with crystallized intelligence. More research is required however, before public health recommendations can be given.

## Introduction

Micronutrient malnutrition impairs children's cognitive performance and developmental potential (1). Single micronutrient interventions have shown that iodine and iron, and possibly other micronutrients such as zinc and B-vitamins, may benefit children's mental development (2). Because micronutrient deficiencies often coexist and synergistic effects of micronutrients on physical functions may indirectly affect cognition, supplementing children with multiple micronutrients could have advantages over single micronutrient supplementation. For example, iron and vitamin A have been shown to influence thyroid metabolism in addition to iodine (3;4) and B-vitamins and vitamin A are required for erythropoeisis in addition to iron (5;6). In contrast, micronutrients might also have antagonistic effects, affecting their bioavailability and their functioning in physiological processes that may lead to impaired cognitive functioning. Because iron and zinc, as well as copper and manganese compete for intestinal uptake, a high dosage of one of these minerals may limit the absorption of the others (7).

In 2001, Benton (8) reviewed 13 studies investigating the role of multiple micronutrients on cognition in children aged 6-16 years of which the majority reported a positive effect of the micronutrient supplementation, mostly with non-verbal measures. The author postulated that performance on non-verbal tests results, at least in part, from basic biological functions and could be influenced by diet. In contrast, verbal intelligence comprises the acquired knowledge which was thought not to be affected by nutrition on the shorter term. Some limitations of the review were that no strict selection criteria were applied for inclusion or exclusion of studies and that the results of the studies were not pooled to quantify the effect of micronutrients on cognition. Furthermore, it remained unclear whether there are other specific cognitive domains, beyond non-verbal intelligence that could be influenced by micronutrient supplementation and whether the effects would depend on other factors, such as age, nutritional and socio-economic status. Since Benton's review, more trials have been published in literature, most of which were conducted in developing countries. Children in developing countries have, in general, a more monotonous diet and may have a higher risk of micronutrient deficiencies. Hence, these children might benefit more from micronutrient supplementation than their peers in developed countries.

In the present study, we aimed to systematically review the current literature and to perform a meta-analysis to quantify the effect of multiple micronutrient interventions on cognitive performance in children, from infancy until late adolescence, i.e. 0-18 years of age. Moreover, because we expected heterogeneity among the studies, we explored whether factors such as age, country, nutritional status, duration and type of micronutrient supplementation would predict the effects of micronutrients on cognition.

## Methods

### Identification of trials

We searched in titles and abstracts of trials published between 1970-2008 in databases of the Institute for Scientific Information Web of Knowledge, Cochrane (CENTRAL), EmBase, Australasian Medical Index, Chinese Biomedical Literature, Latin American Caribbean Health Sciences Literature and Japan Information Centre of Science and Technology File on Science, Technology and Medicine. A search string consisting of a combination of the following terms was used: trace element, vitamin, carotenoid, micronutrient, mineral, multiple micronutrient; with cognition, memory, mental performance, child development, infant development, school performance, academic achievement, psychomotor; and with child, adolescent, infant, pre-schooler, toddler. Additionally, we performed a lateral search, checked lists of references of publications found and searched the past three issues of conference proceedings of the International Union of Nutritional Sciences (IUNS) and the International Life Sciences Institute (ILSI) conferences.

### Selection criteria

*Type of trials:* All randomized (also cluster randomized) placebo-controlled trials evaluating micronutrient supplementation of children aged 0-18 years for a period of ≥4 weeks and its effect on cognitive performance, regardless of language and publication status were considered for inclusion. Also, trials using a factorial design with multiple intervention groups were eligible.

*Type of participants*: Trials conducted in apparently healthy children were included. Trials conducted in selected subjects with learning disabilities, neuro-psychiatric disorders or chronic psychiatric health conditions were excluded.

*Types of intervention:* We included trials in which children were supplemented with at least three different micronutrients compared with a placebo, as well as studies that compared foods fortified with ≥3 micronutrients with non-fortified foods. Moreover, trials that had given other interventions along with the multiple micronutrients, such as deworming tablets or omega-3 fatty acids were also included provided that the addition of multiple micronutrients was the sole difference between the intervention and control group. When studies used supplements which contained also bio-active substances such as flavonoids and carotenoids, which are commonly found in micronutrient supplements, these studies were included provided that the supplement predominantly consisted of micronutrients. A combination of ≥3 B-vitamins only was not considered to qualify as a multiple micronutrient intervention, as B-vitamins are involved in similar biological processes. Studies in which (omega-3) fatty acids, proteins or herbals were part of the micronutrient mix were excluded when the intervention was compared with a placebo.

*Type of outcomes:* Studies reporting cognitive performance as primary or secondary outcome measure of the intervention were included.

**Data handling**

Data were extracted by two independent people (AE and TG), using a pre-defined data extraction sheet. Possible relevant publications in languages other than English were translated. If needed and wherever possible, authors were contacted to retrieve missing data.

For each trial we tried to retrieve scores on all cognitive subtests measured rather than combined scores. The cognitive tests were grouped by two independent people (TG and CT) into the cognitive domains defined by Carroll (9) including fluid intelligence, crystallized intelligence, short-term memory (referred to as learning and memory in the original model), visual perception, retrieval ability, and cognitive processing speed (consisting of the two domains cognitive speediness and processing speed in the original model), presented here in descending order of correlation with intelligence. We added the cognitive domains sustained attention and motor skills as these domains are frequently found in neuropsychological scales, and we added academic performance. Fluid intelligence comprises reasoning abilities and is generally measured through tasks of analogies and series completions. Crystallized intelligence reflects a set of acquired skills and knowledge, and assessed by tasks of verbal comprehension, vocabulary and evaluation of semantic relationships. It is seen as more dependent on experience and education within a culture than fluid intelligence. Following a priori decision, a meta-analysis was performed at the cognitive domain level, provided that ≥3 trials assessing that domain were available.

**Statistical analysis**

The primary outcome was the difference in change in cognitive test scores from baseline to trial termination, and sensitivity analysis end scores were also evaluated. If the number of trials reporting end scores was higher than trials reporting change scores, then the end scores were used as primary outcome variable for that subset. For trials assessing one cognitive domain with more than one cognitive test, we computed a standardized mean difference (SMD) by taking the mean of the standardized scores of the cognitive tests used. We pooled data reported for subgroups (e.g. boys and girls) and intervention groups (e.g. different dose of micronutrients) that were a priori decided not to be of interest for investigation of heterogeneity. To avoid multiple counting of the control group in trials with multiple intervention groups and a single control group, we divided the sample size of the control group equally between the number of intervention groups, while retaining the mean change and its SD (10). This method will reduce the probability of type 1 error.

The following principles were employed when variables required for the meta-analysis were not stated in the publication or available from authors: 1) within a group, the lower of the two stated sample sizes in the beginning or the end of a trial was assumed to be the sample size for the change; 2) the mean change in outcome

variable was computed as the difference of mean post and pre-intervention levels; 3) wherever feasible, standard deviations were imputed (back calculated) from the stated standard errors (SE), *t, F* or *P* values; or if not possible, standard deviations were computed assuming a correlation of 0.5 between the pre-intervention and post-intervention variances (11); 4) the mean age of subjects was computed as the average of the stated range.

Effect sizes for individual trials were calculated by dividing the difference between the mean change in intervention and control group by the pooled SD. The overall mean effect size was calculated using the random effects model. The presence of bias in the extracted data was evaluated graphically using the funnel plot and Begg and Egger's regression tests(12;13). The $I^2$ (variation in overall effect size attributable to heterogeneity) and Cochran Q statistics were computed to test for heterogeneity of the effect sizes.

Stratified analyses, specified in advance, were conducted for the following variables: quality of methods (concealment of allocation (adequate compared with unclear, inadequate, or not used); blinding (double blinding compared with single blinding, no blinding, or unclear); attrition (<10% compared with ≥10% or unknown)), comparability of intervention groups at baseline (yes compared with no or unclear), compliance to intervention (adequate ≥70% compared with others), type of analysis (intention-to-treat compared with others), country development status (medium compared with high rank on Human Development Index (HDI) defined by the United Nations Development Programme), supplementation vehicle (fortification compared with tablet), number of micronutrients provided (≤20 compared with >20) duration of supplementation (≤12 weeks compared with >12 weeks) and, inclusion of either iron or iodine in intervention (yes compared with no). Because of the limited number of trials in children <5 years, the meta-analysis was only performed on trials conducted in children ≥5 years. This prevented us from including age in subgroup analysis. To further explore heterogeneity between the studies, a meta-regression analysis was conducted to investigate effects of these variables and mean age of subjects. Because of the limited number of trials available, no sensitivity analyses were conducted for the cognitive domains other than fluid and crystallized intelligence. All analyses were conducted with STATA™ (version 9.2; StataCorp, College Station, TX) software.

## Results

Nineteen publications described 20 trials that were eligible for inclusion in the meta-analysis (see **Figure 1**), of which three were conducted in children <5 years of age (14-16) (see **Table 1** for an overview of the trial characteristics). Because only two of these studies measured gross motor development, and the third study conducted more general tests of development, it was not possible to classify the outcomes in the cognitive domains for the children >5 years, and there were too

few trials to conduct a separate meta-analyses for these trials. Therefore, we briefly review them here. Faber et al (15) investigated the effect of a fortified compared with unfortified porridge on gross motor development of South-African infants aged 6-12 months. After 6 months of intervention, infants receiving the fortified porridge achieved significantly higher motor development scores than infants receiving the unfortified porridge. Similarly, Olney et al (16) showed that infants aged 5-11 months (*n*=90) from Zanzibar who received iron, folate and zinc for 1 year achieved walking unassisted 1 month earlier than infants receiving a placebo (*n*=103). Dhingra et al (14) conducted a trial in 633 young children aged 1-3 years from India who received fortified or unfortified milk. After 1 year of supplementation, no significant differences between the two groups were found on the Bayley's mental and motor development scores, behavior and language development.

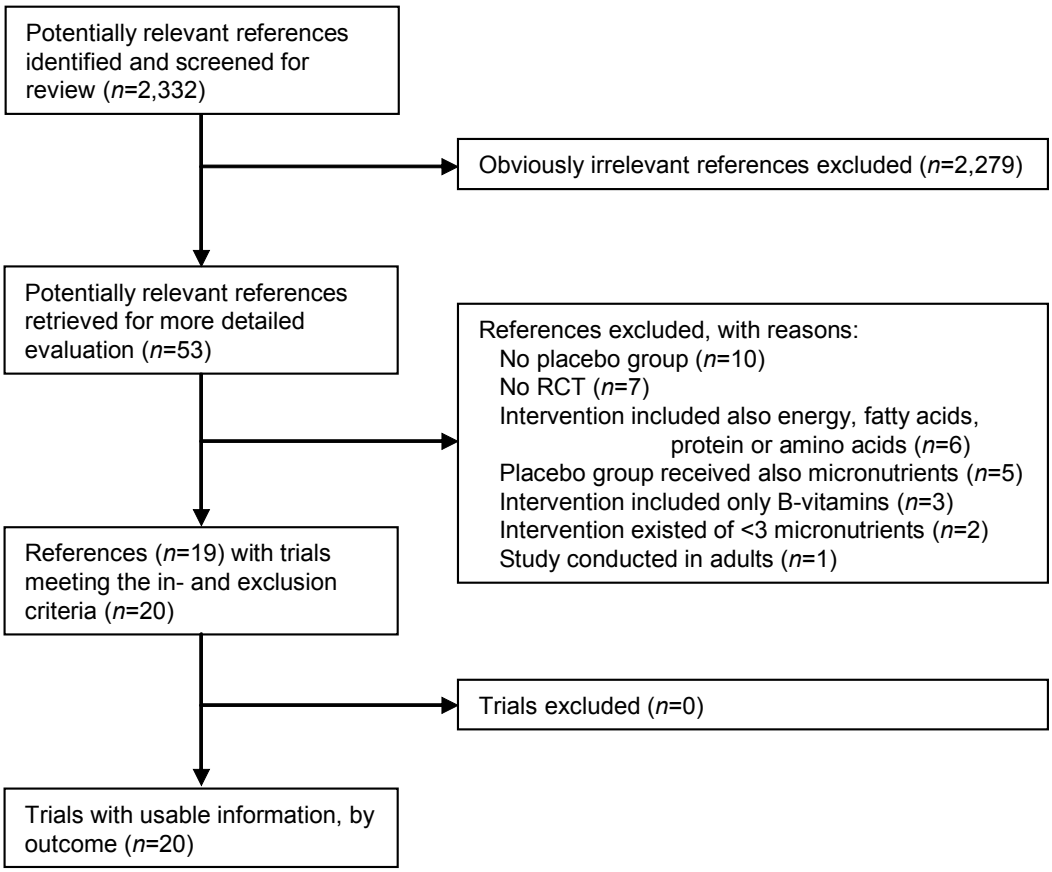


**Figure 1**. Flow diagram for inclusion of randomized controlled trials (RCT).

Sixteen publications with trials in children ≥5 years of age were found (17-32) (see **Table 1**), of which one trial (25) was reported as an abstract. One publication (24) described two trials following the same design but conducted in two different countries, hence a total of 17 trials were identified. Six trials were conducted in developing countries (21;22;24;30-32). Nine trials were conducted in children

**Table 1**. Overview of trial characteristics[1]

| Author, country, year (reference) | Initial sample size (*n*) | Attrition rate (%) | Mean age (y) | Prevalence of stunting (%)[2] | Mean baseline hemoglobin concentration (µg/L) | Intervention[3] | Duration (wk)[4] | Main cognitive outcomes |
|---|---|---|---|---|---|---|---|---|
| *Trials in children aged <5 years* | | | | | | | | |
| Dhingra et al, India, 2004 (14)[5] | 633 | NA | 2.0 | NA | NA | Vitamins: 330 µg RE vit A, 48 mg vit C, 8.1 mg vit E<br>Minerals: 0.3 mg Cu, 9.6 mg Fe, 9.6 mg Zn | 26 | No significant differences between groups |
| Faber et al, South Africa, 2005 (15) | 361 | 19 | 0.7 | 11.5 | 111 | Vitamins: 0.4 mg $B_2$, 0.15 mg $B_6$, 0.25 µg $B_{12}$, 56 mg vit C, 2.5 mg vit E, 3 mg β-carotene<br>Minerals: 110 µg Cu, 11 mg Fe, 10 µg Se, 3 mg Zn | 26 | Positive effect of micronutrient treatment on motor development ($P$=0.007) |
| Olney et al, Zanzibar, 2006 (16)[5] | 435 | 67 | 0.7 | NA | 94 | Vitamins: 50 µg folate<br>Minerals: 12.5 mg Fe, 10 mg Zn | 52 | Positive effect of micronutrient on time to walk unassisted ($P$=0.035) |
| *Trials in children aged 5-16 years* | | | | | | | | |
| Benton & Roberts, UK, 1988 (17) | 64 | NA | 12.5 | NA | NA | Vitamins: 375 µg vit A, 3.9 mg $B_1$, 5 mg $B_2$, 50 mg $B_3$, 50 mg $B_5$, 12 mg $B_6$, 100 µg biotin, 100 µg folate, 10 µg $B_{12}$, 500 mg vit C, 3 µg vit D, 70 IU vit E, 100 µg vit K<br>Minerals: 100 mg Ca, 0.2 mg Cr, 1.3 mg Fe, 50 µg I, 7.6 mg Mg, 1.5 mg Mn, 0.1 mg Mo, 10 mg Zn<br>Other: 50 mg bioflavanoids, 70 mg choline bitartrate, 30 mg inositol, 10 mg PABA | 35 | Positive effect of micronutrient treatment on non-verbal IQ ($P$<0.01) |
| Benton & Buts, Belgium, 1990 (18)[5,6] | 167 | NA | 13.0 | NA | NA | Vitamins: 5000 IU vit A, 1.5 mg $B_1$, 1.7 mg $B_2$, 20 mg $B_3$, 2 mg $B_6$, 400 µg folate, 6 µg $B_{12}$, 60 mg vit C, 400 IU vit D, 15 IU vit E<br>Minerals: 1.6 mg Ca, 2 mg Cu, 18 mg Fe, 25 mg Mg, 1 mg Mn, 10 mg Zn | 22 | Positive effect of micronutrient treatment on non-verbal IQ in boys with diet low in micronutrients (~<50% RDA) ($P$<0.02) |
| Benton & Cook, US, 1991 (19) | 47 | 6 | 6.5 | NA | NA | Vitamins: 300 µg vit A, 0.7 mg $B_1$, 0.9 mg $B_2$, 10 mg $B_3$, 25 mg $B_5$, 1.3 mg $B_6$, 10 µg biotin, 200 µg folate, 2.5 µg $B_{12}$, 70 mg vit C, 6 mg vit E, 10 µg vit D, 100 µg vit K<br>Minerals: 30 mg Ca, 30 µg Cu, 100 µg Cr, 2.4 mg Fe, 50 µg I, 8 mg Mg, 1 mg Mn, 0.1 mg Mo, 4 mg Zn<br>Other: 20 mg bioflavonoids, 35 mg choline bitartrate, 15 mg inositol, 5 mg PABA | 7 | Positive effect of micronutrient treatment on total IQ and non-verbal tests ($P$<0.005) |
| Crombie et al, UK, 1990 (20) | 94 | 9 | 12.3 | NA | NA | Vitamins: 375 µg vit A, 3.9 mg $B_1$, 5 mg $B_2$, 50 mg $B_3$, 50 mg $B_5$, 12 mg $B_6$, 100 µg biotin, 100 µg folate, 10 µg $B_{12}$, 500 mg vit C, 3 µg vit D, 70 IU vit E, 100 µg vit K<br>Minerals: 100 mg Ca, 0.2 mg Cr, 1.3 mg Fe, 50 µg I, 7.6 mg Mg, 1.5 mg Mn, 0.1 mg Mo, 10 mg Zn<br>Other: 50 mg bioflavanoids, 70 mg choline bitartrate, 30 mg inositol, 10 mg PABA | 30 | No significant differences between groups |

| Author, country, year (reference) | Initial sample size (*n*) | Attrition rate (%) | Mean age (y) | Prevalence of stunting (%)[2] | Mean baseline hemoglobin concentration (μg/L) | Intervention[3] | Duration (wk)[4] | Main cognitive outcomes |
|---|---|---|---|---|---|---|---|---|
| Jinabhai et al, South Africa, 2001 (21) | 386 | 14 | 9.0 | 7.3 | NA | Vitamins: 350 μg vit A, 0.25 mg vit B,<br>Minerals: 200 mg Ca, 5 mg Fe, 2.5 mg Zn | 16 | No significant differences between groups |
| Manger et al, Thailand, 2008 (22) | 569 | 11 | 9.2 | 10.4 | 120 | Vitamins: 270 μg RE vit A<br>Minerals: 5 mg Fe, 50 μg I, 5 mg Zn | 31 | Positive effect of micronutrient treatment on visual recall test (0.5 more items 95% CI: 0.1, 0.9) |
| Nelson et al, UK, 1990, 7-10 years (23)[5] | 51 | 2 | 8.5 | NA | NA | Vitamins: 1000 μg vit A, 2.2 mg $B_1$, 2.8 mg $B_2$, 24 mg $B_3$, 50 mg $B_5$, 2 mg $B_6$, 100 μg biotin, 450 μg folate, 5 μg $B_{12}$, 50 mg vit C, 10 mg vit E, 15 μg vit D, 100 μg vit K<br>Minerals: 100 mg Ca, 200 μg Cr, 2 mg Cu, 15 mg Fe, 100 μg I, 25 mg Mg, 1.5 mg Mn, 150 μg Se, 15 mg Zn | 4 | No significant differences between groups |
| Nelson et al, UK, 1990, 11-12 years (23)[5] | 176 | 9 | 11.5 | NA | NA | Vitamins: 1000 μg vit A, 2.2 mg $B_1$, 2.8 mg $B_2$, 24 mg $B_3$, 50 mg $B_5$, 2 mg $B_6$, 100 μg biotin, 450 μg folate, 5 μg $B_{12}$, 50 mg vit C, 10 mg vit E, 15 μg vit D, 100 μg vit K<br>Minerals: 100 mg Ca, 200 μg Cr, 2 mg Cu, 15 mg Fe, 100 μg I, 25 mg Mg, 1.5 mg Mn, 150 μg Se, 15 mg Zn | 4 | No significant differences between groups |
| Osendarp et al, Australia, 2007 (24) | 396 | 30 | 8.7 | NA | 130 | Vitamins: 400 μg RE vit A, 1 mg $B_6$, 150 μg folate, 1.5 μg $B_{12}$, 45 mg vit C<br>Minerals: 10 mg Fe, 5 mg Zn | 52 | Positive effect of micronutrient treatment on verbal learning and memory factor (effect size, 0.23; 95% CI: 0.01, 0.46) |
| Osendarp et al, Indonesia, 2007 (24) | 384 | 4 | 8.2 | NA | 128 | Vitamins: 400 μg RE vit A, 1 mg $B_6$, 150 μg folate, 1.5 μg $B_{12}$, 45 mg vit C<br>Minerals: 10 mg Fe, 5 mg Zn | 52 | Positive effect of micronutrient treatment on verbal learning and memory factor in girls (effect size, 0.32; 95% CI: -0.01, 0.64) |
| Penland et al, USA, 1999 (25)[5-7] | 240 | NA | 7.5 | NA | NA | MN: Vitamins: 2500 IU vit A, 0.9 mg $B_1$, 1.1 mg $B_2$, 12 mg $B_3$, 1.1 mg $B_6$, 35 μg folate, 1 μg $B_{12}$, 400 IU vit D, 7 mg vit E, 20 μg vit K<br>Minerals: 1 mg Cu, 30 μg Cr, 1 mg F, 90 μg I, 1.5 mg Mn, 30 μg Mo, 20 μg Se<br>MN+Zn: MN + 20 mg Zn<br>MN+Fe: MN + 24 mg Fe | 10 | Positive effect of MN+Zn treatment on reasoning test ($P<0.05$) |
| Schoenthaler et al, USA, 1991 (26)[8] | 615 | 7 | 14.0 | NA | NA | Vitamins: 5000 IU vit A, 1.5 mg $B_1$, 1.7 mg $B_2$, 20 mg $B_3$, 10 mg $B_5$, 2 mg $B_6$, 300 μg biotin, 400 μg folate, 6 μg $B_{12}$, 60 mg vit C, 400 IU vit D, 30 IU vit E, 50 μg vit K<br>Minerals: 200 mg Ca, 2 mg Cu, 0.10 mg Cr, 18 mg Fe, 150 μg I, 80 mg Mg, 2.5 mg Mn, 0.25 mg Mo, 0.10 mg Se, 15 mg Zn | 13 | Positive effect of micronutrient treatment with 100% RDA on non-verbal IQ ($P=0.01$) |

| Author, country, year (reference) | Initial sample size (*n*) | Attrition rate (%) | Mean age (y) | Prevalence of stunting (%)[2] | Mean baseline hemoglobin concentration (μg/L) | Intervention[3] | Duration (wk)[4] | Main cognitive outcomes |
|---|---|---|---|---|---|---|---|---|
| Schoenthaler et al, USA, 2000 (27) | 468 | 16 | 8.9 | NA | NA | Vitamins: 750 μg vit A, 0.75 mg $B_1$, 0.85 mg $B_2$, 10 mg $B_3$, 5 mg $B_5$, 1 mg $B_6$, 200 μg folate, 3 μg $B_{12}$, 40 mg vit C, 20 mg vit E, 5 μg vit D<br>Minerals: 50 μg Cr, 1 μg Cu, 9 mg Fe, 1.25 μg Mn, 0.12 μg Mo, 50 μg Se, 7.5 mg Zn | 13 | Positive effect of micronutrient treatment on non-verbal IQ (2.5 IQ points, 95% CI: 1.85, 2.15) |
| Snowden, UK, 1997 (28) | 30 | 40 | 9.5 | NA | NA | Vitamins: 375 μg vit A, 3.9 mg $B_1$, 5 mg $B_2$, 50 mg $B_3$, 50 mg $B_5$, 12 mg $B_6$, 100 μg biotin, 100 μg folate, 10 μg $B_{12}$, 500 mg vit C, 3 μg vit D, 70 IU vit E, 100 μg vit K<br>Minerals: 100 mg Ca, 0.2 mg Cr, 1.3 mg Fe, 50 μg I, 7.6 mg Mg, 1.5 mg Mn, 0.1 mg Mo, 10 mg Zn<br>Other: 50 mg bioflavanoids, 70 mg choline bitartrate, 30 mg inositol, 10 mg PABA | 10 | Positive effect of micronutrient treatment on non-verbal IQ ($P$=0.02) |
| Solon et al, Philippines, 2003 (29) | 851 | 13 | 9.9 | NA | 119 | Vitamins: 420 μg vit A, 0.92 mg $B_2$, 5 mg $B_3$, 1.0 mg $B_6$, 120 μg folate, 1.0 μg $B_{12}$, 150 mg vit C, 5 mg TE vit E<br>Minerals: 9.6 mg Fe, 96 μg I, 7.5 mg Zn | 16 | Positive effect of micronutrient treatment in iodine and iron deficient subjects on non-verbal ability ($P$=0.03) and verbal ability ($P$=0.02) |
| Southon et al, UK, 1994 (30)[6] | 54 | 6 | 13.6 | NA | 140 | Vitamins: 1 mg $B_1$, 1.4 mg $B_2$, 16 mg $B_3$, 4 mg $B_5$, 1.8 mg $B_6$, 100 μg biotin, 400 μg folate, 3 μg $B_{12}$, 25 mg vit C, 10 μg vit D<br>Minerals: 2 mg Cu, 50 μg Cr, 12 mg Fe, 150 μg I, 2.5 mg Mn, 50 μg Se, 15 mg Zn | 16 | No significant differences between groups |
| Vazir et al, India, 2006 (31)[9] | 869 | 30 | 10.7 | 11.5 | 118 | Vitamins: 400 μg RE vit A, 0.7 mg $B_1$, 1.6 mg $B_2$, 0.9 mg $B_3$, 2 mg $B_6$, 200 μg folate, 1 μg $B_{12}$, 80 mg vit C and 2.5 μg vit D<br>Minerals: 400 mg Ca, 14 mg Fe, 75 μg I, 2.3 mg Zn | 60 | Positive effect of micronutrient treatment on attention-concentration test ($P$<0.05) |
| Wang et al, China, 2003 (32)[5] | 220 | 0 | 10.0 | NA | NA | Vitamins: 400 μg RE vit A, 1 mg $B_1$, 1 mg $B_2$, 1 mg $B_6$, 100 μg folate, 50 mg vit C, 5 μg vit D<br>Minerals: 400 mg Ca 8 mg Fe, 20 μg Se, 10 mg Zn | 26 | Positive effect of micronutrient treatment on reading speed, learning capacity and mathematics |

[1] Trials were individually randomized double blind; NA, not available.
[2] Stunting was defined as <-2SD height-for-age *z*-scores based on National Center for Health Statistics/World Health Organization growth reference data.
[3] All placebo groups received no micronutrients.
[4] All cognitive outcomes were measured at the end of the duration of the intervention.
[5] Procedure of randomization is unclear.
[6] Procedure of blinding is unclear.
[7] Trial included three intervention groups: MN, micronutrients, MN+Zn, micronutrients + zinc, MN+Fe, micronutrients + iron.
[8] Trial had initially three intervention groups providing, 50, 100 or 200% of the RDA of the micronutrients. For the meta-analysis the three groups were pooled, and the amounts of micronutrients of the 100% RDA group are presented.
[9] Trial had a cluster randomized design.

5-12 years of age, four trials in children 12-16 years of age and four trials in children 5-16 years of age. The duration of the interventions varied from 4 to 60 weeks. The number of micronutrients provided ranged from 5 to 25. The range of doses of micronutrients was 1.2-24 mg/d (4-133% Recommended Dietary Allowance (RDA)) for iron, 50-100 µg/d (40-110% of the RDA) for iodine, 2.5-20 mg/d (20-180% of the RDA) for zinc, 35-450 µg/d (12-150% of the RDA) for folate and 0.45-10 µg/d (25-420% of the RDA) for vitamin $B_{12}$. Subjects received micronutrient supplements in 11 trials and, a micronutrient fortified beverage, biscuit or seasoning powder in six trials. The cognitive tests used in the trials were clustered into the cognitive domains as shown in **Table 2**.

**Fluid intelligence**

Twelve trials assessed the domain of fluid intelligence, of which 10 measured this domain by a combined score of subtests for non-verbal intelligence (17-20;23;26-29;31) and two by individual tests (25;30). The funnel plot looked somewhat asymmetrical (see **Figure 2**), however there was no evidence for publication bias by Begg (*P*=0.14) and Egger (*P*=0.15) tests.

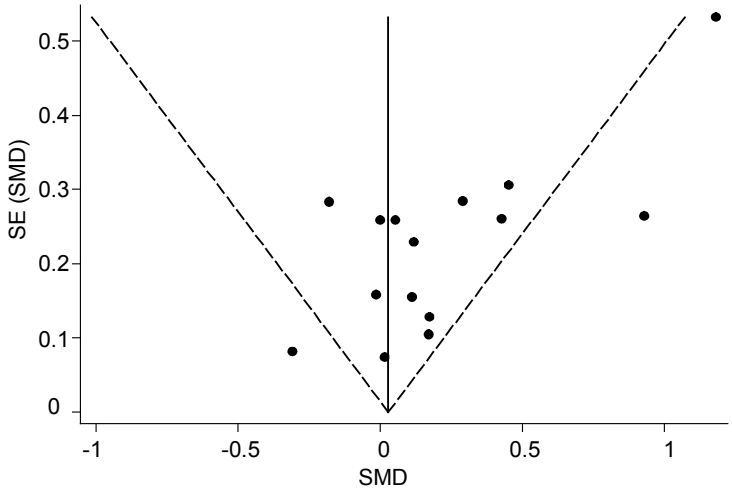


**Figure 2.** Funnel plot for fluid intelligence with 95% confidence limits. SMD, standardized mean difference.

The heterogeneity among trials was significant (*P*<0.001) and the overall effect size of multiple micronutrients on fluid intelligence was 0.14 SD (95% CI: -0.02, 0.29), *P*=0.083 (see **Figure 3**). On influence analysis, the effect size did not change significantly when trials were omitted one at a time. Subgroup analyses revealed that compliance, method of randomization, method of analysis, country development status, supplementation vehicle and number of micronutrients provided were significant predictors of heterogeneity (see **Table 3**). Only country development status, supplementation vehicle and number of micronutrients provided were also significant in univariate regression analysis (**Table 4**). In multivariate regression analysis using a model with country development status and number of micronutrients, these variables were no longer significant (Table 4).

**Table 2.** Overview of clustering of cognitive tests into cognitive domains according to Carroll's model[1]

| | Fluid intelligence | Crystallized intelligence | Short-term memory | Visual perception | Long-term memory and retrieval | Cognitive processing speed | Sustained attention | Academic Performance |
|---|---|---|---|---|---|---|---|---|
| Benton & Roberts (1988) | Calvert non-verbal | Verbal Cognitive Abilities Test | | | | | | |
| Benton & Buts (1990) | Calvert non-verbal + DG or OOHMT | | | | | | | |
| Benton & Cook (1991) | BAS Matrices | BAS Similarities<br>BAS Vocabulary | BAS Recall of digits | | | | Shakow's paradigm | |
| Crombie et al (1990) | Calvert non-verbal<br>Cattell<br>Heim AH4 part 2<br>Raven's matrices | Verbal Cognitive Abilities Test<br>Heim AH4 part 1 | | | | | | |
| Jinabhai et al (2001) | | GMT | | | RAVLT | SDMT | | |
| Manger et al (2008) | | | | | | | | Mathematics<br>Thai<br>English<br>Science |
| Nelson et al (1990) 7-10 years | Heim AH1X/Y | | WISC-R Digit span | | | WISC-R Coding | | |
| Nelson et al (1990) 11-12 years | Heim AH4 part 2 | Heim AH4 part 1 | WISC-R Digit span | | | WISC-R Coding | | |
| Osendarp et al (2007) Australia | | WISC-III Vocabulary<br>WIAT Mathematical reasoning<br>WIAT Reading comprehension<br>WIAT Spelling | WISC-III Digit span<br>RAVLT immediate recall | WISC-III /WAIS Block design | RAVLT | WISC-III Coding<br>NEPSY Fluency structured<br>NEPSY Fluency random | NEPSY Visual attention 2 | |
| Osendarp et al (2007) Indonesia | | WISC-III Vocabulary<br>WIAT Mathematical reasoning<br>NAR Reading comprehension | WISC-III Digit span<br>RAVLT immediate recall | WISC-III /WAIS Block design | RAVLT | WISC-III Coding<br>NEPSY Fluency structured<br>NEPSY Fluency random | NEPSY Visual attention 2 | |
| Penland et al (1999) | CPAS-R Oddity task | | | | | | | |
| Schoenthaler et al (1991) | WISC-R non-verbal<br>Matrix Analogies Test | WISC-R verbal | | | | | | CTBS |

| | Fluid intelligence | Crystallized intelligence | Short-term memory | Visual perception | Long-term memory and retrieval | Cognitive processing speed | Sustained attention | Academic Performance |
|---|---|---|---|---|---|---|---|---|
| Schoenthaler et al (2000) | WISC-R non-verbal | | | | | | | |
| Snowden (1997) | Calvert non-verbal | Verbal Cognitive Abilities Test | | | | | | |
| Solon et al (2003) | PMAT-FC non-verbal | PMAT-FC verbal | | | | | | |
| Southon et al (1994) | WISC-R picture arrangement | WISC-R Information<br>WISC-R Similarities<br>WISC-R Vocabulary<br>WISC-R Comprehension | WISC-R Digit span | WISC-R Picture completion<br>WISC-R Block design<br>WISC-R Object assembly | | WISC-R Coding | | |
| Vazir et al (2006) | MISIC Performance IQ | MISIC Verbal IQ | | Knox Cube Imitation test | PGI memory scale | Letter Cancel-lation test | | Mathematics<br>Science<br>Social studies |
| Wang et al (2003) | | | | | | | | Learning capacity<br>Mathematics<br>Reading speed<br>Language |

[1] Includes all cognitive domains according to Carroll's model, with the exception of auditory processing, which has not been measured by any of the trials. BAS, Brisith Ability Scale; DG, Differentiele Geschiktheidsbatterij; CPAS-R, Cognitive Psychomotor Assessment System-Revised; CTBS, Comprehensive Test of Basic Skills; GMT, Group Mathematics Test; MISIC, Malin's Intelligence Scale for Children; NAR, Neale Analysis of Reading; OOHMT, Otis Ottawa d'Habitele Mentale Test; PGI, Post Graduate Institute (India); PMAT-FC, Primary Mental Abilities Test for Filipino Children; NEPSY, Developmental Neuropsychological Assessment; RAVLT, Rey Auditory Verbal Learning Test; SDMT, Symbol Digit Modalities Test; WAIS, Wechsler Adult Intelligence Scale; WIAT, Wechsler Individual Achievement Test Screener; WISC-III/R, Wechsler Intelligence Scale for Children-III/Revised, IQ, Intelligence Quotient.

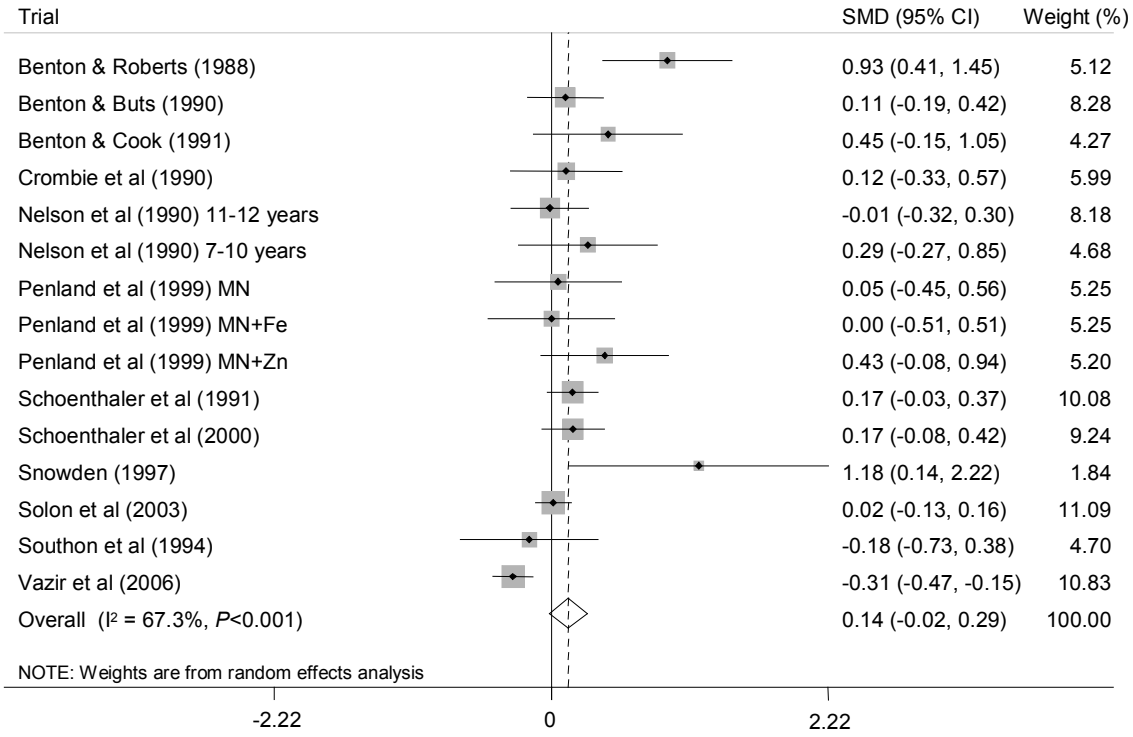


**Figure 3**. Forest plot for fluid intelligence, SMD, standardized mean difference.

## Crystallized intelligence

Crystallized intelligence was assessed in 12 trials, of which eight measured this domain by a combined score of subtests for verbal intelligence (17;19;20;23;26;28;29;31), and three trials by 3-4 subtests (24;30) and one trial by one subtest (21). From statistical tests by Begg ($P$=0.95) and Egger ($P$=0.77) there was no evidence for publication bias, but the funnel plot looked slightly asymmetrical (see **Figure 4**). There was significant heterogeneity between the

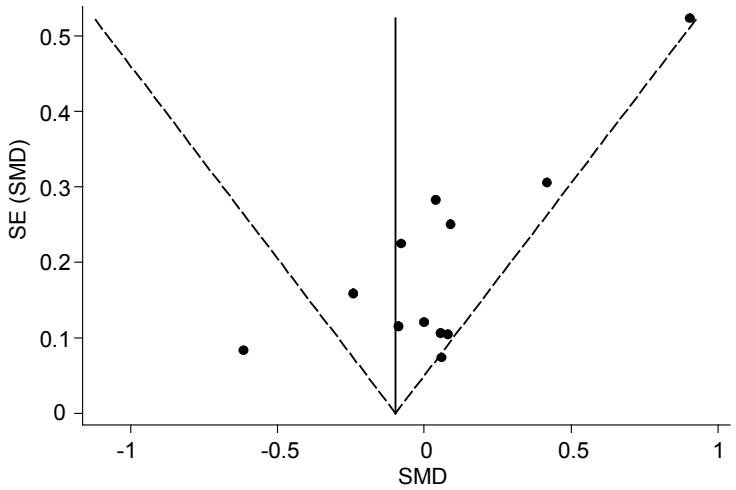


**Figure 4**. Funnel plot for crystallized intelligence with 95% confidence limits. SMD, standardized mean difference.

trials (*P*<0.001). Overall, there was no significant effect (-0.03 SD, 95% CI: -0.21, 0.15; *P*=0.74) of multiple micronutrients on crystallized intelligence (see **Figure 5**). When trials were omitted one at a time, the overall effect size did not change significantly (data not shown). Significant predictors of heterogeneity on subgroup analyses were allocation concealment, compliance, method of randomization, method of analysis, and inclusion of iodine (Table 3), whereas no variables significantly explained heterogeneity in univariate analyses (Table 4).

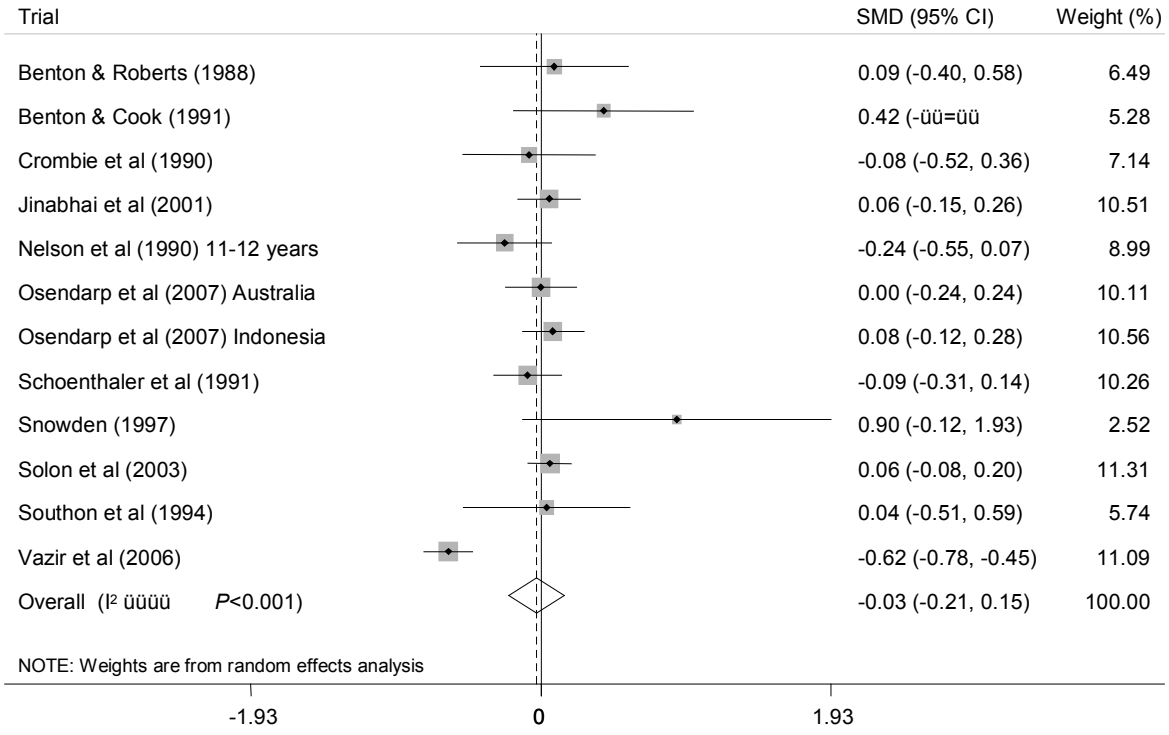


**Figure 5**. Forest plot for crystallized intelligence, SMD, standardized mean difference.

## Other cognitive domains

For the other cognitive domains ≤7 trials were available. For all domains, except short-term memory and sustained attention, there was significant heterogeneity between the trials. The overall effect sizes are presented in Table 5. A significant positive overall effect of micronutrient supplementation was found on academic performance (0.30 SD, 95% CI: 0.01, 0.58; *P*=0.044) based on four trials (22;26;31;32). These studies had a relatively large sample size of 220-869 subjects, and three of them (22;31;32) were conducted in developing countries. There were no significant overall effects on the domains of short-term memory, visual perception, retrieval ability, cognitive processing speed and sustained attention.

**Table 3**. Sensitivity and subgroup analyses for fluid and crystallized intelligence

| | Fluid intelligence | | | | Crystallized intelligence | | | |
|---|---|---|---|---|---|---|---|---|
| Stratification variable | No of trials | Overall effect size[1] (95% CI); *P*-value | Tests for heterogeneity $I^2$(%); Q (*P*-value) | *P* for hetero-geneity between subgroups | No of trials | Overall effect size[1] (95% CI); *P*-value | Tests for heterogeneity $I^2$(%); Q (*P*-value) | *P* for hetero-geneity between subgroups |
| Change scores | 15 | 0.14 (-0.02, 0.29); 0.083 | 67.3; 42.8 (<0.001) | NA | 12 | -0.03 (-0.21, 0.15); 0.741 | 80.8; 57.2 (<0.001) | NA |
| End scores | 7 | 0.35 (0.11, 0.59); 0.004 | 44.9; 10.9 (0.920) | | 9 | 0.00 (-0.11, 0.11); 0.957 | 14.6; 9.4 (0.312) | |
| Allocation concealment | | | | | | | | |
| Adequate | 7 | 0.27 (-0.05, 0.58); 0.097 | 84.4; 38.5 (<0.001) | 0.665[2] | 9 | -0.01 (-0.25, 0.23); 0.928 | 84.1; 50.4 (<0.001) | 0.050[2] |
| Others | 8 | 0.05 (-0.06, 0.15); 0.400 | 0.0; 4.1 (0.773) | | 3 | -0.03 (-0.21, 0.17); 0.789 | 32.8; 3.0 (0.226) | |
| Blinding | | | | | | | | |
| Double Blinded | 8 | 0.20 (-0.04, 0.43); 0.106 | 81.9; 38.6 (<0.001) | 0.451[2] | 10 | -0.01 (-0.22, 0.20); 0.929 | 84.0; 56.1 (<0.001) | 0.565[2] |
| Others | 7 | 0.08 (-0.08, 0.24); 0.307 | 0.0; 3.7 (0.720) | | 2 | -0.17 (-0.45, 0.10); 0.208 | 0.0; 0.75 (0.386) | |
| Attrition rate | | | | | | | | |
| <10% | 6 | 0.13 (-0.02, 0.27); 0.085 | 0.0; 3.6 (0.607) | 0.120 | 6 | -0.03 (-0.16, 0.09);0.618 | 0.0; 4.9 (0.423) | 0.208[2] |
| Others | 9 | 0.17 (-0.06, 0.39); 0.152 | 78.3; 36.8 (<0.001) | | 6 | -0.03 (-0.34, 0.29); 0.878 | 90.1; 50.7 (<0.001) | |
| Comparability of groups at baseline | | | | | | | | |
| Yes | 11 | 0.14 (-0.05, 0.33); 0.139 | 75.0; 40.0 (<0.001) | 0.285 | In all trials, groups were comparable at baseline | | | |
| Others | 4 | 0.14 (-0.07, 0.35); 0.204 | 0.0; 1.7 (0.646) | | | | | |
| Compliance | | | | | | | | |
| Adequate (≥70%) | 6 | -0.01 (-0.24, 0.22); 0.921 | 69.5; 16.4 (0.006) | 0.005 | 7 | -0.15 (-0.39, 0.10); 0.247 | 83.7; 36.8 (<0.001) | <0.001[2] |
| Others | 9 | 0.25 (0.05, 0.44); 0.014 | 56.5; 18.4 (0.018) | | 5 | 0.08 (-0.03, 0.20); 0.150 | 0.0; 3.8 (0.429) | |
| Randomization method | | | | | | | | |
| Individual | 10 | 0.19 (0.03, 0.35); 0.023 | 53.1; 19.2 (0.024) | 0.001 | 11 | 0.03 (-0.05, 0.10); 0.539 | 0.0; 9.2 (0.517) | <0.001 |
| Others | 5 | 0.01 (-0.27, 0.30); 0.930 | 68.2; 12.57 (0.014) | | 1 | -0.62 (-0.78, -0.45);<0.001 | NA | |
| Method of analyses | | | | | | | | |
| Intention to treat | 4 | -0.06 (-0.36, 0.25); 0.708 | 74.1; 11.6 (0.009) | 0.001 | 5 | -0.13 (-0.48, 0.22); 0.463 | 88.7; 35.3 (<0.001) | <0.001[2] |
| Others | 11 | 0.20 (0.05, 0.36); 0.012 | 48.9; 19.6 (0.034) | | 7 | 0.02 (-0.11, 0.15); 0.767 | 30.4; 8.6 (0.196) | |
| HDI rank of countries[3] | | | | | | | | |
| High | 13 | 0.20 (0.06, 0.34); 0.005 | 31.7; 17.6 (0.129) | <0.001 | 8 | -0.03 (-0.16, 0.11); 0.702 | 9.8; 7.8 (0.354) | 0.215 |
| Medium | 2 | -0.15 (-0.46, 0.17); 0.372 | 88.6; 8.8 (0.003) | | 4 | -0.11 (-0.46, 0.25); 0.551 | 47.9; 93.7 (<0.001) | |
| Supplementation vehicle | | | | | | | | |
| Tablets | 13 | 0.20 (0.06, 0.34); 0.005 | 31.7; 17.6 (0.129) | <0.001 | 7 | -0.02 (-0.20, 0.16); 0.831 | 21.7; 7.7 (0.264) | 0.426[2] |
| Fortification | 2 | -0.15 (-0.46, 0.17); 0.372 | 88.6; 8.8 (0.003) | | 5 | -0.09 (-0.38, 0.20); 0.556 | 91.8; 48.9 (<0.001) | |

| | Fluid intelligence | | | | Crystallized intelligence | | | |
|---|---|---|---|---|---|---|---|---|
| Stratification variable | No of trials | Overall effect size[1] (95% CI); *P*-value | Tests for heterogeneity $I^2$(%); Q (*P*-value) | *P* for hetero-geneity between subgroups | No of trials | Overall effect size[1] (95% CI); *P*-value | Tests for heterogeneity $I^2$(%); Q (*P*-value) | *P* for hetero-geneity between subgroups |
| No of micronutrients | | | | | | | | |
| 5-20 | 8 | 0.01 (-0.16, 0.18); 0.913 | 62.8; 18.8 (0.009) | 0.001 | 6 | -0.07 (-0.34, 0.20); 0.590 | 89.8; 49.2 (<0.001) | 0.505[2] |
| >20 | 7 | 0.32 (0.07, 0.57); 0.013 | 56.3; 13.7 (0.033) | | 6 | -0.01 (-0.22, 0.20); 0.918 | 33.9; 7.6 (0.182) | |
| Duration | | | | | | | | |
| ≤12 weeks | 6 | 0.24 (-0.02, 0.50); 0.067 | 32.3; 7.4 (0.194) | 0.088 | 3 | 0.24 (-0.41, 0.89); 0.475 | 71.9; 7.1 (0.029) | 0.625[2] |
| >12 weeks | 9 | 0.09 (-0.10, 0.27); 0.365 | 75.4; 32.5 (<0.001) | | 9 | -0.07 (-0.27, 0.14); 0.526 | 83.9; 49.8 (<0.001) | |
| Iron in intervention | | | | | | | | |
| Yes | 13 | 0.13 (-0.04, 0.29); 0.137 | 70.3; 40.4 (<0.001) | 0.241 | | | All trials included iron | |
| No | 2 | 0.24 (-0.13, 0.61); 0.201 | 4.2; 1.1 (0.307) | | | | | |
| Iodine in intervention | | | | | | | | |
| Yes | 13 | 0.14 (-0.04, 0.32); 0.121 | 70.7; 41.0 (<0.001) | 0.188 | 9 | -0.05 (-0.30, 0.21); 0.720 | 83.6; 48.9 (<0.001) | 0.005[2] |
| No | 2 | 0.15 (-0.05, 0.34); 0.132 | 0.0; 0.1 (0.762) | | 3 | 0.05 (-0.08, 0.17); 0.437 | 0.0; 0.26 (0.879) | |

[1] Obtained by using a random effects model.
[2] Considerable heterogeneity observed in one or more sub-groups, test for heterogeneity between sub-groups likely to be invalid.
[3] HDI, Human Development Index, countries were classified according the classification by United Nations Development Programme into HDI ranks high, medium or low.
NA=not applicable.

**Table 4**. Meta-regression analysis for fluid and crystallized intelligence[1]

| | Fluid intelligence | | | | Crystallized intelligence | |
|---|---|---|---|---|---|---|
| | Univariate analysis | | Multivariate analysis[2] | | Univariate analysis | |
| Trial characteristic | β (95% CI) | *P*-value | β (95% CI) | *P*-value | β (95% CI) | *P*-value |
| Trial quality | | | | | | |
| Allocation concealment (others vs adequate) | 0.13 (-0.23, 0.50) | 0.44 | | | 0.02 (-0.43, 0.46) | 0.93 |
| Blinding (others vs double blind) | 0.82 (-0.29, 0.45) | 0.64 | | | 0.12 (-0.44, 0.67) | 0.65 |
| Attrition (others <10%) | -0.02 (-0.40, 0.36) | 0.91 | | | 0.04 (-0.35, 0.43) | 0.83 |
| Comparability at baseline (others vs yes) | -0.008 (-0.44, 0.42) | 0.97 | | | In all trials groups were comparable at baseline | |
| Compliance (others ≥70%) | -0.26 (-0.54, 0.02) | 0.07 | | | -0.31 (-0.67, 0.05) | 0.09 |
| Randomization method (others vs individual) | -0.21 (-0.50, 0.09) | 0.17 | | | -0.32 (-0.41, -0.22) | <0.001 |
| Method of analysis (others vs intention to treat) | -0.28 (-0.63, 0.71) | 0.11 | | | -0.19 (-0.56, 0.17) | 0.26 |
| Mean age (increase per year) | -0.02 (-0.09, 0.06) | 0.69 | | | -0.04 (-0.13, 0.04) | 0.29 |
| HDI rank (medium vs high)[3] | -0.34 (-0.58, -0.09) | 0.007 | -0.07 (-0.54, 0.40) | 0.76 | 0.13 (-0.20, 0.47) | 0.45 |
| Duration (increase per week) | -0.01 (-0.02, 0.00) | 0.10 | | | -0.19 (-0.72, 0.34) | 0.45 |
| Supplementation vehicle (fortification vs tablets)[‡] | -0.34 (-0.58, -0.09) | 0.007 | | | -0.12 (-0.51, 0.27) | 0.52 |
| Increase per micronutrient in intervention | 0.04 (0.01, 0.06) | 0.005 | 0.03 (-0.01, 0.08) | 0.15 | 0.00 (-0.02, 0.03) | 0.73 |
| Iron included in intervention (no vs yes) | 0.12 (-0.38, 0.62) | 0.65 | | | All trials included iron | |
| Iodine included in intervention (no vs yes) | 0.01 (-0.40, 0.41) | 0.97 | | | -0.13 (-0.47, 0.21) | 0.46 |

[1] Multiple regression analysis was only conducted for those variables that were significant ($P<0.05$) on univariate regression analyses.
[2] Obtained by using a model with HDI (continuous), supplementation vehicle (tablets vs fortification), increase per micronutrient in intervention (continuous), and dose of iron (continuous) as covariates.
[3] HDI, Human Development Index, countries were classified according the classification by United Nations Development Programme into HDI ranks high, medium or low. For fluid intelligence, trials with a high HDI also provided micronutrients in tablet form and trials with medium HDI provided micronutrients in fortified products; therefore only HDI of countries was taken into account in the multivariate analysis.

**Table 5**. Overall effect size estimates per cognitive domain

| | | Overall effect size[1] | | Tests for heterogeneity | |
|---|---|---|---|---|---|
| Cognitive domain | No of trials | (95% CI) | *P*-value | $I^2$(%); Q | *P*-value |
| Short-term memory | 6 | 0.05 (-0.11, 0.21) | 0.55 | 24.7; 6.6 | 0.25 |
| Visual perception | 4 | 0.14 (-0.28, 0.56) | 0.51 | 91.7; 36.1 | <0.001 |
| Long term memory | 4 | 0.01 (-0.15, 0.17) | 0.89 | 58.9; 7.3 | 0.06 |
| Cognitive processing speed | 7 | -0.20 (-0.61, 0.22) | 0.35 | 94.3; 105.2 | <0.001 |
| Sustained attention | 3 | 0.13 (-0.11, 0.36) | 0.30 | 49.1; 3.9 | 0.14 |
| Academic performance[2] | 4 | 0.30 (0.01, 0.58) | 0.04 | 86.9; 22.9 | <0.001 |

[1] Obtained by using a random effects model.
[2] Effect size estimated from end scores.

## Discussion

This meta-analysis suggests the possibility of a small positive effect of multiple micronutrient supplementation on fluid intelligence (reasoning ability), which was not statistically significant (*P*=0.083) and, a positive effect on academic performance (based on a limited number of four trials, *P*=0.044) in children 5-16 years of age. There were no effects on crystallized intelligence (acquired knowledge) and other cognitive domains.

### Strengths and limitations of the review

We conducted an up-to-date systematic review employing rigorous selection methodology, including studies from both developed and developing countries. Furthermore, we performed a meta-analysis at the level of the different cognitive domains, which has not been shown before in literature. We realize that our definition of multiple micronutrients is arbitrary, however others have used the same definition as ours (33-35). Furthermore, micronutrient interventions varied across trials; hence, results should be interpreted accordingly. We found no evidence for publication bias among trials assessing the domains of fluid and crystallized intelligence and influence analyses did not indicate that any of the trials had a significant impact on the overall effect sizes for these domains. In the cluster randomized trial (31), a correction for design effects was not possible. We conducted a large number of subgroup and meta-regression analyses and given the increased risk of false positive results, significant findings should be interpreted with caution. Unfortunately, we lacked data on indicators of nutritional status to explore whether malnourished children would benefit more from multiple micronutrients than well-nourished children. However, our main conclusions regarding fluid and crystallized intelligence did not change after performance of the sensitivity analyses.

### Choice of outcome measures

We included trials that measured cognitive performance as primary or secondary outcome. Although the cognitive test batteries differed by trial, most of them comprised subtests from reliable and valid test batteries such as the Wechsler's Intelligence Scale for Children or validated adapted versions (29;31). In addition,

measuring a similar domain with different subtests can be viewed as a more robust assessment of that particular domain (36). However, the lack of availability of data on subtest level for most of the older trials (published in the 1990s) hampered the initial aim to classify subtests into cognitive domains and therefore combined scores were used for classification.

**Predictors of heterogeneity**

There was significant heterogeneity among the trials. For fluid intelligence, heterogeneity could be explained by the difference in development status of the countries, supplementation vehicle and number of micronutrients in both subgroup and univariate meta-regression analyses. Our findings suggest that development status of countries, supplementation vehicle and number of micronutrients provided, could each individually explain the different findings of the different studies in the meta-analysis, as suggested by the univariate regression analysis. However, in the multiple regression analysis, including all of these variables in one model together with the treatment, these variables were not significant predictors anymore probably due to confounding (i.e. dependency in the model).

Contrary to our expectations, for trials conducted in developed countries with high HDI rank and presumingly a better nutritional status, which also provided micronutrient in tablets, the overall effect on fluid intelligence was significantly larger compared to trials conducted in developing countries, with an estimated lower nutritional status, using fortified products. Differences between developed and developing countries may be explained by the fact that most of the cognitive test batteries originated from developed countries and were validated in Western populations, and may therefore be more sensitive than adapted test batteries used in developing countries. Also, an overall lack of energy and protein in diets of children in developing countries may have overruled the effect of micronutrients. This may also explain why provision of micronutrients in fortified foods containing energy and protein may not have resulted in clear benefits of micronutrients only. Furthermore, a higher number of different vitamins and minerals may lead to larger improvements in cognitive performance; however we lack data to clarify what type and dose of micronutrients would be most effective.

**Comparisons with earlier reviews**

Our results for fluid and crystallized intelligence seem to confirm the observations from the earlier review (8), indicating that non-verbal or fluid intelligence, but not verbal or crystallized intelligence, might be improved by micronutrient interventions. Several mechanisms have been described how micronutrients can influence cognition. For instance, iron is required for myelination and neurotransmitter neurochemistry (37) and neurotransmitter processes are impaired in the presence of iron deficiency anemia, which is most probably caused by a reduction in oxygen

availability (38). Zinc is as coenzyme required for neurogenesis, neuronal migration, and synaptogenesis (39) and B-vitamins are required for neurotransmitter synthesis and functioning (40-44), brain energy metabolism (45) and myelination of the spinal cord and brain (44).

There are several hypotheses why the impact of micronutrients might be beneficial to fluid, but not crystallized intelligence. Possibly, the cognitive tests assessing fluid intelligence and the lower cognitive abilities would be more sensitive to pick up subtle differences compared to those for crystallized intelligence. In addition, it may take more time, may be several years, to show significant differences in acquired skills and knowledge following nutritional interventions than for the other cognitive domains, and the duration of the trials may have been too short to demonstrate effects. Furthermore, environmental factors, such as education, parenting styles and socio-economic status may be more important determinants of crystallized intelligence than nutrition.

The overall effect of multiple micronutrients on fluid intelligence (0.14 SD) found in the current meta-analysis is smaller compared to that of iron supplementation alone on mental development (0.41 SD) in children ≥8 years (46). This may be attributed to the fact that doses of iron provided in the meta-analysis on iron only were higher (30-100 mg/d) compared to doses of iron in multiple micronutrient interventions in our meta-analysis (1.2-24 mg/d). In addition, whereas trials on iron only used iron tablets, the studies on multiple micronutrients used food as delivery system. The extra energy and protein provided in the food matrix may have overruled the effects of the micronutrients in these studies. In addition, perhaps single iron supplementation may be as or even more effective for children's development than multiple micronutrients. However, two studies in infants have shown that zinc in addition to iron and folate was equal or more beneficial for their development than iron and folate alone (16;47). Therefore, future, properly designed studies are recommended to determine whether multiple micronutrients are more advantageous than single micronutrients.

**Implications for public health policy and recommendations for future research**

This meta-analysis suggests the possibility of a marginal positive effect of micronutrients on reasoning abilities and academic performance (limited data) of children. However, the evidence is currently not robust enough to recommend routine multiple micronutrient supplementation for improving cognitive performance. To decide whether multiple micronutrients should be recommended for optimal mental development in children, more research is needed, taking into account the observed heterogeneity among the studies, to assess whether multiple micronutrient supplementation has advantages over that of single micronutrients in randomized controlled trials. Furthermore, because of limited data, more trials in developing

countries are needed to investigate whether micronutrients improve cognition and to assess whether perhaps protein and energy in addition to micronutrients would be required to optimize intellectual development of malnourished children. Future trials should include various cognitive domains, such as short-term memory, visual perception, retrieval ability, sustained attention and cognitive processing speed to investigate whether multiple micronutrients affect the cognitive domains in different ways.

## Conclusions

In conclusion, this meta-analysis suggests that multiple micronutrient supplementation in healthy school-age children may be associated with a small increase in fluid intelligence, while crystallized intelligence seems unaffected. However, development of public health guidelines specifically designed to improve cognitive performance of children through routine micronutrient interventions is currently considered premature.

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## References

1. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B. Child development in developing countries 1 - Developmental potential in the first 5 years for children in developing countries. Lancet 2007;369:60-70.
2. Bryan J, Osendarp S, Hughes D, Calvaresi E, Baghurst K, van Klinken JW. Nutrients for cognitive development in school-aged children. Nutr Rev 2004;62:295-306.
3. Zimmermann M, Adou P, Torresani T, Zeder C, Hurrell R. Persistence of goiter despite oval iodine supplementation in goitrous children with iron deficiency anemia in Cote d'Ivoire. Am J Clin Nutr 2000;71:88-93.
4. Zimmermann MB, Jooste PL, Mabapa NS et al. Vitamin A supplementation in iodine-deficient African children decreases thyrotropin stimulation of the thyroid and reduces the goiter rate. Am J Clin Nutr 2007;86:1040-4.
5. Zimmermann MB, Biebinger R, Rohner F et al. Vitamin A supplementation in children with poor vitamin A and iron status increases erythropoietin and hemoglobin concentrations without changing total body iron. Am J Clin Nutr 2006;84:580-6.
6. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr 2004;24:105-31.
7. Sandstrom B. Micronutrient interactions: effects on absorption and bioavailability. Br J Nutr 2001;85 Suppl 2:S181-S185.
8. Benton D. Micro-nutrient supplementation and the intelligence of children. Neurosci Biobehav Rev 2001;25:297-309.
9. Carroll JB. Human cognitive abilities: A survey of factor-analytic studies. New York, NY, US: Cambridge University Press, 1993.
10. Higgins JPT, Deeks JJ, Altman DG. Chapter 16. Special topics in statistics. In: Higgins JPT, Green S, eds. Cochrane Handbook for Systematic Reviews of Interventions. Chichester (UK): John Wiley & Sons 2008.
11. Follmann D, Eliott P, Suh I, Cutler J. Variance imputation for overviews of clinical trials with continuous response. Journal of Clinical Epidemiology 1992;45:769-73.
12. Sterne JAC, Egger M, Smith GD. Investigating and dealing with publication and other biases. In: Egger M, Smith GD, Altman DG, eds. Systematic Reviews in Health Care: Meta-analysis in Context. London: BMJ Publishing 2001:189-208.
13. Sterne JAC, Bradburn MJ, Egger M. Meta-analysis in STATA™. In: Egger M, Smith GD, Altman DG, eds. Systematic Reviews in Health Care: Meta-analysis in Context. London: BMJ Publishing 2001:347-69.
14. Dhingra P, Menon VP, Sazawal S et al. Effect of fortification of milk with zinc and iron along with vitamins C, E, A and selenium on growth, iron status and development in preschoolchildren - A community based double-

masked randomized trial. Pediatr Gastroenterol 2004;53-7.
15. Faber M, Kvalsvig JD, Lombard CJ, Benade AJS. Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. Am J Clin Nutr 2005;82:1032-9.
16. Olney DK, Pollitt E, Kariger PK et al. Combined iron and folic acid supplementation with or without zinc reduces time to walking unassisted among Zanzibari infants 5- to 11-mo old. J Nutr 2006;136:2427-34.
17. Benton D, Roberts G. Effect of vitamin and mineral supplementation on intelligence of a sample of schoolchildren. Lancet 1988;1:140-3.
18. Benton D, Buts JP. Vitamin/mineral supplementation and intelligence. Lancet 1990;335:1158-60.
19. Benton D, Cook R. Vitamin and Mineral Supplements Improve the Intelligence Scores and Concentration of 6-Year-Old Children. Personal Individ Diff 1991;12:1151-8.
20. Crombie IK, Todman J, Mcneill G, Florey CD, Menzies I, Kennedy RA. Effect of Vitamin and Mineral Supplementation on Verbal and Nonverbal Reasoning of Schoolchildren. Lancet 1990;335:744-7.
21. Jinabhai CC, Taylor M, Coutsoudis A, Coovadia HM, Tomkins AM, Sullivan KR. A randomized controlled trial of the effect of antihelminthic treatment and micronutrient fortification on health status and school performance of rural primary schoolchildren. Ann Trop Paediatr 2001;21:319-33.
22. Manger MS, McKenzie JE, Winichagoon P et al. A micronutrient-fortified seasoning powder reduces morbidity and improves short-term cognitive function, but has no effect on anthropometric measures in primary schoolchildren in northeast Thailand: a randomized controlled trial. Am J Clin Nutr 2008;87:1715-22.
23. Nelson M, Naismith DJ, Burley V, Gatenby S, Geddes N. Nutrient intakes, vitamin-mineral supplementation, and intelligence in British schoolchildren. Br J Nutr 1990;64:13-22.
24. Osendarp SJ, Baghurst KI, Bryan J et al. Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia. Am J Clin Nutr 2007;86:1082-93.
25. Penland JG, Sandstead HH, Egger N et al. Zinc, iron and micronutrient supplementation effects on cognitive and psychomotor function of Mexican-American schoolchildren. FASEB J 1999;13:A921.
26. Schoenthaler SJ, Amos SP, Eysenck HJ, Peritz E, Yudkin J. Controlled trial of vitamin-mineral supplementation: effects on intelligence and performance. Personal Individ Diff 1991;12:351-62.
27. Schoenthaler SJ, Bier ID, Young K, Nichols D, Jansenns S. The effect of vitamin-mineral supplementation on the intelligence of American schoolchildren: A randomized, double-blind placebo-controlled trial. J Altern Complement Med 2000;6:19-29.
28. Snowden W. Evidence from an analysis of 2000 errors and omissions made in IQ tests by a small sample of schoolchildren, undergoing vitamin and mineral supplementation, that speed of processing is an important factor in IQ performance. Personal Individ Diff 1997;22:131-4.
29. Solon FS, Sarol JN, Bernardo ABI et al. Effect of a multiple-micronutrients-fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of schoolchildren in the Philippines. Food Nutr Bull 2003;24:S129-S140.
30. Southon S, Wright AJA, Finglas PM, Bailey AL, Loughridge JM, Walker AD. Dietary-Intake and Micronutrient Status of Adolescents - Effect of Vitamin and Trace-Element Supplementation on Indexes of Status and Performance in Tests of Verbal and Nonverbal Intelligence. Br J Nutr 1994;71:897-918.
31. Vazir S, Nagalla B, Thangiah V, Kamasamudram V, Bhattiprolu S. Effect of micronutrient supplement on health and nutritional status of schoolchildren: mental function. Nutrition 2006;22:S26-S32.
32. Wang Y, Yin S, Zhao X et al. [Study on the effect of micronutrients supplementation on health status of children]. Wei Sheng Yan Jiu 2003;32:455-8.
33. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. Cochrane Database Syst Rev 2006;CD004905.
34. Ramakrishnan U, Neufeld LM, Flores R, Rivera J, Martorell R. Multiple micronutrient supplementation during early childhood increases child size at 2 y of age only among high compliers. Am J Clin Nutr 2009;89:1125-31.
35. Allen LH, Peerson JM, Olney DK. Provision of multiple rather than two or fewer micronutrients more effectively improves growth and other outcomes in micronutrient-deficient children and adults. J Nutr 2009;139:1022-30.
36. Isaacs E, Oates J. Nutrition and cognition: assessing cognitive abilities in children and young people. Eur J Nutr 2008;47 Suppl 3:4-24.
37. Beard JL, Connor JR. Iron status and neural functioning. Ann Rev Nutr 2003;23:41-58.
38. Agarwal KN. Iron and the brain: neurotransmitter receptors and magnetic resonance spectroscopy. British Journal of Nutrition 2001;85:S147-S150.
39. Bhatnagar S, Taneja S. Zinc and cognitive development. British Journal of Nutrition 2001;85:S139-S145.
40. Bettendorff L. Thiamine in excitable tissues - Reflections on a non-cofactor role. Metabolic Brain Disease 1994;9:183-209.
41. Guilarte TR. Vitamin B6 and cognitive development: recent research findings from human and animal studies. Nutr Rev 1993;51:193-8.
42. Hutto BR. Folate and cobalamin in psychiatric illness. Compr Psychiatry 1997;38:305-14.
43. Sugden C. One-carbon metabolism in psychiatric illness. Nutr Res Rev 2006;19:117-36.
44. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev 2008;66:250-5.
45. Westermarck T, Antila E. Diet in relation to the nervous system. In: Garrow JS, James WPT, Ralph A, eds. Human nutrition and dietetics. London: Churchill Livingstone 2000:715-30.
46. Sachdev HPS, Gera T, Nestel P. Effect of iron supplementation on mental and motor development in children:

systematic review of randomised controlled trials. Public Health Nutr 2005;8:117-32.
47. Black MM, Baqui AH, Zaman K et al. Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. Am J Clin Nutr 2004;80:903-10.

# Chapter 3

## Effects of omega-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: a review of human studies

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## Abstract

The present paper evaluates the most recent randomized controlled trials assessing the efficacy of omega-3 long chain polyunsaturated fatty acid (LCPUFA) supplementation (with or without omega-6 LCPUFA) during pregnancy, lactation, infancy and childhood on visual and cognitive development.

Available evidence suggests a beneficial effect of maternal omega-3 LCPUFA supplementation during pregnancy and lactation on cognitive development of infants and children, but not for visual development. Evidence for an effect of LCPUFA supplementation of preterm and term infants on cognitive development of infants remains inconclusive. However, supplementing term infants with daily doses of 100 mg docosahexaenoic acid plus 200 mg arachidonic acid improves visual development as measured by electrophysiological tests. Evidence for benefits of omega-3 LCPUFA on cognitive development in healthy children older than two years of age is too limited to allow a clear conclusion.

Taken together, the evidence for potential benefits of LCPUFA supplementation is promising but yet inconclusive.

## Introduction

There is considerable interest in the role of certain long chain polyunsaturated fatty acids (LCPUFA), in visual and cognitive development throughout childhood. The omega-3 fatty acid docosahexaenoic acid (DHA) and the omega-6 fatty acid arachidonic acid (AA) are the major LCPUFA in the brain (1). DHA and AA are rapidly incorporated in the nervous tissue of retina and brain during the brain's growth spurt, which mainly takes place from the last trimester of pregnancy up to 2 years of age (1-4). Beyond development of the central nervous system, omega-3 and omega-6 fatty acids may influence brain function throughout life by modifications of neuronal membrane fluidity, membrane activity-bound enzymes, number and affinity of receptors, function of neuronal membrane ionic channels, and production of neurotransmitters and brain peptides (5). Although DHA and AA are the major structural components of the central nervous system, there is currently no consensus whether dietary supplementation of LCPUFA has benefits for visual and cognitive development of infants. Two Cochrane reviews conclude that for preterm infants, there are no positive long-term effects (>6 months of age) of LCPUFA on visual or intellectual development, while evidence for a beneficial effect of LCPUFA supplementation on early visual development of preterm infants (<6 months of age) is inconclusive (6). For term infants there is little evidence for a benefit of LCPUFA supplementation on visual or general development (7). Recently, McCann & Ames (8) reviewed evidence from human and animal studies to assess whether DHA is required for development of normal brain function. They concluded that evidence is too inconsistent to conclude that infant formula should be supplemented with DHA.

Since publication of the Cochrane reviews and review by McCann & Ames, several new human intervention studies on this topic appeared in literature. Additionally, new research focused on the effect of maternal omega-3 LCPUFA supplementation during pregnancy and lactation on visual and cognitive function of infants. In this paper, we therefore aim to re-evaluate the currently available evidence on the effect of LCPUFA on visual and cognitive function during infancy and later childhood. We focus on human nutrition intervention studies on LCPUFA supplementation during pregnancy, lactation, infancy and childhood.

## Methodology

### Literature search

For assessment of the effects of LCPUFA supplementation on visual and cognitive development of children, we divided the literature into four topic areas:

1. Supplementation of mothers during pregnancy and lactation and effects on visual and cognitive development of their infants.
2. Supplementation of preterm infants during the first two years of life and effects on their visual and cognitive development.

3. Supplementation of term infants during the first two years of life and effects on their visual and cognitive development.
4. Supplementation of children older than 2 years of age and effects on their cognitive development.

For identification of studies, we searched the literature databases of Web of Science (Institute for Scientific Information). The search string consisted of combinations of the following terms: long chain polyunsaturated fatty acids, docosahexaenoic acid, eicosapentaenoic acid, linolenic acid, arachidonic acid, linoleic acid, omega-3 fatty acids, omega-6 fatty acids; with infants, children, preschoolers, toddlers, complementary feeding, neonates, offspring, babies; and with cognition, development, mental, learning, brain, visual acuity, neurology. In addition, lists of references in the identified publications were checked. We restricted this review to randomized controlled trials that supplemented subjects for periods of at least four weeks, and in which supplementation with LCPUFA was the sole variable differing between treatment and control groups. Therefore, comparisons of groups fed supplemented formula with groups fed human milk, as in some of the trials, are beyond the scope of this review and will not be discussed. When less than three controlled human trials one of the five selected topics were available, we also searched for observational data. This was the case for supplementation of children older than two years. For studies in preterm and full term infants, we restricted our review to studies published after the two Cochrane reviews on effects of LCPUFA on development (6;7).

**Definitions and methods of investigation of visual and cognitive development**

Two major outcomes that may be influenced by omega-3 LCPUFA supplementation are visual and cognitive development.

Visual development reflects maturation of visual (cerebral) function and retinal (sensory) function. Retinal function can be assessed by electroretinography (ERG) which measures the electrical responses of sensory cells in the retina. Visual acuity is a measure of clearness of vision. Visual acuity can be measured by visual evoked potentials (VEP) which is the electrical response of the brain to a visual stimulus (electrophysiological methods) or by preferential looking techniques which are behavioral reactions to cards presenting different visual contrasts that are compared and assessed by a technician (behavioral methods). Most studies investigating the effects of LCPUFA on visual development measured grating acuity. This is the spatial threshold for resolving dark and light stripes, which can be studied by both electrophysiological and behavioral methods (9). Stereo acuity is another measure of visual function of depth perception, assessing the minimum detectable binocular disparity (9).

The term cognition is broad and covers various high-level psychological

processes, such as memory, learning, reasoning, attention and language. Cognitive development refers to the changes of the cognitive processes observed over longer periods of time (months or years) and is usually assessed in children by batteries of performance tests assessing specific cognitive abilities. Mental or cognitive development of infants and young children is multidimensional and nonlinear. It is a result of physical growth, neurological maturation, interactions with the environment and the integration of stimuli provided by immediate caregivers and broader social and economic context. Therefore, the assessment of mental development of infants requires the examination of multiple domains and multiple sources of information; the comparison of developmental milestones with standardized population is essential. The Bayley Scales of Infant Development (BSID) is used infants and young children and measures general development. The BSID has strong technical characteristics, because it has been standardized on a very large population. It has, however, a poor predictive validity for later intelligence (10). More specific cognitive functions, such as gross motor skills, parent-child interaction, language comprehension, sleep-wake behavior, and attention can be assessed in infants and young children with more precise methods. However, these assessments are rarely used as they require time-consuming observation, specific experimental settings and multiple sources of information.

In older children (>3 years), intelligence is usually assessed by sets of short tests measuring various cognitive abilities all correlated with a general factor of intelligence, such as the Kaufman Assessment Battery for Children (K-ABC) and Wechsler tests (WISC) (11).

## Results

### Maternal omega-3 LCPUFA supplementation and effect on visual and cognitive development of their neonates

Six studies have been found in literature assessing the effect of maternal supplementation during pregnancy and/or lactation with omega-3 LCPUFA on visual and cognitive development of their infants. The characteristics of these trials are shown in **Table 1**.

#### *Supplementation during pregnancy*

Three trials investigated maternal supplementation before birth, when the fetus is developing, and measured effects on visual and/or cognitive development after birth. One of these trials was excluded because no results on effects of DHA supplementation of mothers on cognitive development of the infants were reported (12). Another trial by Malcolm et al (13;14) assessed the effect of fish oil supplementation (0.2 g DHA/d) to mothers from week 15 of pregnancy to delivery, on infant retinal and visual development. No differences were found, neither in maturity of the retina at 1 week of age, nor in visual function measured

**Table 1**. Overview of randomized controlled trials on effect of DHA supplementation in pregnant and lactating women on visual and cognitive development of infants

| Reference and location | N | Supplementation to mothers | | Functional measurements: age at assessment | Significant effects of supplementation | |
|---|---|---|---|---|---|---|
| | | Period | Dose/d | | Functional | Biochemical DHA status |
| Malcolm et al. (13;14), UK | 63 | Wk 15 to delivery | I: 200 mg DHA + 36 mg EPA<br>C: 400 mg oleic acid | Flash VEP: 0-5 d<br>Flash and pattern-reversal VEP: 50, 66 wk PCA<br>ERG: 0-7 d | None | Maternal: *<br>Infant: none |
| Tofail et al. (15), Bangla-desh | 249 | Wk 25 to delivery | I: 1200 mg DHA +1800 mg EPA<br>C: 2250 mg LA + 270 mg ALA | BSID: 10 mo | None | ND |
| Helland et al. (17), Norway | 341 | Wk 17-19 to 3 mo post partum | I: 1183 mg DHA + 803 mg EPA + 160 mg LA<br>C: 8.3 mg DHA + 4747 mg LA | EEG: 2 d, 3 mo<br>FT: 27, 39 wk | None | Infant: *<br>HM: * |
| Helland et al. (18), Norway | 90 | Wk 17-19 to 3 mo post partum | I: 1183 mg DHA +803 mg EPA + 160 mg LA<br>C: 8.3 mg DHA + 4747 mg LA | IQ (K-ABC): 4 y | Positive: IQ 4.1 points higher in I versus C | Infant: *<br>HM: *<br>Child 4 y: ND |
| Gibson et al. (19), Australia | 52 | 12 wk post partum | I1: 0 g DHA<br>I2: 0.2 g DHA<br>I3: 0.4 g DHA<br>I4: 0.9 g DHA<br>I5: 1.3 g DHA | VEP: 12, 16 wk<br>BSID: 1, 2 y | None | Infant: *<br>HM: * |
| Lauritzen et al. (20; 21), Dan-mark | 97 | 16 wk post partum | I: 1.3 g DHA+EPA+ DPA<br>C: olive oil | VEP: 2, 4 mo<br>Motor function: 2, 4, 9 mo<br>MPS: 9 mo<br>MACDI: 12, 24 mo | Positive: MPS intention scores 2.0 point higher in I versus C in girls<br>Negative: MACDI vocabulary com-prehension 17 point lower in I ver-sus C; 33 point lower in I versus C in boys; sentence complexity 3.0 point lower in I versus C in boys | Infant: *<br>HM: *<br>Infant >4mo: ND |
| Jensen et al. (22), USA | 160 | 4 mo post partum | I: 200 mg DHA<br>C: soy+corn oil | TAC, VEP: 4, 8 mo<br>GGM, CLAMS, CAT: 12, 30 mo<br>BSID: 30 mo | Positive: BSID-PDI 8.4 point higher in I versus C | Infant: *<br>HM: * |

I, intervention group; C, control group; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; ALA, $\alpha$-linolenic acid; DPA, docosapentaenoic acid; VEP, visual evoked potential; PCA, post conceptional age; ERG, electroretinography; BSID, Bayley Scales of Infant Development; EEG, electroencephalogram; FT, Fagan Test of Infant Intelligence, IQ, Intelligence Quotient; K-ABC, Kaufman Assessment Battery for Children; MPS, Means-end Problem Solving; MACDI, MacArthur Communicative Development Inventories; TAC, Teller Acuity Card procedure; GGM, Gesell Gross Motor; CLAMS, Clinical Linguistic and Auditory Milestone Scale; CAT, Clinical Adaptive Test; PDI, Psychomotor Development Index; ND, not determined; HM, human milk.
* Increased levels

by VEP to flash and pattern reversal stimuli at birth and at 10 and 26 weeks of age, between neonates born to mothers supplemented with fish oil versus those from mothers supplemented with high-oleic acid sunflower oil. A third trial by Tofail et al (15) investigated the effect of supplementing 249 pregnant Bengalese women with either fish oil (1.2 g DHA + 1.8 g EPA/d) or soy bean oil (2.25 g LA + 0.27 g ALA/d) from the 25$^{th}$ week of pregnancy until delivery on cognitive outcomes of her infant. Cognitive development was measured using the BSID when infants were 10 months of age. Furthermore, measurements of infant's behavior, quality of stimulation at home (Home Observation for Measurement of Environment) and socio-economic indicators were used to control outcomes of the intervention (15). The mean Mental Development Index (MDI) and Psychomotor Development Index (PDI) sub-scores of the BSID did not differ significantly between the two groups.

The studies by Malcolm and Tofail did not detect an effect of maternal omega-3 LCPUFA supplementation during pregnancy on visual function or cognitive development in the first year of life. Explanations for this could be that the dose and/or duration of the DHA supplementation were not sufficient. In fact, the dose used in the study by Malcolm et al (13;14) was low (0.2 g DHA/d) and did not significantly increase the infant's DHA status (16).

Tofail et al (15) used a high dose of DHA (1.2 g/d) for a supplementation period that was 6-10 weeks shorter than in the study by Malcolm et al (13;14), but no biochemical parameters were measured to confirm that the DHA status of the infant increased. Cross-sectional analyses of the data of Malcolm et al (13;14) showed that maturity of the retina at 1 week, and visual acuity at 10 and 26 weeks of age were positively associated with the DHA status of infants at birth. This indicates that the outcome measures in Malcolm et al (13;14) were sufficiently sensitive to detect effects on retinal and visual function, while there is no such evidence for the BSID measurement in the study by Tofail et al (15). Because of the differences and limitations in the designs of these two studies, it is yet uncertain whether omega-3 LCPUFA supplementation during pregnancy can have a beneficial effect on visual or cognitive development of the infants.

***Supplementation during pregnancy and lactation***

One study by Helland et al (17) investigated the effect of supplementing mothers with either cod liver oil (1.2 g DHA/d) or corn oil (4.7 g linoleic acid (LA)/d) from week 17-19 of pregnancy until delivery and continued for three months during lactation. No treatment differences between the groups were found in electroencephalogram (EEG) maturity of the two-day old neonates, EEG maturity at 3 months of age and for novelty preference at 6 and 9 months of age. When these children were 4 years of age, however, IQ estimated by the Mental Processing Composite of the K-ABC was significantly (4 points) higher in the cod liver oil group than in the control group (18). It is unclear whether this can be ascribed to supplementation

during pregnancy, during lactation or both. The dose of DHA used was effective in increasing infant' DHA status, and the Mental Processing Composite score was significantly positively correlated with infants' DHA status at 4 weeks of age (18). No positive correlations were found between EEG maturity at 3 months and novelty preference and DHA umbilical plasma, except for EEG maturity at 2 days of age (17). Outcomes of this study suggest that effects of DHA supplementation during pregnancy and lactation may appear later in life, when cognitive function is more mature and cognitive psychometric tests have higher discriminative power.

***Supplementation during lactation***

Three randomized controlled trials have assessed the effect of supplementing lactating mothers with omega-3 LCPUFA, on cognitive development of their children. Gibson et al (19) investigated the effect of supplementing lactating women with one of five increasing doses of DHA (0-1.3 g/d) from algae oil for the first 12 weeks postpartum on visual and cognitive development of their infants. No significant effects of DHA supplementation were found on visual acuity at 12 and 16 weeks of age and on MDI and PDI sub-scores of the BSID at 1 and 2 years of age. Nevertheless, a small but statistically significant association of infant DHA status and breast milk DHA concentration at 12 weeks of age with the MDI measured at 1 year was found. This positive correlation disappeared, however, when the children were 2 years old. No relationships with infant DHA status or breast milk DHA were observed for either the PDI or for visual acuity. In a study in Danish lactating women with low fish intake, Lauritzen et al (20) found no significant effects of fish oil supplementation on visual acuity of infants at 2 and 4 months (1.3 g omega-3 LCPUFA/d for 16 weeks) as compared to olive oil supplementation. However, in a cross-sectional analysis of the fish oil supplemented infants, a significant positive association was demonstrated between infant DHA status and visual acuity at 4 months, but not at 2 months of age.

Infants were followed to investigate the effects of the supplementation on motor function at 2, 4 and 9 months, problem solving ability at the age of 9 months and linguistic development at 1 and 2 years of age (21). Fish oil supplementation had a significant positive effect on problem solving in girls at 9 months, while no such effect was detected in boys and the total group. In contrast, fish oil supplementation resulted in a significantly negative effect on vocabulary comprehension at 1 year in the total group and in boys, and on sentence complexity in boys. However, no differences between groups were found on vocabulary tests when children were 2 years of age. Correlation of infant DHA status at 4 months with cognitive tests yielded a significant positive relationship with problem solving in girls at 9 months and a significant negative relationship with vocabulary comprehension in boys at 12 months. No significant correlations were found for any of the other outcome measures or at other ages. In the third study, Jensen et al (22) investigated the effect

of supplementing lactating mothers with either DHA from algae oil (200 mg DHA/d) or vegetable oil for 4 months on visual function and neurodevelopment of their infants. Visual function was assessed with the Teller Acuity Card procedure, and by measuring sweep and transient VEP at 4 and 8 months of age. Neurodevelopment status was evaluated by the BSID at 30 months. At 12 and 30 months of age, gross motor development (Gesell Development Inventory), language development (Clinical Linguistic and Auditory Milestone Scale), and visual-motor problem-solving (Clinical Adaptive Test) were also determined. No significant effects of intervention were observed, with the exception of significantly higher scores on the PDI sub-score of the BSID in 30 months old infants of mother supplemented with DHA compared to controls. No significant correlations were found between infant DHA status and any of the functional outcome measures.

In the three randomized controlled trials summarized above, DHA supplementation during 4 months increased the DHA content of human milk and that of infant blood DHA status in a dose-dependent way. However, none of the three trials found significant effects of supplementation on any of the indicators of visual development. It could be speculated that supplementation during 3-4 months is too short to have a beneficial effect on visual development and should even start during pregnancy. However, it may also be DHA supplementation during lactation has no relevant effect on visual development.

For cognitive development, the effects of DHA supplementation during lactation are inconsistent. The study by Gibson et al (19) with only 52 children was considered too small to detect any effect. Jensen et al (22) and Lauritzen et al (21) had adequate power and found mixed effects of DHA supplementation at 9-30 months of age. No effects of the interventions were observed on motor development before the age of 12 months, but Jensen et al (22) showed a large positive effect of DHA supplementation on psychomotor development at 30 months. This finding indicates that effects of supplementation on motor development may appear later in life. No positive effects of DHA supplementation were shown on any of the measures of general intelligence in the three studies, except for problem solving ability in girls at 9 months in the study by Lauritzen et al (21). Effects of DHA supplementation on language development were not consistent between the studies by Lauritzen et al (21) and Jensen et al (22). This could be explained by use of different test methodologies, the higher dose of DHA and higher sensitivity of mothers with low fish intake to fish oil supplementation in the study by Lauritzen et al (21). There is no good explanation why some positive effects on cognitive development were only found in girls and why negative but transient effects on language development were more pronounced in boys.

In conclusion, DHA supplementation during lactation seems not effective for visual development of breastfed infants, but there is some evidence for a beneficial effect on psychomotor and cognitive development of the infant. An important

question is whether these positive effects would be sustainable in later childhood.

**Effect of LCPUFA supplementation on visual and cognitive development in preterm infants**

Preterm born infants have significantly lower LCPUFA concentrations than infants born after a full term pregnancy, because preterm infants miss the major period in utero of DHA and AA accretion in the brain, which is during the third trimester of gestation (4). Simmer & Patole (6) concluded in a Cochrane review of nine studies published between 1990 and 2002, that there were no positive long-term effects (>6 months of age) of LCPUFA supplementation on visual or intellectual development in preterm infants. Evidence for a beneficial effect of LCPUFA on early visual development (<6 months of age) was inconclusive (6). Since that review, two large randomized controlled trials and one smaller trial assessing the effect of LCPUFA supplemented formula on visual and general development of preterm infants were published (**Table 2**). Fewtrell et al (23) investigated the effects of supplementing 204 preterm infants of tuna oil in combination with borage oil rich in $\gamma$-linoleic acid (GLA) (0.5 g DHA and 0.9 g GLA/100g fat) on mental and psychomotor development and neurological status at 9 and 18 months after term. GLA is a precursor of AA; it is readily converted to AA in human body (23). No significant differences in MDI and PDI sub-scores of the BSID were found between the groups. However, pre-planned subgroup analysis showed that LCPUFA supplemented boys, but not girls, had significantly higher mental development scores at 18 months after term than controls. Authors suggested that boys may be more sensitive to the effects of suboptimal early nutrition on neurodevelopment (24). In another recent study, Clandinin et al (25) found significantly higher MDI and PDI sub-scores of the BSID at 18 months after term in 179 preterm infants who received LCPUFA supplemented formula (0.32 g DHA from algae or fish oil plus 0.67 g AA from fungi oil/100 g fat), compared to the control group. Subgroup analyses to assess effects in boys or girls were not conducted.

Fang et al (26) showed in a small study of 27 preterm infants a significant beneficial effect of LCPUFA supplemented formula containing 0.05 g DHA and 0.10 g AA/100 g fat on the PDI and MDI sub-scores of the BSID at 6 and 12 months of age compared to the controls. The source of DHA and AA was not reported. No differences were found in the different measures of visual development at 4 and 6 months of age. Formula was supplemented from birth to 6 months. Infants were less premature (born at 30-37 wk of gestation) and had a higher birth weight (nearly 2000 g) than infants in the studies by Fewtrell and Clandinin (born <35 wk of gestation, birth weight <2000 g). The dosage LCPUFA was low and duration of the intervention was short compared to the other two studies. Other limitations of the study were the small sample size and a complete description of the composition of the formula was lacking. Therefore, these outcomes should be interpreted with

**Table 2**. Overview of randomized controlled trials on effect of LCPUFA supplementation and cognition in premature infants

| Reference and location | N | Subjects | Supplementation | | Functional measurements: age at assessment after term | Significant effects of supplementation | |
|---|---|---|---|---|---|---|---|
| | | | Period | Dose (% of total fatty acids) | | Function | Biochemical DHA status |
| Fewtrell et al. (23), UK | 204 | Infants born <35 wk and ≤2000 g | Birth to 9 mo after term | F1: 0.1% EPA+ 0.5% DHA+ 0.9% GLA<br>F2: no LCPUFA | KPS: 9 mo<br>NI: 9, 18 mo<br>BSID: 18 mo | Positive in subgroup analysis: F1 boys 5.7 point higher BSID-MDI score versus F2 boys | ND |
| Clandinin et al. (25), Australia | 179 | Infants born ≤ 35 wk | Birth to 92 wk PMA (12 mo after term) | F1: 0.32% DHA-algae+ 0.64% AA<br>F2: 0.32% DHA-algae+ 0.10% EPA+ 0.64% AA<br>F3: no LCPUFA | BSID: 18 mo | Positive: F1 and F2 had 6 and 10 point higher BSID-MDI scores versus F3<br>F1 and F2 had 6 point higher BSID-PDI scores than F3 | ND |
| Fang et al. (26), Taiwan | 27 | Infants born 32-37 wk. Age at enrollment >32 wk and >2000 g body weight | Birth to 6 mo | F1: 0.05% DHA+ 0.10% AA<br>F2: no LCPUFA | VEP: 4, 6 mo<br>L&HH: 4, 6 mo<br>BSID: 6, 12 mo | Positive: F1 had significantly higher scores on BSID-MDI (4.4 and 8.2 points) and BSID-PDI (6.8 and 11.3 points) compared to F2 at 6 and 12 mo respectively | ND |

PMA, postmenstrual age; F1–3, formula group; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; GLA, γ-linoleic acid; LCPUFA, long chain polyunsaturated fatty acids; AA, arachidonic acid; KPS, Knobloch, Passamick, and Sherrards' Developmental Screening Inventory; NI, neurologic impairment diagnosed by a pediatrician; BSID, Bayley Scales of Infant Development; VEP, visual evoked potential; L&HH, Lea grating acuity and Hiding Heidi low contrast "FACE" cards; MDI, Mental Development Index; PDI, Psychomotor Development Index; ND, not determined.

care. Simmer & Patole (6) reviewed five studies that assessed the effect of LCPUFA supplemented formula on cognitive development of preterm infants as measured by BSID and Fagan Test. These studies had mixed results. Five studies assessed cognitive development only when infants were 12 months after term or older. Two of these five studies (27;28) measured also effects of LCPUFA supplementation at 18 and 24 months after term using BSID, but no significant effects on cognitive development of infants were found. However, the duration of supplementation in these studies, 1 (27) and 6 (28) months, was much shorter than the 12 months in the study by Clandinin et al (25).

Taken together, there are indications for a beneficial effect of LCPUFA supplementation on cognitive development of preterm infants. The three studies mentioned above showed positive effects, but evidence is as yet inconclusive. It may be that effects of LCPUFA supplementation are larger when the duration of supplementation is continued until the age of 12 months after term; or that the effect can be better detected at older ages, when cognitive tests are more sensitive and reliable.

**Effect of LCPUFA supplementation on visual and cognitive development in full term infants**

Simmer (7) concluded in a Cochrane review that there was little evidence for a benefit of LCPUFA supplementation on visual or general neural development in the first 36 months of age. This conclusion was based on systematic review of nine randomized controlled trials of term infants fed infant formulas with or without LCPUFA. However, they concluded that supplementation of LCPUFA appeared to be beneficial on information processing during the first year of life (7). SanGiovanni et al (29) performed a meta-analysis of five of these nine studies and assessed the effect of DHA supplementation on visual acuity in term infants. A significant better visual acuity at 2 months of age (0.32 ± 0.09 octaves better acuity in DHA supplemented groups) was found when visual acuity was measured using behavioral tests, but not when measured with electrophysiological tests. At older ages up to 12 months, no significant effects could be detected at all. In a meta-regression analysis of 14 intervention studies, Uauy et al (30) demonstrated a significant positive association ($r^2$=0.35) between the DHA provided by the supplement and higher visual acuity at 4 months of age. These data suggest that when increasing the DHA content of formula with 10% of total fatty acids (28 mg DHA/d), the visual acuity response improves with −0.067 log of the minimum angle of resolution (logMAR). When taking into account also the hypothetical DHA converted from ALA (1, 5 or 10%) and EPA (18%) plus DHA content of the formula (DHA equivalents), then the association was stronger ($r^2$=0.53-0.68) and improvements in visual acuity response were higher. Increasing formula with 28 mg DHA equivalents would result in an improvement of visual acuity response of −0.252 logMAR (30).

After the review by Simmer in 2001 (7), there were nine new randomized controlled trials published on the effect of LCPUFA supplementation in full term infants on visual and cognitive development (Table 3). Four of these nine studies have also recently been reviewed by McCann & Ames (8). Auestad et al (31) found no beneficial effects on visual acuity and mental development in the first 14 months of life after supplementation with infant formula with 0.14% DHA and 0.45% AA from egg-derived triglyceride or from a combination of fish and fungal oil for 12 months. The authors applied multiple measures of general development, including the Fagan Test assessing general information processing and the BSID. Furthermore, they measured language development using MacArthur Communicative Development Inventories and overall behavior and well-being of children by the Infant Behavior Questionnaire and Behavior Rating sub-score of the BSID. In the follow-up of an earlier supplementation study (32), Auestad et al (33) also observed no significant effects of infant formula on visual acuity, visual-motor function, IQ and language assessments when children were 39 months of age (32). These children were initially fed formula with either 0.14% DHA and 0.45% AA from egg-derived phospholipids, or with 0.23% DHA only from fish oil, or control formula without added LCPUFA during their first year of life. Bouwstra et al assessed the effect of infant formula supplemented with 0.23% DHA, 0.39% AA, and 0.18% GLA during the first two months of life on neurodevelopment as reflected by general movements (34) and the Hempel test and BSID (35). The source of LCPUFA was a combination of egg-yolk, tuna and fungal oil which resembled the composition of LCPUFA in human milk. LCPUFA supplementation reduced the occurrence of mildly abnormal general movements of infants at 3 months of age compared to control formula (34). However, no significant effects were observed on neurodevelopment when infants were 18 months of age (35). Similarly, Ben et al (36) found no significant effects of supplementing Chinese infants with formula containing 0.18% DHA and 0.18% AA (source unknown) for a period of 6 months on MDI and PDI sub-scores of the BSID when infants were 3 and 6 months of age. In contrast, two trials by Birch et al (37;38) demonstrated significant improvement in visual acuity and visual stereo-acuity throughout the first year of life in LCPUFA supplemented infants compared to controls. Infants received formula supplemented with 0.36% DHA and 0.72% AA from single-cell oil or control formula for 12 months. Also, Ünay et al (39) showed a significantly more rapid maturation of the auditory brainstem response as measured by changes in auditory evoked potentials in infants who were supplemented with 0.5% DHA from unknown source during the first 16 weeks of life compared to controls (39).

Two studies conducted by Hoffman et al assessed the effects of formula supplemented with 0.36% DHA plus 0.72% AA from single-cell oil (40) or baby food with egg yolk enriched with 83 mg DHA versus non-enriched egg-yolk (41) on visual function in infants. Supplementation occurred only during the complementary

**Table 3**. Overview of randomized controlled trials on effect of LCPUFA supplementation and cognition in full term infants

| Reference and location | N | Subjects | Supplementation | | Cognitive measurements: age of assessment | Significant effects of supplementation | |
|---|---|---|---|---|---|---|---|
| | | | Period | Dose (% of total fatty acids) | | Functional | Biochemical DHA status |
| Auestad et al. (31), USA | 404 | Healthy infants gestational age 37-42 wk | Birth to 1 y | F1: 0.45% AA + 0.14% DHA<br>F2: 0.46% AA + 0.13 % DHA<br>F3: no LCPUFA | TAC: 2, 4, 6, 12 mo<br>FT: 6, 9 mo<br>BSID: 6, 12 mo<br>MACDI: 9, 14 mo<br>IBQ: 6, 12 mo | None | Infant: * |
| Birch et al. (37), USA | 65 | Healthy infants gestational age 37-40 wk; HM up to 6 wk | From 6-52 wk of age | F1: 0.72% AA + 0.36% DHA<br>F2: no LCPUFA | VEP: 6, 17, 26, 52 wk<br>SA: 17, 26, 39, 52 wk | Positive: VEP at 17 wk -0.11 logMAR[a], at 26 wk -0.15 logMAR and and at 52 wk -0.16 logMAR better in F1 versus F2; SA was at 17 wk 0.5 logsec[b] better in F1 versus F2 | Infant: * |
| Auestad et al. (33), USA | 157 | Healthy infants followed up at 39 mo | Birth to 1 y | F1: 0.45% AA + 0.12% DHA<br>F2: 0.23% DHA<br>F3: no LCPUFA | SBIS: 39 mo<br>PPVTR: 39 mo<br>MLU: 39 mo<br>BVMI: 39 mo<br>TAC: 39 mo | None | Infant: ND<br>Child 39 mo: none |
| Bouwstra et al. (34; 35), the Netherlands | 472 | Healthy infants gestational age 37-42 wk | Birth to 2 mo | F1: 0.39% AA + 0.23% DHA + 0.18% GLA<br>F2: no LCPUFA | QGM: 3 mo<br>H: 18 mo<br>BSID: 18 mo | Positive on QGM: Significant less mildly abnormal general movements in F1 (31%) versus F2 (19%) | ND |
| Hoffman et al. (40), USA | 61 | Healthy infants, HM up to 4/6 mo | From 4/6 to 12 mo of age | F1: 0.72% AA + 0.36% DHA<br>F2: no LCPUFA | VEP: 4/6, 12 mo<br>SA: 4/6, 12 mo | Positive: VEP 0.1 logMAR better in F1 versusF2 | Infant: * |
| Ben et al. (36), China | 245 | | Birth to 6 mo | F1: 0.18% AA + 0.18% DHA<br>F2: no LCPUFA | BSID: 3, 6 mo | None | Infant: * |
| Hoffman et al. (41), USA | 51 | Healthy infants HM up to 6 mo | From 6-12 mo of age | I: 83 mg DHA enriched egg yolk<br>C: no DHA | VEP: 6, 9, 12 mo<br>SA: 6, 12 mo | Positive: VEP at 9 mo -0.14 logMAR and VEP at 12 mo -0.16 logMAR better in F1 versus F2 | Infant: * |
| Ünay et al. (39), Turkey | 80 | Healthy infants | Birth to 16 wk | F1: 0.5% DHA<br>F2: no DHA | BAEP: 1, 16 wk | Positive: More rapidly maturation of auditory brainstem at 16 wk in F1 than F2 | Infant: * |
| Birch et al. (38), USA | 71 | Healthy infants gestational age 37-40 wk | Birth to 1 y | F1: 0.72% AA + 0.36% DHA<br>F2: no LCPUFA | VEP: 6, 17, 39, 52 wk<br>SA: 17, 39, 52 wk | Positive: VEP -0.12 logMAR better in F1 at 17, 39 and 52 wk than F2; SA was 0.46 logsec better in F1 at 17 wk than to F2 | Infant: * |

HM, human milk; F1–3, formula group; AA, arachidonic acid; DHA, docosahexaenoic acid; GLA, γ-linoleic acid; LCPUFA, long chain polyunsaturated fatty acids; TAC, Teller Acuity Cards; FT, Fagan Test of Infant Intelligence; BSID, Bayley Scales of Infant Development; MACDI, MacArthur Communicative Development Inventories; IBQ, Infant Behavior Questionnaire; VEP, visual evoked potential; SA, stereoacuity; SBIS, Stanford Binet Intelligence Scale; PPVTR, Peabody Picture Vocabulary Test Revised, MLU, Mean Length of Utterance; BVMI, Beery Visual-Motor Index; QGM, Quality of General Movements; H, Hempel; BAEP, Auditory Brainstem Evoked Potential; ND, not determined. * Increased levels; [a] LogMAR corresponds to the log of the minimum angle of resolution; [b] Logsec, log seconds expresses look duration.

feeding period from 4-6 months until 12 months of age following exclusive breastfeeding. Both studies showed that visual acuity at age 9 and 12 months, but not visual stereo-acuity, was significantly more mature in LCPUFA supplemented infants. The studies by Hoffman suggest that DHA supplementation may be of benefit during the period of weaning when infants receive complementary foods next to breast feeding or infant formula, as these foods may be low in omega-3 LCPUFA.

Data from four studies by the groups of Birch and Hoffman, all showing positive effects of LCPUFA supplementation on visual development, were combined by Morale et al (42) to assess the effect of duration of supplementation. This analysis demonstrated that longer duration of LCPUFA supplementation by either human milk or formula was associated with higher improvement in visual acuity at 52 weeks of age, i.e. –0.002 logMAR improvement per week of LCPUFA supply.

Four out of five trials mentioned above showed beneficial effects of LCPUFA on visual development. All these studies used higher doses of LCPUFA compared to the study that did not find a benefit. Also, all four studies measured visual acuity by the more sensitive electrophysiological tests (9). This is consistent with findings from earlier studies reviewed by Simmer (7), of which Makrides et al (43) and Birch et al (44) found significant effects on VEP using formula with 0.36% DHA, while Auestad et al (45) found no effects using a formula with 0.12% DHA. Also, no major effects of LCPUFA supplementation were found when visual acuity was assessed with behavioral methods in the studies reviewed by Simmer (7). However, these findings are in contrast with earlier findings of the meta-analysis by SanGiovanni et al (29), who found no significant effects of DHA supplementation on visual acuity measured with behavioral tests only.

Taken together, above studies suggest that LCPUFA supplementation to formula in high dose of 0.36% DHA plus 0.72% AA and prolonged supplementation up to 12 months benefits visual development of infants. These high dosages of LCPUFA compare with a daily intake of approximately 100 mg DHA plus 200 mg AA; based on a 4% lipid content of formula, a 92.5% fatty acid content in milk total lipids, and a volume intake of 750 mL/d as proposed by Uauy et al (30).

In agreement with the conclusions by Simmer based on results of earlier studies (7), most of the more recent studies on LCPUFA supplementation in term infants failed to demonstrate effects on various aspects of cognitive development. Studies seemed to have sufficient power to detect relevant effects. Studies by Auestad et al (31;33) used a broad assessment of various tests that have been conducted at standardized circumstances, which are expected to be sufficiently sensitive to detect effects. Possible explanations for not finding effects are that doses in these studies were relatively low (<0.23% DHA), or that effects of LCPUFA supplementation appear at older ages, or that LCPUFA supplementation does not materially affect cognitive development. Future studies are needed to assess the effect of LCPUFA

supplementation on cognitive development at high dose (100 mg DHA plus 200 mg AA) and prolonged duration (preferably 12 months); conditions under which significant effects on visual development were found.

**Omega-3 fatty acid status and cognitive performance in older children**

There are no randomized controlled trials published in literature investigating the potential effects of omega-3 fatty acids supplementation after the age of 2 years on cognitive performance in children. We found one prospective Dutch cohort study by Bakker et al (46) assessing the relationship of DHA status at birth and 7 years of age with cognitive performance (K-ABC ) measured when children were 7 years old. No significant relationships were found. Zhang et al (47) studied the association between PUFA intake and psychosocial and cognitive performance of children aged 6-16 years in a cross-sectional analysis on data from the Third National Health and Nutrition Survey in the USA. Higher intake of total omega-3 and omega-6 PUFA (10 g/day increase) was associated with better performance on the digit span test, but not with performance on block design, arithmetic, and reading comprehension tests. These data suggest that higher PUFA intake could be beneficial for children's working memory. However, no data were available on dietary intakes of omega-3 and omega-6 fatty acids separately.

In contrast to the limited data in healthy children, there is more evidence for a beneficial effect of PUFA supplementation in children with diagnosed mental disorders and children with phenylketonuria (PKU). Three (48-50) out of five (48-52) double blind randomized controlled trials showed promising results of supplementation with a combination of omega-3 and omega-6 fatty acids on diminishing some behavioral symptoms in school-age children with attention-deficit hyperactivity disorder (ADHD). Supplementation with omega-3 fatty acids alone (DHA, EPA) seemed not effective (51;52).These studies mostly failed to show any improvement of attention or cognitive performance in controlled conditions. Only one of the five studies demonstrated significant improvements of PUFA supplementation on spelling and reading using tests that could also be applied in healthy children (50). However, outcomes of this study can not be extrapolated to healthy children as significant diminishing of symptoms of hyperactivity and inattention are likely the underlying factors of improvement on spelling and reading. Children with PKU consume diets restricted in animal foods and therefore have a low intake of AA and DHA resulting in a poor LCPUFA status which may negatively influence their development (53). A randomized controlled and an uncontrolled trial in children aged 1-12 years with PKU have demonstrated that supplementation with omega-3 fatty acids (10-15 mg DHA/kg bodyweight) improved their visual function as measured by VEP (54;55). Although these children were diagnosed for PKU, the studies suggest that visual function can be affected by DHA supplementation in children older than 2 years of age with a poor LCPUFA status.

In conclusion, there are no trial data and only one prospective cohort study on the effect of omega-3 fatty acid supplementation on cognitive performance in healthy children older than two years of age. However, it remains conceivable that omega-3 fatty acid supplementation could be beneficial in these children, in particular when intake of omega-3 fatty acids is low and nutritional status is poor. This should be addressed in future randomized controlled trials.

## Discussion and conclusion

We reviewed the available data from randomized controlled trials that investigated effects on visual and cognitive development of omega-3 fatty acid supplementation to pregnant and lactating women, infants and children. With the exception of studies in term infants, the total number of randomized controlled trials on this topic is still very limited, especially for supplementation in older children. It is difficult to directly compare studies, as they assessed visual and cognitive development by many different outcome measures, and also had multiple differences in design that could influence the outcomes and interpretation. In particular, studies differed in the type and dose of fatty acids supplied to the intervention and control groups, duration of supplementation, age of subjects at cognitive assessment, and methodology used for assessment of cognitive outcomes. Thus, there is a large heterogeneity among studies. Nevertheless, we can conclude from these studies that:

- For supplementing pregnant and or lactating women with DHA, there is currently no supporting evidence for a beneficial effect on visual development, but there is suggestive evidence for a beneficial effect of supplementation during pregnancy and lactation or lactation only on mental development and on longer-term cognition.
- For supplementing preterm infants with DHA and AA, evidence for benefits on visual development at <6 months of age remains inconclusive, while there are indications from two studies for a beneficial effect of supplementation in early in life on cognitive development at >12 months of age.
- For supplementing term infants, with LCPUFA in high doses (100 mg DHA and 200 mg AA per day), there is consistent evidence for a beneficial effect on visual development during the first year of life, while there is hardly such evidence for beneficial effects on cognitive development.
- For supplementing healthy children older than two years of age with DHA, there is no evidence for beneficial effect on cognitive performance.

Although the totality of the evidence is still inconclusive, there are promising indications that supplementation with DHA and other omega-3 fatty acids, either or not in combination with omega-6 fatty acids, during pregnancy, lactation and infancy may benefit for visual and cognitive development early in life.

As the incorporation of DHA and AA in the developing brain is particularly high in the prenatal period (3;4), supplementation during pregnancy would theoretically

be expected to have the largest impact on visual and cognitive development of infants. However, the evidence from supplementation studies in pregnant women is currently too limited to support this notion. The benefit of DHA supplementation on cognition in older children has not been investigated yet, but the potential for beneficial effects is expected to be smaller than in younger infants or the fetus, as brain development and the potential incorporation of DHA becomes slower after the age of 2 years (56). However, it remains possible that an increased supply of DHA to the brain in this period results in changes in fatty acid composition of brain tissue or that DHA has other metabolic effects that could affect brain function (5). Obviously, such effects are very difficult or impossible to measure in humans.

When all intervention studies are taken together, most positive effects of the LCPUFA supplementation on cognitive development were detected by general tests of intelligence, such as the BSID. Motor function was less frequently improved by LCPUFA supplementation and language development may even be negatively affected.

The difficulty of measuring mental development during infancy may partly explain why studies have yielded conflicting results. The central nervous system of infants is developing and therefore all the various cognitive skills have not been differentiated yet. Therefore, it is hard to reliably measure cognitive development at young ages. Methodologies should be both sensitive and both general, assessing a broad spectrum of cognitive functions to detect effects during early life. Measurements should take place in a standardized way, preferably at the same age and under the exact same conditions, which requires a laboratory setting. Alternatively, the effect of supplementation may be measured when children are older (≥3 years of age), when the brain is more differentiated and different test can be applied that assess more specific aspects of cognition. Also, new methodologies such as brain imaging techniques could help in future trials to detect subtle effects on brain structure and function (57).

The available data do not allow formulation of specific recommendations for optimal dosages, types, forms and ratio of omega-6 and omega-3 fatty acids for optimal development. Optimal brain development during infancy and childhood and maintenance of brain function throughout life requires an adequate and balanced supply of the EFA, LA and $\alpha$-linolenic acid (ALA) in the diet (5;58). It is generally assumed that LA and ALA will at least partly be converted into DHA and AA, respectively. However, it is presently unclear whether conversion of dietary EFA is under all circumstances sufficient to meet optimal DHA and AA requirements. A limited number of studies in human adults show that conversion of ALA to EPA and DHA synthesis is only marginal (59-62), with an overall conversion rate of at most some 4% (63). For infants, conversion rates could be higher to cover the needs for brain development (30), but quantitative data on ALA conversion in infants and children are lacking. However, a few studies demonstrated that supplementing

preterm infants with ALA (64) improved retinal function in early life, suggesting that ALA is converted to such an extent that DHA content of the retina can be influenced.

A relatively high ratio of omega-6 to omega-3 fatty acids in diet could theoretically impair the conversion of ALA to DHA in vivo (65), as ALA and LA compete for the same desaturation enzymes needed for the conversion of ALA to EPA and DHA and for conversion of LA to AA. Studies on LCPUFA supplementation and visual and cognitive development in children investigated effects of omega-3 fatty acids alone or omega-3 fatty acids in combination with omega-6 fatty acids, but there are no studies specifically investigating the role of omega-6 fatty acids alone on development and function of the central nervous system. The rationale for adding AA or other omega-6 fatty acids to omega-3 fatty acid supplementation is to prevent a decline in AA status (66) and prevent infants from possible growth faltering (67). Although evidence from human studies is lacking, it is believed that sufficient supply of omega-3 fatty acids is more important for visual and cognitive development and function. Therefore, it would be of value to investigate how mental development in infants and children may be affected by using different types of omega-3 fatty acids and at different omega-6:omega-3 fatty acid ratio's in the diet.

More recently, research has focused on the bioavailability of DHA from diet to the brain. Animal studies have suggested that DHA in phospholipid form would be more effective in crossing blood-brain barrier compared to DHA in triglyceride form (68;69). In the intervention studies reviewed DHA was mostly delivered in the triglyceride form or a combination of both. The studies by Auestad (31;33) have investigated different forms of DHA but did not show beneficial effects on cognitive development, but dosages may have been too low.

In conclusion, there is still limited and inconsistent evidence that supplementing mothers, infants or children with longer chain omega-3 fatty acids (possibly with additional omega-6 fatty acids) can improve visual and cognitive development of infant or child. However, there are promising indications for effects of supplementing pregnant and lactating mothers with omega-3 LCPUFA on cognitive development of their children, that warrant further studies in these and other target groups.

Future studies should specifically address the dose, type, form and ratio of omega-6 and omega-3 fatty acids and duration of supplementation required for optimal visual and cognitive development. These studies should be designed with high enough statistical power to detect small but relevant effects on visual and cognitive function by using standardized, sensitive test methodologies for measuring visual function and specific cognitive abilities, and measure effects over a longer period of child development. New techniques for investigating nutritional influences on the developing brain structures, such as brain imaging techniques, should also be explored.

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## References
1. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129-S138.
2. Dobbing J, Sands J. Quantitative growth and development of human brain. Arch Dis Child 1973;48:757-67.
3. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. Early Hum Dev 1980;4:131-8.
4. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Hum Dev 1980;4:121-9.
5. Yehuda S. Omega-6/omega-3 ratio and brain-related functions. World Rev Nutr Diet 2003;92:37-56.
6. Simmer K and Patole S. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev. 1, CD000375. 2004.
7. Simmer K. Longchain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database Syst Rev.(4), CD000376. 2001.
8. McCann JC, Ames BN. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. Am J Clin Nutr 2005;82:281-95.
9. Neuringer M, Jeffrey BG. Visual development: Neural basis and new assessment methods. J Pediatr 2003;143:S87-S95.
10. Flanagan DP, Harrison PL. Contemporary Intellectual Assessment, Theories, Tests and Issues. New York: The Guilford Press, 2005.
11. Carroll JB. Human cognitive abilities: A survey of factor-analytic studies. New York, NY, US: Cambridge University Press, 1993.
12. Colombo J, Kannass KN, Shaddy DJ et al. Maternal DHA and the development of attention in infancy and toddlerhood. Child Dev 2004;75:1254-67.
13. Malcolm CA, McCulloch DL, Montgomery C, Shepherd A, Weaver LT. Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial. Archives of Disease in Childhood 2003;88:F383-F390.
14. Malcolm CA, Hamilton R, McCulloch DL, Montgomery C, Weaver LT. Scotopic electroretinogram in term infants born of Mothers supplemented with docosahexaenoic acid during pregnancy. Invest Ophthalmol Vis Sci 2003;44:3685-91.
15. Tofail F, Kabir I, Hamadani JD et al. Supplementation of fish oil and soy oil during pregnancy and psychomotor development of infants. J Health Pop Nutr 2006;24:48-56.
16. Montgomery C, Speake BK, Cameron A, Sattar N, Weaver LT. Maternal docosahexaenoic acid supplementation and fetal accretion. Br J Nutr 2003;90:135-45.
17. Helland IB, Saugstad OD, Smith L et al. Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. Pediatrics 2001;108:art-e82.
18. Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003;111:art-e39.
19. Gibson RA, Neumann MA, Makrides M. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr 1997;51:578-84.
20. Lauritzen L, Jorgensen MH, Mikkelsen TB et al. Maternal fish oil supplementation in lactation: Effect on visual acuity and n-3 fatty acid content of infant erythrocytes. Lipids 2004;39:195-206.
21. Lauritzen L, Jorgensen MH, Olsen SF, Straarup EM, Michaelsen KF. Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants. Reprod Nutr Dev 2005;45:535-47.
22. Jensen CL, Voigt RG, Prager TC et al. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. Am J Clin Nutr 2005;82:125-32.
23. Fewtrell MS, Abbott RA, Kennedy K et al. Randomized, double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in preterm infants. J Pediatr 2004;144:471-9.
24. Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. Br Med J 1998;317:1481-7.
25. Clandinin MT, Van Aerde JE, Merkel KL et al. Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. J Pediatr 2005;146:461-8.
26. Fang PC, Kuo HK, Huang CB, Ko TY, Chen CC, Chung MY. The effect of supplementation of docosahexaenoic acid and arachidonic acid on visual acuity and neurodevelopment in larger preterm infants. Chang Gung Med J 2005;28:708-15.
27. Fewtrell MS, Morley R, Abbott RA et al. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. Pediatrics 2002;110:73-82.
28. Wezel-Meijler G, van der Knaap MS, Huisman J, Jonkman EJ, Valk J, Lafeber HN. Dietary supplementation of long-chain polyunsaturated fatty acids in preterm infants: effects on cerebral maturation. Acta Paediatrica 2002;91:942-50.

29. SanGiovanni JP, Berkey CS, Dwyer JT, Colditz GA. Dietary essential fatty acids, long-chain polyunsaturated fatty acids, and visual resolution acuity in healthy fullterm infants: a systematic review. Early Hum Dev 2000;57:165-88.
30. Uauy R, Hoffman DR, Mena P, Llanos A, Birch EE. Term infant studies of DHA and ARA supplementation on neurodevelopment: Results of randomized controlled trials. J Pediatr 2003;143:S17-S25.
31. Auestad N, Halter R, Hall RT et al. Growth and development in term infants fed long-chain polyunsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study. Pediatrics 2001;108:372-81.
32. Scott DT, Janowsky JS, Carroll RE, Taylor JA, Auestad N, Montalto MB. Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? Pediatrics 1998;102:E59.
33. Auestad N, Scott DT, Janowsky JS et al. Visual, cognitive, and language assessments at 39 months: A follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. Pediatrics 2003;112:E177-E183.
34. Bouwstra H, Dijck-Brouwer DA, Wildeman JA et al. Long-chain polyunsaturated fatty acids have a positive effect on the quality of general movements of healthy term infants. Am J Clin Nutr 2003;78:313-8.
35. Bouwstra H, Dijck-Brouwer DAJ, Boehm G, Boersma ER, Muskiet FAJ, Hadders-Algra M. Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants. Acta Paediatrica 2005;94:26-32.
36. Ben XM, Zhou XY, Zhao WH et al. Growth and development of term infants fed with milk with long- chain polyunsaturated fatty acid supplementation. Chin Med J 2004;117:1268-70.
37. Birch EE, Hoffman DR, Castaneda YS, Fawcett SL, Birch DG, Uauy RD. A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. Am J Clin Nutr 2002;75:570-80.
38. Birch EE, Castaneda YS, Wheaton DH, Birch DG, Uauy RD, Hoffman DR. Visual maturation of term infants fed long-chain polyunsaturated fatty acid-supplemented or control formula for 12 mo. Am J Clin Nutr 2005;81:871-9.
39. Unay B, Sarici SU, Ulas UH, Akin R, Alpay F, Gokcay E. Nutritional effects on auditory brainstem maturation in healthy term infants. Arch Dis Child Fetal Neonatal Ed 2004;89:F177-F17µ.
40. Hoffman DR, Birch EE, Castaneda YS et al. Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: A randomized clinical trial. J Pediatr 2003;142:669-77.
41. Hoffman DR, Theuer RC, Castaneda YS et al. Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. J Nutr 2004;134:2307-13.
42. Morale SE, Hoffman DR, Castaneda YS, Wheaton DH, Burns RA, Birch EE. Duration of long-chain polyunsaturated fatty acids availability in the diet and visual acuity. Early Hum Dev 2005;81:197-203.
43. Makrides M, Neumann M, Simmer K, Pater J, Gibson R. Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 1995;345:1463-8.
44. Birch EE, Hoffman DR, Uauy R, Birch DG, Prestidge C. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatr Res 1998;44:201-9.
45. Auestad N, Montalto MB, Hall RT et al. Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. Pediatr Res 1997;41:1-10.
46. Bakker EC, Ghys AJA, Kester ADM et al. Long-chain polyunsaturated fatty acids at birth and cognitive function at 7y of age. Eur J Clin Nutr 2003;57:89-95.
47. Zhang J, Hebert JR, Muldoon MF. Dietary fat intake is associated with psychosocial and cognitive functioning of school-aged children in the United States. J Nutr 2005;135:1967-73.
48. Richardson AJ, Puri BK. A randomized double-blind, placebo-controlled study of the effects of supplementation with highly unsaturated fatty acids on ADHD-related symptoms in children with specific learning difficulties. Prog Neuro-Psychopharmacol Biol Psychiatry 2002;26:233-9.
49. Stevens L, Zhang W, Peck L et al. EFA supplementation in children with inattention, hyperactivity, and other disruptive behaviors. Lipids 2003;38:1007-21.
50. Richardson AJ, Montgomery P. The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. Pediatrics 2005;115:1360-6.
51. Voigt RG, Llorente AM, Jensen CL, Fraley JK, Berretta MC, Heird WC. A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. Journal of Pediatrics 2001;139:189-96.
52. Hirayama S, Hamazaki T, Terasawa K. Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder - a placebo-controlled double-blind study. Eur J Clin Nutr 2004;58:467-73.
53. Infante JP, Huszagh VA. Impaired arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acid synthesis by phenylalanine metabolites as etiological factors in the neuropathology of phenylketonuria. Mol Genet Metab 2001;72:185-98.
54. Agostoni C, Massetto N, Biasucci G et al. Effects of long-chain polyunsaturated fatty acid supplementation on fatty acid status and visual function in treated children with hyperphenylalaninemia. J Pediatr 2000;137:504-9.
55. Beblo S, Reinhardt H, Muntau AC, Mueller-Felber W, Roscher AA, Koletzko B. Fish oil supplementation improves visual evoked potentials in children with phenylketonuria. Neurology 2001;57:1488-91.
56. Paus T. Mapping brain maturation and cognitive development during adolescence. Trends Cogn Sci 2005;9:60-

8.
57. Thomas KM. Assessing brain development using neurophysiologic and behavioral measures. J Pediatr 2003;143:S46-S53.
58. Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE. Essential fatty acids in visual and brain development. Lipids 2001;36:885-95.
59. Emken EA, Adlof RO, Gulley RM. Dietary Linoleic-Acid Influences Desaturation and Acylation of Deuterium-Labeled Linoleic and Linolenic Acids in Young-Adult Males. Biochimica et Biophysica Acta-Lipids and Lipid Metabolism 1994;1213:277-88.
60. Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. Journal of Lipid Research 2001;42:1257-65.
61. Salem N, Pawlosky R, Wegher B, Hibbeln J. In vivo conversion of linoleic acid to arachidonic acid in human adults. Prostaglandins Leukotrienes and Essential Fatty Acids 1999;60:407-10.
62. Vermunt SHF, Mensink RP, Simonis AMG, Hornstra G. Effects of age and dietary n-3 fatty acids on the metabolism of [C-13]-alpha-linolenic acid. Lipids 1999;34:S127.
63. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. Current Opinion in Clinical Nutrition and Metabolic Care 2002;5:127-32.
64. Udell T, Gibson RA, Makrides M. The effect of alpha-linolenic acid and linoleic acid on the growth and development of formula-fed infants: A systematic review and meta-analysis of randomized controlled trials. Lipids 2005;40:1-11.
65. Blank C, Neumann MA, Makrides M, Gibson RA. Optimizing DHA levels in piglets by lowering the linoleic acid to alpha-linolenic acid ratio. J Lipid Res 2002;43:1537-43.
66. Hornstra G. Importance of polyunsaturated fatty acids of the n-6 and n-3 families for early human development. Eur J Lipid Sci Technol 2001;103:379-89.
67. Carlson SE, Cooke RJ, Werkman SH, Tolley EA. First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. Lipids 1992;27:901-7.
68. Werner A, Havinga R, Kuipers F, Verkade HJ. Treatment of EFA deficiency with dietary triglycerides or phospholipids in a murine model of extrahepatic cholestasis. Am J Physiol Gastrointest Liver Physiol 2004;286:G822-G832.
69. Wijendran V, Huang MC, Diau GY, Boehm G, Nathanielsz PW, Brenna JT. Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates. Pediatr Res 2002;51:265-72.

# Chapter 4

## Undernutrition, fatty acid and micronutrient status in relation to cognitive performance in Indian schoolchildren: a cross-sectional study

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## Abstract

**Background**: While undernutrition and anemia have previously been linked to poor development of children, relatively little is known about the role of B-vitamins and fatty acids on cognition.

**Objective**: The present study aims to explore the associations between indicators of body size, fatty acid and micronutrient status on cognitive performance in 598 Indian schoolchildren aged 6-10 years.

**Methods**: Baseline data of a clinical study were used to assess these associations by analyses of variance adjusting for age, gender, school, maternal education and cognitive tester. The Kaufman Assessment Battery for Children II was used to measure four cognitive domains, including fluid reasoning, short-term memory, retrieval ability and cognitive speediness. Scores were combined into an overall measure, named Mental Processing Index (MPI).

**Results**: Body size indicators and hemoglobin concentrations were significantly positively related to cognitive domains and MPI, such that increases of 1 SD in height-for-age and weight-for-age *z*-scores would each translate into a 0.09 SD increase in MPI, *P*=0.0006 and *P*=0.002, respectively. A 10 g/L increase in hemoglobin concentrations would translate into a 0.08 SD increase in MPI, *P*=0.0008. Log-transformed vitamin $B_{12}$ concentrations were significantly inversely associated with short-term memory, retrieval ability and MPI (β=-0.124; 95% CI: -0.224, -0.023, *P*=0.02). Other indicators of iron, iodine, folate and fatty acid status were not significantly related to cognition.

**Conclusions**: Our findings for body size, fatty acids and micronutrients are in agreement with previous observational studies. The inverse association of vitamin $B_{12}$ with mental development was unexpected and needs further study.

## Introduction

Low intakes of energy, protein and other nutrients, together with high infection rates and poor socio-economic status may lead to linear growth retardation and impaired child development (1). Cross-sectional studies have linked stunting (short stature for age) and low weight for age to poor development and school achievement in infants and children (2). The detrimental effects of undernutrition early in life (<2 years of age) on intellectual development seem irreversible and remain apparent during childhood and adolescence (3;4).

The omega-3 fatty acid DHA and the omega-6 fatty acid arachidonic acid are important structural components of the human central nervous system (5) and play a role in brain functioning through their involvement in aspects of neuron function and of neurotransmitter synthesis (6). These two fatty acids can be synthesized by the human body from the $\alpha$-linolenic acid (ALA) and linoleic acid. However, dietary intake of the omega-3 fatty acid ALA in children is considered to be low (7;8), which possibly limits adequate cognitive functioning. In fact, high fish intake during pregnancy has been associated with better cognitive development of infants (9-11) and maternal and infant DHA supplementation may benefit visual, motor and mental development of infants and young children (12-18). For healthy children >2 years of age such evidence is currently limited (19).

Among the micronutrients, iron and iodine interventions have been shown to improve intelligence scores of children (20;21). Iron is needed for the formation of hemoglobin for adequate oxygen transport in the human body. In the brain, iron is required for myelination and neurotransmitter synthesis (22). Iodine is an important component of the thyroid hormones, thyroxin and triiodothyronine, which play a major role in the growth and development, function and maintenance of the central and peripheral nervous system (23). For the B-vitamins, however, little research has been conducted to investigate whether these vitamins are of influence on mental development in children. Vitamin $B_{12}$ (cobalamin) deficiency has been associated with lower scores on cognitive tests in Guatemalan (24) and Dutch (25) children. Folate is important for closure of the neural tube during fetal development (26), but no studies have investigated the role of folate on cognitive functioning in children after birth. In the brain, folate is required for neurotransmitter production and myelination (27;28). Because of the interactions with folate metabolism, vitamin $B_{12}$ is indirectly involved in neurotransmitter synthesis. Furthermore, the vitamin $B_{12}$ cofactors adenosylcobalamin and methylcobalamin are involved in myelination of the spinal cord and brain (29).

The primary objective of the current study is to investigate the associations between indicators of body size, fatty acid status, and iron, iodine and B-vitamin status on overall cognitive performance in 598 Indian school-age children. Secondary, we will explore the relationships of the nutritional parameters with specific cognitive domains known to be sensitive to differences in nutritional

status in children. We hypothesize that the indicators of body size, fatty acid and micronutrient status (iron, iodine, folate, and vitamin $B_{12}$) will be positively related to overall cognitive performance and specific cognitive domains.

## Experimental Methods

The CHAMPION (Children's Health And Mental Performance Influenced by Optimal Nutrition) study was designed to investigate the efficacy of foods fortified with omega-3 fatty acids and micronutrients on improving intellectual performance and growth in Indian schoolchildren (30). The baseline data of this study, collected in the period between November 2005 and February 2006, were used to assess the associations between height-for-age and weight-for-age *z*-scores, hemoglobin concentration and indicators of omega-3 and omega-6 fatty acid, iron, iodine, folate and vitamin $B_{12}$ status and cognitive performance. These nutritional parameters were selected based on their possible relationship with children's mental development.

### Subjects

Two primary schools serving children from a poor socio-economic background in Bangalore city, India were selected for participation in the study. Almost all children living in the surrounding communities attended these schools, where they were taught in the local Kannada language. Before study start, parents or caretakers of all children aged 6-10 years, attending grades 2-5 of these schools were invited for a meeting during which the study procedures were explained to them. Informed, written consent from the parents and verbal assent from their children was obtained from 645 parent-child pairs. Children were included in the study if they were: 1) apparently healthy, without any chronic illness and physical/mental handicaps; 2) not severely anemic (hemoglobin <80 g/L); 3) not severely undernourished (<-3 SD for weight-for-age (WAZ) and height for age (HAZ) *z*-scores of National Health Centre for Statistics/World Health Organization standards (NCHS/WHO) (31)); 4) not intending to use micronutrients supplements during the study; and 5) planning to reside in the study area during the next 12 months. Children who were frequently absent from school (>40 days during 6 months prior to start of the study) and children who took micronutrient supplements in the period of 3 months prior to the study start were excluded. A total of 598 children were enrolled in the study. Details on the enrolment, including a flow chart of children recruited in the study have been published elsewhere (30).

### Socio-demographic information

Socio-demographic information on household composition, parental education, income, and use of fortified foods was collected by a structured questionnaire which was administered to the mother or primary caretaker of the subjects. The age of the children was verified by the school records.

## Cognitive performance

Cognitive performance was evaluated using age-appropriate, validated psychometric tests that were administered by seven masters-level psychologists in Kannada language. The psychologists were trained extensively during three weeks before the study to ensure standardization in the test administration and scoring procedures. The cognitive test battery was administered to each child on a single day over three sessions of which two took place in the morning and one in the afternoon. Care was taken to ensure all children had breakfast before testing began in the morning since omitting breakfast is known to impair cognitive performance (32). The cognitive test battery consisted of 11 subtests, including six core tests of the Kaufman Assessment Battery for Children, second edition (KABC-II) for children 3-18 y (pattern reasoning, triangles, rover, number recall, word order, atlantis) (33), two tests from Wechsler Intelligence Scale for Children-Revised and Wechsler Intelligence Scale for Children-4 (picture arrangement, coding) and three additional tests from Rey Auditory-Verbal Learning Test (auditory-verbal learning test), Neuropsychological Assessment tool (verbal fluency), and number cancellation, which was specifically designed for the study. The 11 subtests covered four cognitive domains as specified in Carroll's model as described in the KABC-II manual (33), including fluid reasoning, short-term memory, retrieval ability and cognitive speediness (see **Figure 1**).

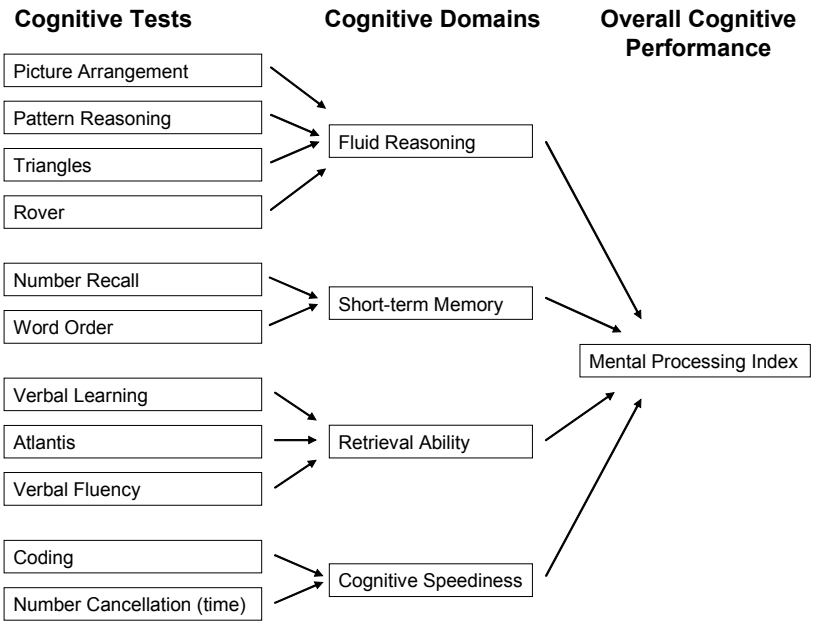


**Figure 1**. Clustering of cognitive tests in domain scores.
Fluid reasoning involves basic processes of reasoning and other mental activities that depend only minimally on learning and acculturation; short-term memory is an ability that requires apprehending and holding information in immediate awareness briefly and then using that information within a few seconds; retrieval ability comprises the capacity to store information in long-term memory and to retrieve that information fluently and efficiently; cognitive speediness measures the ability of rapid cognitive processing of information involving attention.

These domains were chosen because they have shown to be influenced by previous nutritional interventions (34). The test battery underwent an extensive adaptation process to ensure its suitability in the local cultural context (35). For each subtest, a sum score was calculated and converted into a standardized *z*-score. The domain score was composed by taking the average of standardized *z*-scores for the tests constituting a domain. The average of the domain scores named the Mental Processing Index (MPI) was a composite measure of overall cognitive performance based on the KABC-II manual (33). Our model of clustering of individual sum scores to form a composite score in four separate cognitive domains showed good validity assessed by structural equation modeling techniques (36).

**Anthropometry**

Anthropometric measurements were conducted in duplicate according to standard techniques (37) by trained research assistants. Height was recorded to the nearest 0.1 cm using a locally made stadiometer (Biorad, Chennai, India) that was fixed to a wall. Body weight was recorded to the nearest 0.1 kg using a digital weighing scale (Breuer, Germany). During the measurements, children wore their school uniform and no shoes, caps or hats. Height-for-age *z*-scores (HAZ) and weight-for-age *z*-scores (WAZ) were computed by data on height, weight, age and gender using the NCHS/WHO growth reference data (31). Children with HAZ and WAZ <-2 SD of this reference median were classified as stunted and underweight, respectively. We did not include weight-for-height *z*-scores, because NCHS/WHO reference data are lacking for children >10 years of age, which concerned 57 children aged 10-11 years in our study.

**Biochemical indicators**

A whole blood sample (10 mL) was collected in the morning from non-fasted children by venipuncture in an EDTA vacutainer. A spot urine sample was also collected in a sterile plastic container, and the samples were transported to the laboratory on ice. Care was taken to limit the exposure of the samples to light. Hemoglobin concentrations were determined within 4 hours of collection using an AcT Diff2 Counter (Beckman Coulter Inc, Fullerton, CA, USA). One aliquot of whole blood for erythrocyte folate estimation was immediately treated with freshly prepared 1% ascorbic acid. The remaining blood was immediately centrifuged (3000 rpm, 10 min, 4 ºC), and the plasma was stored in 2 mL eppendorf tubes at –80 ºC until analysis. One milliliter of erythrocytes was washed with 5 mL saline containing EDTA (1 L normal saline + 0.00324 g Disodium EDTA), flushed under nitrogen and stored at –80 ºC until analysis for fatty acid content. Serum ferritin was measured by an enzyme immunoassay (Access® 2 Beckman Coulter autoanalyser, Brea, CA, USA (38) against an external 3-level control material (WHO Standard 80/578; Ramco Laboratories Inc., Houston, TX, USA). Serum soluble transferrin receptor

(sTfR) was measured by using an enzyme immunoassay (Ramco Laboratories Inc.) with two-level control materials provided by the manufacturer. C-reactive protein (CRP) was analyzed by a turbidimetric method (Roche Hitachi 902, Indianapolis, IN, USA (38). Plasma vitamin B12, and red blood cell folate were analyzed using a Chemiluminescence System (ACS:180, Bayer Diagnostics, Tarrytown, USA (39;40). Fatty acid content of erythrocyte membrane phospholipids was analyzed using gas chromatography with a flame ionization detector (Varian 3800, Palo Alto, CA, USA). The procedure involved the extraction of total lipids, isolation of phospholipid fraction by thin- layer chromatography and transmethylation of phospholipids (41-43). The fatty acid methyl esters were separated by chain length and degree of saturation by injection onto a 50 m × 0.2 mm capillary column (FAME, Varian, Palo Alto, CA, USA) with nitrogen as carrier gas. Urinary iodine was measured using the Sandell-Kolthoff reaction as modified by Pino et al. (44). Satisfactory agreement in urinary iodine was obtained on urine samples at four different concentrations measured and the EQUIP-Network (Ensuring the Quality of Urinary Iodine Procedures, Centers for Disease Control and Prevention, Atlanta, GA). The following criteria were used to define micronutrient deficiencies: anemia: hemoglobin <115 g/L (45); iron deficiency: serum ferritin <15 mg/L and/or sTfR >7.6 mg/L (46); folate deficiency: erythrocyte folate <305 nmol/L (47); vitamin B12 deficiency: plasma vitamin B12 <148 pmol/L (48) and; iodine deficiency: urinary iodine <100 µg/L) (49).

**Statistical analyses**

Values for serum ferritin concentrations from subjects with elevated CRP (>10 mg/L) were excluded from statistical analyses. Body iron stores were calculated from serum ferritin and sTfR concentrations using the formula by Cook et al (50). Differences in mean cognitive outcomes between boys and girls, schools and different levels of education of the mother were assessed by *t*-tests. Distributions of parameters of fatty acid status, serum ferritin and sTfR, erythrocyte folate, plasma vitamin $B_{12}$ and urinary iodine were normalized by natural logarithm (ln) transformation before analysis. Associations between the nutritional parameters and the cognitive scores were analyzed using analysis of variance (SAS General Linear Modeling procedure) taking into account age, gender, school, maternal education level and assessor of cognitive tests as covariates. All available data were analyzed, missing values were not replaced. All analyses were performed using SAS version 9.1 statistical software package (SAS Institute Inc, Cary, NC).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committees at St John's National Academy of Health Sciences, Bangalore, India and Wageningen University, the Netherlands. Written informed consent was obtained from the parents of all subjects and verbal assent from all subjects.

Verbal consent was witnessed and formally recorded.

## Results

Five hundred and ninety eight children completed the baseline measurements on cognitive performance, anthropometry and hemoglobin concentrations. Data on biochemical indicators of micronutrient and fatty acid status were available for at least 529 and 541 children, respectively. The socio-demographic characteristics and nutritional status of subjects are presented in **Table 1**.

**Table 1**. Characteristics of the study population

| Characteristic | N | |
|---|---|---|
| *Sociodemographic parameters* | | |
| Gender (% males) | 598 | 49 |
| Age (y) | 598 | 8.7 ± 1.2[1] |
| Family income (Indian rupees/month) | 598 | 2700 (2000, 3800)[2] |
| Uneducated mothers (%) | 571 | 45.5 |
| *Anthropometric measures* | | |
| Height (m) | 598 | 122.9 ± 7.8 |
| Weight (kg) | 598 | 21.4 ± 3.8 |
| *Erythrocyte phospholipid fatty acid status (% of total fatty acids (wt/wt))* | | |
| Linoleic acid | 541 | 14.12 (13.07, 15.21) |
| Arachidonic acid | 541 | 14.61 (12.75, 16.18) |
| $\alpha$-Linolenic acid | 541 | 0.21 (0.17, 0.25) |
| EPA | 541 | 0.16 (0.13, 0.20) |
| DHA | 541 | 3.21 (2.70, 3.65) |
| *Micronutrient status* | | |
| Blood hemoglobin (g/L) | 598 | 127.8 ± 9.9 |
| Serum ferritin (µg/L) | 535 | 23.6 (14.9, 34.2) |
| Serum soluble transferrin receptors (mg/L) | 538 | 5.7 (4.9, 6.8) |
| Total body iron stores (mg/kg) | 535 | 3.22 ± 3.20 |
| Erythrocyte folate (nmol/L) | 529 | 515 (371, 745) |
| Plasma vitamin $B_{12}$ (pmol/L) | 533 | 197 (151, 266) |
| Urinary iodine (µg/L) | 542 | 108 (66, 200) |

[1] Mean ± SD (all such values).
[2] Median ($25^{th}$, $75^{th}$ percentiles) (all such values).

Mean age of the children was 8.7 ± 1.2 years and 49% of them were boys. Nearly half of the mothers were uneducated and median family income was 2700 Indian rupees per month, which is close to the poverty line of US$2 per day. Twenty-two percent of the children were stunted and 30% were underweight. The prevalence of anemia was 9%, while that of iron, folate, vitamin $B_{12}$ and iodine deficiencies were 31%, 17%, 23% and 47%, respectively.

### Associations of covariates with cognitive performance

Age was significantly positively related with all cognitive outcomes (β=0.31; 95% CI: 0.27, 0.35), $P<0.0001$ for MPI). Mean cognitive scores for boys and girls are presented in **Table 2**. Scores on the domains of retrieval ability and cognitive speediness, and MPI were significantly lower in boys compared to girls

(*P*<0.001). These findings did not change when scores were corrected for age (data not shown). There was a significant difference in performance on short-term memory and retrieval ability between the two schools (data not shown). Children of mothers with <6 y of education had significantly lower MPI scores compared to children of mothers with ≥6 y of education (mean ± SD were -0.05 ± 0.66 vs 0.07 ± 0.63, *P*=0.03, respectively).

**Table 2**. Cognitive domain scores for boys and girls (mean ± SD)[1]

| | Boys (*n*=293) | Girls (*n*=305) |
|---|---|---|
| Mental Processing Index | -0.09 ± 0.64[2] | 0.09 ± 0.64 |
| Fluid reasoning | 0.01 ± 0.81 | -0.01 ± 0.77 |
| Short-term memory | -0.06 ± 0.92 | 0.05 ± 0.88 |
| Retrieval ability | -0.12 ± 0.76[2] | 0.11 ± 0.75 |
| Cognitive speediness | -0.21 ± 0.88[2] | 0.20 ± 0.83 |

[1] Domain scores are expressed in z-scores.
[2] Scores between boys and girls were significantly different, *t*-test *P*<0.001.

## Associations of nutritional parameters with cognitive performance

**Table 3** provides an overview of the associations between the nutritional parameters and the indicators of cognitive performance. Scatter plots of the correlations between the MPI and HAZ, WAZ, hemoglobin and vitamin $B_{12}$ concentrations are shown in **Figure 2**. HAZ scores were significantly positively related to all cognitive domains and MPI. WAZ were significantly positively associated with all cognitive parameters, except cognitive speediness. The associations of HAZ and WAZ would in theory mean that an increase of 1 SD in HAZ and WAZ would correspond with 0.09 SD increase in MPI, *P*=0.0006 and *P*=0.002, respectively.

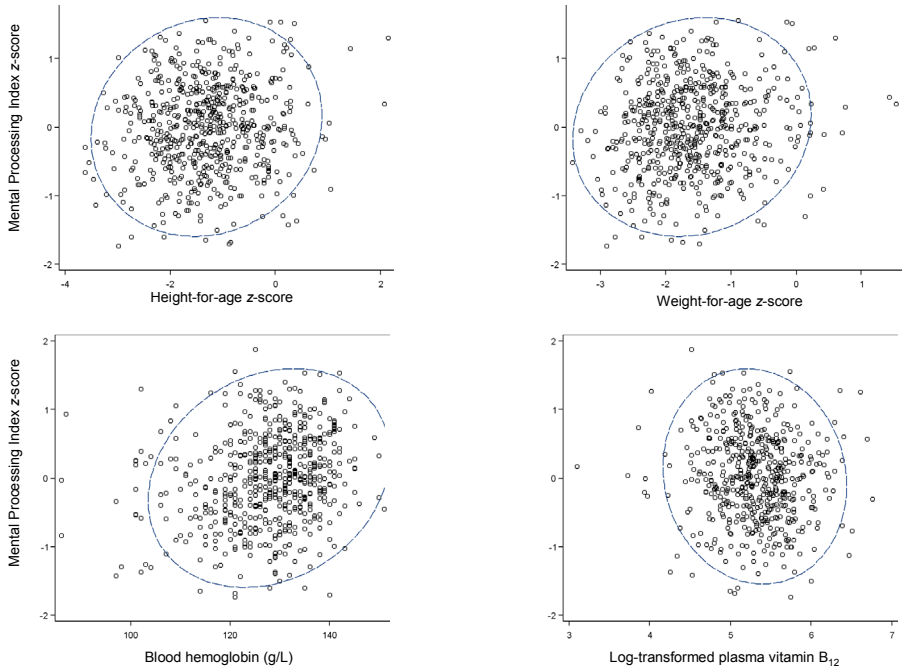


**Figure 2**. Scatterplots of correlations between the Mental Processing Index and height-for-age *z*-score (*r*=0.10, *P*=0.012), weight-for-age *z*-score (*r*=0.11, *P*=0.007), hemoglobin concentration (*r*=0.21, *P*<0.0001) and log-transformed vitamin $B_{12}$ (*r*=-0.09, *P*=0.046).

**Table 3**. Overview of associations of nutritional factors with cognitive performance[1]

| | N | Mental Processing Index | Cognitive domains | | | |
|---|---|---|---|---|---|---|
| | | | Fluid reasoning | Short-term memory | Retrieval ability | Cognitive speediness |
| Height-for-age *z*-score | 570 | 0.085 (0.037, 0.133)[2] | 0.114 (0.056, 0.172) | 0.079 (-0.002, 0.160) | 0.061 (0.000, 0.123) | 0.086 (0.019, 0.152) |
| Weight-for-age *z*-score | 570 | 0.091 (0.033, 0.150) | 0.123 (0.054, 0.193) | 0.115 (0.018, 0.213) | 0.077 (0.003, 0.152) | 0.049 (-0.032, 0.130) |
| Linoleic acid[3,4] | 513 | 0.007 (-0.251, 0.265) | 0.200 (-0.112, 0.511) | -0.053 (-0.484, 0.378) | 0.017 (-0.304, 0.337) | -0.133 (-0.488, 0.222) |
| Arachidonic acid[3,4] | 513 | -0.053 (-0.211, 0.105) | 0.047 (-0.143, 0.237) | -0.039 (-0.267, 0.260) | -0.067 (-0.263, 0.129) | -0.187 (-0.343, 0.453) |
| α-Linolenic acid[3,4] | 513 | -0.097 (-0.195, 0.000) | -0.079 (-0.196, 0.046) | -0.124 (-0.286, 0.038) | -0.075 (-0.196, 0.046) | -0.112 (-0.245, 0.021) |
| EPA[3,4] | 513 | -0.006 (-0.122, 0.110) | 0.041 (-0.099, 0.018) | 0.026 (-0.168, 0.219) | -0.087 (-0.230, 0.057) | -0.004 (-0.164, 0.155) |
| DHA[3,4] | 513 | -0.061 (-0.204, 0.083) | -0.032 (-0.204, 0.140) | -0.010 (-0.250, 0.230) | -0.038 (-0.216, 0.140) | -0.164 (-0.360, 0.032) |
| Blood hemoglobin | 570 | 0.007 (0.003, 0.012) | 0.005 (0.000, 0.010) | 0.009 (0.002, 0.016) | 0.008 (0.002, 0.013) | 0.008 (0.002, 0.014) |
| Serum ferritin[3] | 507 | 0.031 (-0.035, 0.096) | 0.020 (-0.059, 0.099) | 0.040 (-0.068, 0.149) | 0.034 (-0.047, 0.115) | 0.028 (-0.062, 0.118) |
| Serum soluble transferrin receptor[3] | 510 | -0.096 (-0.258, 0.065) | -0.106 (-0.300, 0.088) | 0.027 (-0.241, 0.295) | -0.295 (-0.494, -0.097) | -0.010 (-0.232, 0.212) |
| Total body iron stores | 507 | 0.006 (-0.009, 0.020) | 0.006 (-0.011, 0.023) | 0.003 (-0.021, 0.027) | 0.011 (-0.007, 0.029) | 0.003 (-0.017, 0.022) |
| Erythrocyte folate[3] | 501 | -0.038 (-0.112, 0.036) | -0.101 (-0.191, -0.011) | -0.108 (-0.230, 0.015) | 0.053 (-0.038, 0.144) | 0.003 (-0.099, 0.105) |
| Plasma vitamin $B_{12}$[3] | 497 | -0.124 (-0.224, -0.023) | -0.029 (-0.151, 0.094) | -0.212 (-0.380, -0.044) | -0.195 (-0.319, -0.071) | -0.058 (-0.195, 0.079) |
| Urinary iodine[3] | 515 | -0.007 (-0.057, 0.043) | 0.026 (-0.035, 0.086) | -0.044 (-0.127, 0.039) | 0.009 (-0.054, 0.072) | -0.020 (-0.089, 0.049) |

[1] Using a model adjusted for age, gender, school, maternal education and assessor of cognitive tests. The $R^2$ of the models with the different nutritional indicators ranged from 0.34-0.37 for MPI, 0.37-0.39 for fluid reasoning, 0.08-0.10 for short-term memory, 0.25-0.27 for retrieval ability and 0.34-0.36 for cognitive speediness.
[2] β (95% CI) (all such values).
[3] Variables were normalized by natural logarithm transformation.
[4] Fatty acids were measured in the erythrocyte membranes in the phospholipid fraction.

No significant relationships were detected between linoleic acid, arachidonic acid, EPA and DHA and any of the cognitive parameters. α-Linolenic acid was significantly inversely related to the MPI, but no significant associations were observed with the separate cognitive domains.

Hemoglobin concentrations were significantly positively related to all cognitive domains and MPI. Our findings suggest that an increase of 10 g/L in hemoglobin concentration would translate into a 0.08 SD increase in MPI, *P*=0.0008. There was a significant inverse association between sTfR concentrations and retrieval ability. Other indicators of iron status were not significantly related to cognitive performance. Similarly, there were no significant associations between urinary iodine concentrations and cognitive parameters.

In contrast, significantly inverse relationships were found between erythrocyte folate concentrations and fluid reasoning (β=-0.10; 95% CI: -0.19, -0.01) and short-term memory (β=-0.11; 95% CI: -0.23, 0.02). However, when vitamin $B_{12}$ status was added to the model, these inverse associations were not significant anymore for fluid reasoning (β=-0.07; 95% CI: -0.17, 0.02) and for short-term memory (β=-0.08; 95% CI: -0.21, 0.05)). Vitamin $B_{12}$ concentrations were significantly inverse related to short-term memory and retrieval ability, and the MPI. These associations remained significant after further adjusting for hemoglobin and folate status and HAZ: β=-0.19; 95% CI: -0.36, -0.03 for short-term memory; β=-0.20; 95% CI: -0.33, -0.08 for retrieval ability and; β=-0.12; 95% CI: -0.22, -0.02, *P*=0.02 for MPI).

## Discussion

This study shows that indicators of body size, HAZ and WAZ, and hemoglobin concentrations were significantly positively related to various cognitive domain scores and MPI, while plasma vitamin $B_{12}$ concentrations were significantly inversely associated with short-term memory and retrieval ability and the MPI. Other indicators of iron, folate, iodine and fatty acid status were not significantly related to cognitive performance.

Strengths of this cross-sectional study were the availability of biochemical parameters of micronutrient and fatty acid status in a relatively large sample of >500 children from a low socio-economic background. The sample is representative for schoolchildren aged 6-10 years from poor socio-economic classes in Bangalore city and the surrounding peri-urban areas, based on a similar prevalence of anemia measured and similar average heights and weights in our and other studies conducted in children in Bangalore (51). The cognitive test battery was thoroughly adapted to local language and culture and showed good internal and external validity, which is essential to detect any associations between cognitive functioning and nutritional status (52). In addition, we chose to assess the cognitive abilities that have been shown to be influenced by nutritional interventions before (34).

A limitation of a cross-sectional study design is the inability for causal inference. Furthermore, the high number of comparisons made between nutritional and cognitive variables may have yielded false-positive findings (type I error). However, we tried to limit the number of comparisons by the use of composite scores for the cognitive tests. In addition, we aimed to look for patterns among our findings, such as the consistent positive association of HAZ with all cognitive parameters. Another limitation of the study was the finding that our overall model explained only 10%-40% of the variation in cognitive parameters. Genetic variation and environmental factors such as socio-emotional stimulation at home may account for this unexplained variation. In additional analyses we explored whether the interactions of age and gender with the nutritional indicators could explain any variation in cognitive test scores, but the results of these analyses did not yield further insights.

In agreement with our findings, lower HAZ, reflecting longer term undernutrition, has previously been associated with poorer cognitive performance in younger (1-3 years) (53-55) and school-age children (56-58). Moreover, intervention studies have demonstrated that protein-energy supplementation in young children benefits cognitive development on the longer term (4;58;59) and therefore an adequate intake of energy and protein is required for optimal development.

Erythrocyte fatty acid status was unrelated to cognitive performance, which is in line with findings from a cohort study in children aged 7 years of age (60). Possibly, the range in fatty acid status among the children was too narrow to determine effects on cognition. It may also be that erythrocyte or plasma fatty acid status does not resemble brain fatty acid status at school age when most brain growth has been completed. A study in humans estimated that DHA requirements of the brain are rather low and the authors suggested that the liver may synthesize sufficient amounts of DHA to maintain brain DHA concentrations, provided that dietary intake of the precursor $\alpha$-linolenic acid is adequate (61). Moreover, animal studies indicated that synthesis of DHA in the liver is enhanced and the turnover of DHA in the brain is reduced when diets were low in $\alpha$-linolenic acid and free of DHA (61). Thus, intake and erythrocyte concentrations of omega-3 fatty acids may not be related to brain function. Besides, there is some evidence that children with attention-deficit hyperactivity disorders (ADHD) have lower plasma/erythrocyte DHA and higher linoleic and arachidonic acid concentrations than control children (62-65), which could be attributed to differences in fatty acid metabolism (66). Therefore, more research is needed to investigate whether specific subgroups of children may be sensitive to fatty acid interventions and whether fatty acids may predominantly influence certain aspects of behavior, such as attention.

For hemoglobin, we showed a very small but significant positive relationship with mental performance. However, for the other parameters of iron status, no such relationships could be detected. Possibly, this relationship becomes only

apparent when iron deficiency has caused anemia, which was the case in only 6% of our study population. This has also been reported in a review of literature of observational studies showing that (iron deficient) anemic children have poorer cognitive development and school performance than non-anemic children, and it was concluded that it is unclear whether iron deficiency without anemia impairs mental performance (67). In contrast, iron supplementation has been shown to improve mental performance in children >2 years of age in (iron deficient) anemic as well as non-anemic children (20;21), indicating that extra iron may also be beneficial for development of non-anemic children. The higher cognitive scores with increasing hemoglobin concentrations found in our study, suggest that the hemoglobin level for optimal mental performance may be higher than the current definition of anemia (<115 g/L).

Against our expectations, both folate and vitamin $B_{12}$ were inversely associated with some of the cognitive domain scores. For folate these inverse relationships disappeared after controlling for vitamin $B_{12}$ status, while for vitamin $B_{12}$ the inverse associations with memory remained significant even after controlling for folate, hemoglobin and height-for-age. Our findings are in contrast with two earlier observational studies indicating that children with lower plasma vitamin $B_{12}$ concentrations had poorer cognitive test scores (24;25), and could be due to chance. In elderly, however, eight studies did not show significant associations between plasma vitamin $B_{12}$ and cognitive test performance (68) and one study showed an inverse relationship (69). Our finding and the observations in elderly contradict to the overt clinical signs of vitamin $B_{12}$ deficiency of neurological damage. Therefore, it has been questioned whether plasma vitamin $B_{12}$ is a suitable indicator to study effects on cognition (68;70). It is of interest to investigate whether higher plasma homocysteine concentrations are related to poorer mental performance in children, as has been observed in elderly (68;69). In both children and adults, plasma homocysteine concentrations are increasing when folate and vitamin $B_{12}$ intake are low (71) and elevated homocysteine may impair cognitive functioning through neurotoxic and vasotoxic effects (72). Also other indicators of vitamin $B_{12}$ status, such as holotranscobalamin and methylmalonic acid may be worth evaluating in future research (70).

In addition, we could speculate on other confounding factors that influence the relationship between higher plasma vitamin $B_{12}$ concentrations and poorer cognitive performance. Possibly, consumption of animal products infected with pathogens or vegetables contaminated with vitamin $B_{12}$–producing bacteria from manure may improve vitamin $B_{12}$ status (73;74) and simultaneously increase the risk of disease, resulting in poor school attendance and impaired cognition. However, no literature is available to support this hypothesis.

Despite the evidence in literature that iodine deficiency is detrimental to cognitive development (75) and that iodine supplementation improves cognitive

functioning in children (76), we failed to detect any association between urinary iodine concentrations and cognition, which may be due to day-to-day within subject variation in iodine excretion in urine (49).

In conclusion, findings of the current study are in agreement with other observational studies showing that undernutrition (lower HAZ and WAZ) and lower hemoglobin concentrations adversely influence cognitive performance in school-age children, while serum ferritin and sTfR concentrations, and indicators of iodine, folate and fatty acid status were unrelated and an inverse association was found for vitamin $B_{12}$ and memory. Future research is needed to elucidate the role of B-vitamins and homocysteine in cognitive development of children and to investigate whether fatty acid status at school age may be of influence on specific cognitive functions not measured in our study, such as attention.

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## References

1. Martorell R, Mendoza F, Castillo R. Poverty and stature in children. In: Waterlow JC, ed. Linear growth retardation in less developed countries. New York: Raven Press 1988:57-73.
2. Grantham-McGregor S, Baker-Henningham H. Review of the evidence linking protein and energy to mental development. Public Health Nutr 2005;8:1191-201.
3. Chang SM, Walker SP, Grantham-McGregor S, Powell CA. Early childhood stunting and later behaviour and school achievement. J Child Psychol Psychiatry 2002;43:775-83.
4. Walker SP, Chang SM, Powell CA, Grantham-McGregor SM. Effects of early childhood psychosocial stimulation and nutritional supplementation on cognition and education in growth-stunted Jamaican children: prospective cohort study. Lancet 2005;366:1804-7.
5. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129-S138.
6. Yehuda S. Omega-6/omega-3 ratio and brain-related functions. World Rev Nutr Diet 2003;92:37-56.
7. Elmadfa I, Weichselbaum E, Konig J et al. European nutrition and health report 2004. Forum Nutr 2005;1-220.
8. Kris-Etherton PM, Innis S, American DA, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc 2007;107:1599-611.
9. Daniels JL, Longnecker MP, Rowland AS, Golding J. Fish intake during pregnancy and early cognitive development of offspring. Epidemiol 2004;15:394-402.
10. Oken E, Wright RO, Kleinman KP et al. Maternal fish consumption, hair mercury, and infant cognition in a U.S. Cohort. Environ Health Perspect 2005;113:1376-80.
11. Hibbeln JR, Davis JM, Steer C et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 2007;369:578-85.
12. Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003;111:art-e39.
13. Hoffman DR, Theuer RC, Castaneda YS et al. Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. Journal of Nutrition 2004;134:2307-13.
14. Lauritzen L, Jorgensen MH, Olsen SF, Straarup EM, Michaelsen KF. Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants. Reprod Nutr Dev 2005;45:535-47.
15. Jensen CL, Voigt RG, Prager TC et al. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. Am J Clin Nutr 2005;82:125-32.
16. Judge MP, Harel O, Lammi-Keefe CJ. Maternal consumption of a docosahexaenoic acid-containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo. Am J Clin Nutr 2007;85:1572-7.
17. Judge MP, Harel O, Lammi-Keefe CJ. A docosahexaenoic acid-functional food during pregnancy benefits infant

visual acuity at four but not six months of age. Lipids 2007;42:117-22.
18. Agostoni C, Zuccotti CV, Radaelli G et al. Docosahexaenoic acid supplementation and time at achievement of gross motor milestones in healthy infants: a randomized, prospective, double-blind, placebo-controlled trial. Am J Clin Nutr 2009;89:64-70.
19. Eilander A, Hundscheid DC, Osendarp SJ, Transler C, Zock PL. Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: a review of human studies. Prostaglandins Leukot Essent Fatty Acids 2007;76:189-203.
20. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 2001;131:649S-66S.
21. Sachdev HPS, Gera T, Nestel P. Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. Public Health Nutr 2005;8:117-32.
22. Beard JL, Connor JR. Iron status and neural functioning. Ann Rev Nutr 2003;23:41-58.
23. Delange F. The role of iodine in brain development. Proc Nutr Soc 2000;59:75-9.
24. Allen LH, Penland JG, Boy E, DeBaessa Y, Rogers LM. Cognitive and neuromotor performance of Guatemalan schoolers with deficient, marginal, and normal plasma vitamin B-12. FASEB J 1999;13:A544.
25. Louwman MWJ, van Dusseldorp M, van de Vijver FJR et al. Signs of impaired cognitive function in adolescents with marginal cobalamin status. Am J Clin Nutr 2000;72:762-9.
26. Butterworth CE, Jr., Bendich A. Folic acid and the prevention of birth defects. Annu Rev Nutr 1996;16:73-97.
27. Sugden C. One-carbon metabolism in psychiatric illness. Nutr Res Rev 2006;19:117-36.
28. Hutto BR. Folate and cobalamin in psychiatric illness. Compr Psychiatry 1997;38:305-14.
29. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev 2008;66:250-5.
30. Muthayya S, Eilander A, Transler C et al. Effect of fortification with multiple micronutrients and n-3 fatty acids on growth and cognitive performance in Indian schoolchildren: the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. Am J Clin Nutr 2009;89:1766-75.
31. World Health Organization Expert Committee on Physical Status. Physical Status: The Use and Interpretation of Anthropometry. WHO Technical Report Series no. 854. Geneva: World Health Organization, 1995.
32. Grantham-McGregor S. Can the provision of breakfast benefit school performance? Food Nutr Bull 2005;26:S144-S158.
33. Kaufman AS, Kaufman LN. Kaufman Assessment Battery for Children, Second Edition: Manual. Circle Pines, MN: AGS Publishing, 2004.
34. Hughes D, Bryan J. The assessment of cognitive performance in children: Considerations for detecting nutritional influences. Nutrition Reviews 2003;61:413-22.
35. Malda M, van de Vijver FJR, Srinivasan K, Transler C, Sukumar P, Rao K. Adapting a cognitive test for a different culture: An illustration of qualitative procedures. Psychol Sci Q 2008;50:451-68.
36. Malda M, van de Vijver FJR, Srinivasan K, Transler C, Sukumar P. Traveling with cognitive tests: Testing the validity of a KABC-II adaptation in India. Assessment 2009. doi:10.1177/1073191109341445.
37. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Publishers, 1988.
38. Burtis CA, Ashwood ER. Fundamentals of clinical chemistry. Philadelphia, PA: WB Saunders Company, 1996.
39. Miale JB. Laboratory Medicine: Hematology. St. Louis: CV Mosby, 1982.
40. Chen W, Sperling MI, Heminger LA. Vitamin B12. In: Pesce AJ, Kaplan LA, eds. Methods in Clinical Chemistry. St. Louis: CV Mosby 1987:569-73.
41. Rose HG, Oaklander M. Improved procedure for the extraction of lipids from human erythrocytes. Journal of Lipids Research 1965;6:428-31.
42. Gentner PR, Bauer M, Dieterich I. Separation of major phospholipids classes of milk without previous isolation from total lipid extracts. Journal of Chromatography 1981;13:200-4.
43. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipids Research 1964;5:600-8.
44. Pino S, Fang SL, Braverman LE. Ammonium persulfate: a safe alternative oxidizing reagent for measuring urinary iodine. Clin Chem 1996;42:239-43.
45. World Health Organization, United Nations Children's Fund, United Nations University. Iron deficiency anemia. Assessment, prevention and control. A guide for programme managers. Geneva: World Health Organization, 2001.
46. Zimmermann MB, Molinari L, Staubli-Asobayire F. Serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children. Am J Clin Nutr 2005;81:615-23.
47. Institute of Medicine (IOM). Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press, 1998.
48. Jones KM, Ramirez-Zea M, Zuleta C, Allen LH. Prevalent vitamin B-12 deficiency in twelve-month-old Guatemalan infants is predicted by maternal B-12 deficiency and infant diet. J Nutr 2007;137:1307-13.
49. World Health Organization, United Nations Children's Fund, International Council for Control of Iodine Deficiency Disorders. Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. Geneva: World Health Organization, 2001.
50. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359-64.
51. Muthayya S, Thankachan P, Zimmermann MB et al. Low anemia prevalence in school-aged children in Bangalore, South India: possible effect of school health initiatives. Eur J Clin Nutr 2007;61:865-9.
52. Isaacs E, Oates J. Nutrition and cognition: assessing cognitive abilities in children and young people. Eur J Nutr 2008;47 Suppl 3:4-24.

53. Sigman M, Neumann C, Baksh M, Bwibo N, McDonald MA. Relationship between nutrition and development in Kenyan toddlers. J Pediatr 1989;115:357-64.
54. Freeman HE, Klein RE, Kagan J, Yarbrough C. Relations between nutrition and cognition in rural Guatemala. Am J Public Health 1977;67:233-9.
55. Whaley SE, Sigman M, Espinosa MP, Neumann CG. Infant predictors of cognitive development in an undernourished Kenyan population. J Dev Behav Pediatr 1998;19:169-77.
56. Sigman M, Neumann C, Jansen AA, Bwibo N. Cognitive abilities of Kenyan children in relation to nutrition, family characteristics, and education. Child Dev 1989;60:1463-74.
57. Johnston FE, Low SM, de Baessa Y, MacVean RB. Interaction of nutritional and socioeconomic status as determinants of cognitive development in disadvantaged urban Guatemalan children. Am J Phys Anthropol 1987;73:501-6.
58. Grantham-McGregor SM, Walker SP, Chang SM, Powell CA. Effects of early childhood supplementation with and without stimulation on later development in stunted Jamaican children. Am J Clin Nutr 1997;66:247-53.
59. Pollitt E, Watkins WE, Husaini MA. Three-month nutritional supplementation in Indonesian infants and toddlers benefits memory function 8 y later. Am J Clin Nutr 1997;66:1357-63.
60. Bakker EC, Ghys AJA, Kester ADM et al. Long-chain polyunsaturated fatty acids at birth and cognitive function at 7y of age. Eur J Clin Nutr 2003;57:89-95.
61. Rapoport SI, Rao JS, Igarashi M. Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. Prostaglandins Leukot Essent Fatty Acids 2007;77:251-61.
62. Mitchell EA, Aman MG, Turbott SH, Manku M. Clinical characteristics and serum essential fatty acid levels in hyperactive children. Clin Pediatr (Phila) 1987;26:406-11.
63. Stevens L, Zhang W, Peck L et al. EFA supplementation in children with inattention, hyperactivity, and other disruptive behaviors. Lipids 2003;38:1007-21.
64. Chen JR, Hsu SF, Hsu CD, Hwang LH, Yang SC. Dietary patterns and blood fatty acid composition in children with attention-deficit hyperactivity disorder in Taiwan. J Nutr Biochem 2004;15:467-72.
65. Colter AL, Cutler C, Meckling KA. Fatty acid status and behavioural symptoms of attention deficit hyperactivity disorder in adolescents: a case-control study. Nutr J 2008;7:8.
66. Burgess JR, Stevens L, Zhang W, Peck L. Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder. Am J Clin Nutr 2000;71:327S-30S.
67. Nokes C, van den Bosch C, Bundy DAP. The Effects of Iron Deficiency and Anemia on Mental and Motor Performance, Educational Achievement, and Behavior in Children. An Annotated Bibliography. Washington, D.C.: International Nutritional Anemia Consultative Group, 1998.
68. Raman G, Tatsioni A, Chung M et al. Heterogeneity and lack of good quality studies limit association between folate, vitamins B-6 and B-12, and cognitive function. J Nutr 2007;137:1789-94.
69. Durga J, van Boxtel MP, Schouten EG, Bots ML, Kok FJ, Verhoef P. Folate and the methylenetetrahydrofolate reductase 677C-->T mutation correlate with cognitive performance. Neurobiol Aging 2006;27:334-43.
70. Miller JW. Assessing the association between vitamin B-12 status and cognitive function in older adults. Am J Clin Nutr 2006;84:1259-60.
71. Bjorke Monsen AL, Ueland PM. Homocysteine and methylmalonic acid in diagnosis and risk assessment from infancy to adolescence. Am J Clin Nutr 2003;78:7-21.
72. Obeid R, Herrmann W. Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. FEBS Lett 2006;580:2994-3005.
73. Allen LH. Causes of vitamin B12 and folate deficiency. Food Nutr Bull 2008;29:S20-S34.
74. Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. Am J Clin Nutr 2003;78:3-6.
75. Bleichrodt N, Born MP. A meta-analysis of research on iodine and its relationship to cognitive development. In: Stanbury JB, ed. The damaged brain of iodine deficiency. New York, NY: Cognizant Communication Corporation 1994:195-200.
76. Zimmermann MB, Connolly K, Bozo M, Bridson J, Rohner F, Grimci L. Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: a randomized, controlled, double-blind study. Am J Clin Nutr 2006;83:108-14.

# Chapter 5

## Effect of fortification with multiple micronutrients and omega-3 fatty acids on growth and cognitive performance in Indian schoolchildren: the CHAMPION (Children's Health And Mental Performance Influenced by Optimal Nutrition) Study

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## Abstract

**Background:** Fortification with multiple micronutrients has been shown to improve growth and cognitive performance among children in developing countries, but it is unknown whether higher concentrations are more effective than lower concentrations.

**Objective:** We compared the effect of two different concentrations of a combination of micronutrients and omega-3 fatty acids on indicators of growth and cognitive performance in low-income, marginally nourished schoolchildren in Bangalore, India.

**Design:** In a 2x2 factorial, double-blind, randomized controlled trial, 598 children aged 6–10 years were individually allocated to 1 of 4 intervention groups to receive foods fortified with either 100% or 15% of the Recommended Dietary Allowance of micronutrients in combination with either 900 mg $\alpha$-linolenic acid plus 100 mg docosahexaenoic acid or 140 mg $\alpha$-linolenic acid for 12 months. Anthropometric and biochemical assessments were performed at baseline and 12 months. Cognitive performance was measured at baseline and at 6 and 12 months.

**Results:** The high micronutrient treatment significantly improved linear growth at 12 months (0.19 cm; 95% CI: 0.01, 0.36) and short-term memory at 6 months (0.11 SD; 95% CI: 0.01, 0.20) and was less beneficial on fluid reasoning at 6 (-0.10 SD; 95% CI: -0.17, -0.03) and 12 months (-0.12 SD; 95% CI: -0.20, -0.04) than was the low micronutrient treatment, whereas no differences were observed on weight, retrieval ability, cognitive speediness, and overall cognitive performance. No significant differences were found between the omega-3 treatments.

**Conclusions:** The high micronutrient treatment was more beneficial for linear growth than was the low micronutrient treatment. However, with some small differential effects, higher micronutrient concentrations were as effective as lower concentrations on cognitive performance.

## Introduction

Micronutrient deficiencies are a critical concern among children in developing countries affecting their growth and cognitive development (1). They are commonly associated with the consumption of monotonous diets lacking important micronutrients. An effective strategy to combat these deficiencies is the fortification of commonly consumed foods (2). Whereas some studies of multiple micronutrient supplement trials in marginally nourished schoolchildren have reported increases in ponderal (3-6) and in linear (4) growth, others have shown no effects (7;8). Most studies that have assessed the effect of multiple micronutrient fortified foods on cognitive performance in school-age children in developing countries have shown beneficial effects on at least one of the cognitive performance indicators measured (7;9-11) or in subgroups (8;12), with the exception of one study (13). Only one study has thus far been conducted to investigate the different effects of various amounts of micronutrients on growth and intellectual performance in children. The authors investigated the effect of different doses of a multiple micronutrient tablet (50%, 100%, 200% of the Recommended Dietary Allowance (RDA)) on intelligence in US children aged 12-16 years, but results have been inconclusive (14;15). While planning food fortification programs, safety, sensory aspects and cost-effectiveness have to be considered and it is therefore important to know the effect of different concentrations of micronutrients on health outcomes (16).

Essential fatty acids are required for optimal growth and development in children (17). In humans, $\alpha$-linolenic acid (ALA) is converted into the long chain omega-3 fatty acid docosahexaenoic acid (DHA), a major constituent in membranes of the brain and retina (18) and important for the maintenance of optimal brain function (19). Although there is some evidence that supplementation with DHA during infancy may be beneficial for cognitive development (20), such evidence is currently lacking for healthy children older than 2 years of age (21). In populations in developing countries such as in India, vitamin and mineral deficiencies (22) coexisting with a low intake of omega-3 fatty acids (23) could have synergistic adverse effects on the intellectual performance of children. We therefore sought to investigate the efficacy of foods fortified with either low or high concentrations of a micronutrient mix consisting of iodine, iron, riboflavin, vitamin B6, vitamin B12, folate, zinc, calcium, vitamin A and vitamin C in combination with low or high concentrations of omega-3 fatty acids on improving cognitive performance and growth in Indian schoolchildren over one year. We hypothesized that fortification of foods with vitamins and minerals with the addition of omega-3 fatty acids would act synergistically to improve cognitive function and that the higher amounts of micronutrients and omega-3 fatty acids would be more beneficial than lower amounts.

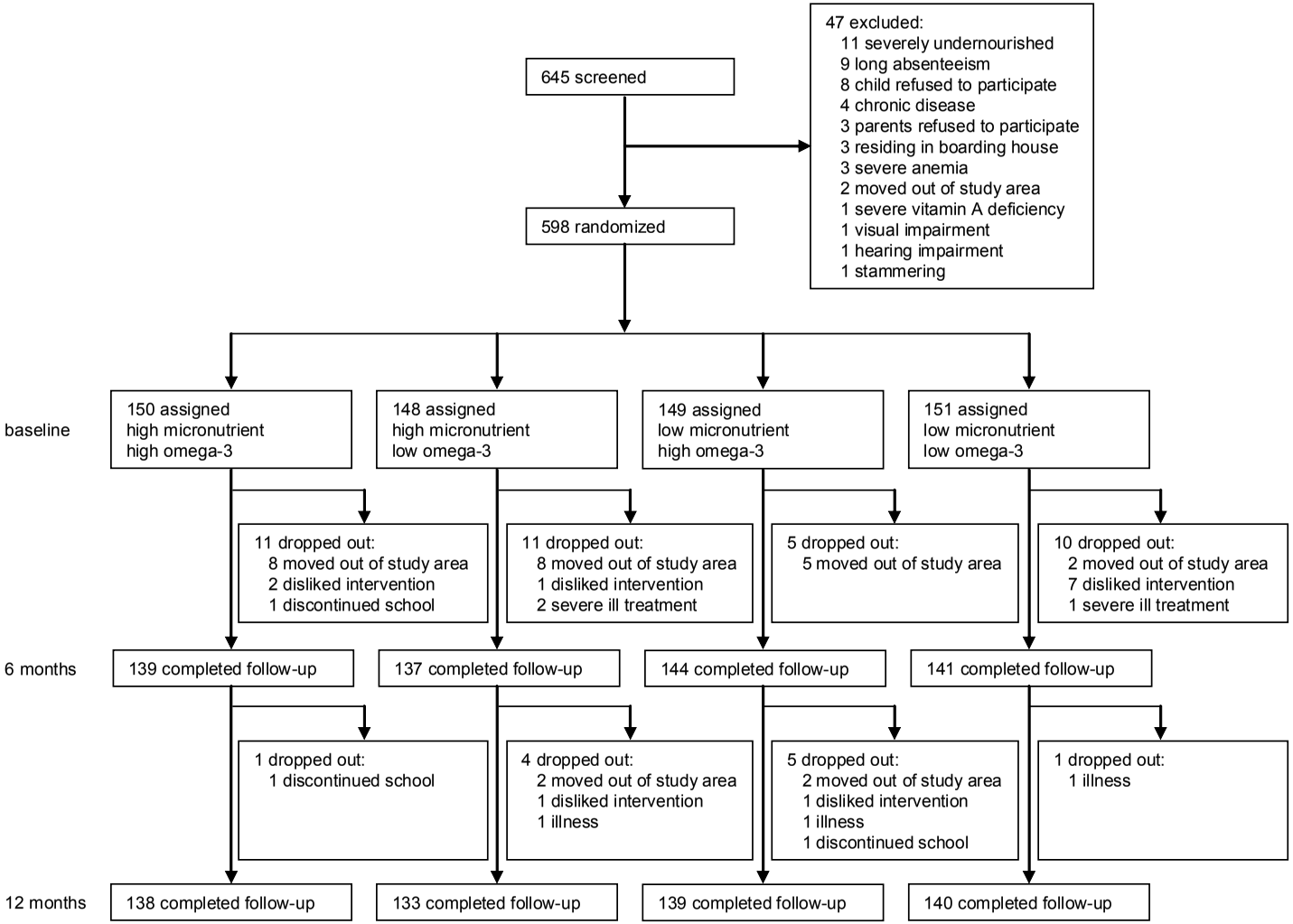


**Figure 1**. Participant flow chart.

## Subjects and methods

### Selection of study sites

The CHAMPION (Children's Health And Mental Performance Influenced by Optimal Nutrition) study was conducted between November 2005 and March 2007 among marginally nourished children attending two primary schools in Bangalore, India, which serve communities from a poor socio-economic background in the vicinity. The schools participated in the local Government's subsidized lunch feeding program, which provided all of the children with a daily meal of cooked rice (250–350 g). Children attending these schools were taught in the local Kannada language. Before the study began, the parents of all children aged 6-10 years, who were attending grades 2-5 in both of the schools, were invited to attend a meeting at which the study procedures were explained. Written informed consent from the parents and verbal assent from their children were obtained (n=645 parent-child pairs). The protocol of the study was approved by the ethical committees at St John's National Academy of Health Sciences, Bangalore, India and Wageningen University, the Netherlands.

### Subjects

The 645 children were eligible for inclusion in the study if they were 1) apparently healthy, with no chronic illness or physical or mental handicaps; 2) not severely anemic (hemoglobin <80 g/L); 3) not severely undernourished (< -3 SD for weight-for-age (WAZ) and height for age (HAZ) *z*-scores of the National Health Centre for Statistics/World Health Organization standards (NCHS/WHO) (24)); 4) not intending to use micronutrient supplements during the study; and 5) planning to reside in the study area during the next 12 months. Children who were frequently absent from school (>40 days during the 6 months before the study began) were also excluded. Severely anemic children received supervised treatment with oral iron tablets (60 mg Fe as ferrous sulfate) 4 d/wk for 12 weeks. Children with other illnesses were referred to a physician. In total, 645 children were screened, 47 children of whom were not eligible because they did not fulfill the inclusion criteria (**Figure 1**) and the remaining 598 children of whom were randomly assigned into the study intervention groups.

### Design

The study used a randomized, double-blind, controlled, 2x2 factorial design, with four parallel treatment groups providing 100% of the RDA (high) or 15% of the RDA (low) of micronutrients and/or high (900 mg ALA plus 100 mg DHA) or low (140 mg ALA) concentrations of omega-3 fatty acids as follows: 1) high micronutrient, high omega-3 fatty acid treatment; 2) low micronutrients, high omega-3 fatty acid treatment; 3) high micronutrients, low omega-3 fatty acid treatment; and 4) low micronutrients, low omega-3 fatty acid treatment. All treatment groups consumed

two fortified products daily: a fruit flavored wheat biscuit with a creamy filling inside and a flavored milk powder drink providing 420 kcal and 13.5 g protein/d. The biscuits were manufactured by Unilever Israel (Arad, Israel) and the drinks by Huijbregts Group, Helmond, the Netherlands (blending) and Budelpack Poortvliet BV, Poortvliet, the Netherlands (packaging).

The 598 children were enrolled into the study in a phased manner in groups of 60-105 children every week over a 3-month period. The enrolled children also completed the study in the same phased manner over 3 months so that all children received the intervention for 12 months. Randomization was performed by means of a computer-generated list in blocks of eight, and the enrolled children were assigned intervention codes in sequence. Before randomization, baseline data for demography and socio-economic status, health status, cognitive and academic performance, dietary intake, blood hematology, blood and urine biochemistry, immune status and intestinal permeability were collected.

**Intervention**

The high micronutrient treatment provided 100% of the RDA of iodine, iron, riboflavin, vitamin $B_6$, vitamin $B_{12}$, folate and vitamin A for children 7-9 years of age (25) (**Table 1**). In order to optimize the bioavailability of iron, zinc was provided at 92% of the RDA based on a molar ratio of 2 (iron) to 1 (zinc), vitamin C at 650% of the RDA based on a molar ratio of 4 (vitamin C) to 1 (iron), and calcium was provided at 33% to minimize possible inhibition of iron absorption (26-28). The low micronutrient treatment delivered micronutrients per kcal proportional to that consumed by the children in their habitual diet (S Muthayya, 2006, unpublished observations). This amounted to 15% of the RDA provided in 420 kcal. The high omega-3 treatment provided 900 mg ALA, which is 50% of the recommended intake of ALA for children aged 2-12 years (29) and 100 mg DHA which corresponds with ~50% of the recommended fish intake for children (30). The low omega-3 treatment provided 140 mg ALA, which is 15% of the amount provided by the high omega-3 treatment, and no DHA. The nutritional composition of each batch of intervention products was assessed before and after consumption of the products by Institut Kuhlmann (Analytik Zentrum Ludwigshafen, Germany) to ensure that designated amounts of the active ingredients were provided.

The drink was prepared daily for each child individually, by mixing 65 g of the powder in 160 mL of boiled water. The fortified products in the four treatment groups were each assigned a specific color code, which was displayed on the package but were otherwise indistinguishable in color, appearance and taste. The investigators, assessors of cognitive tests and participants were blinded until the study was completed, all data were entered, and initial analyses were performed.

At the schools, the treatment assignment of the participating children was identified by using a color-coded personal badge. The children, in groups separated

by color code, consumed the products 6 d/wk at the end of the school day while in a large hall under the direct supervision of the study team. After consumption, visible leftovers of the biscuit and drink were individually estimated and the data recorded. Children were encouraged to consume their habitual diet during the intervention period. Parents were advised not to give their children any fortified products or dietary supplements during the intervention period. During school holidays that lasted >1 week, children consumed the fortified products at home, after clear instructions were given to their parents on how to administer the products to the children. Compliance during holidays was calculated on the basis of the number of sachets issued and returned, and self-report calendars.

**Table 1**. Nutritional composition of the intervention products (total/d)[1]

| | High micronutrient High omega-3 | High micronutrient Low omega-3 | Low micronutrient High omega-3 | Low micronutrient Low omega-3 |
|---|---|---|---|---|
| Vitamin A (µg RE) | 500 | 500 | 75 | 75 |
| Riboflavin (mg) | 0.9 | 0.9 | 0.14 | 0.14 |
| Vitamin $B_6$ (mg) | 1 | 1 | 0.15 | 0.15 |
| Vitamin $B_{12}$ (µg) | 1.8 | 1.8 | 0.27 | 0.27 |
| Folate (µg) | 300 | 300 | 45 | 45 |
| Vitamin C (mg)[2] | 227.1 | 227.1 | 5.25 | 5.25 |
| Calcium[3] | 231 | 231 | 105 | 105 |
| Iodine (µg) | 100 | 100 | 15 | 15 |
| Iron (mg) | 18 | 18 | 2.7 | 2.7 |
| Zinc (mg)[4] | 10.5 | 10.5 | 1.7 | 1.7 |
| Total omega-3 (g) | 1.03 | 0.14 | 1.03 | 0.14 |
| ALA (g)[5] | 0.93 | 0.14 | 0.93 | 0.14 |
| DHA (g)[6] | 0.10 | 0 | 0.10 | 0 |

[1] All intervention products contained 420 kcal, 13.5 g protein, 9.3 g fat (3.7 g omega-6 fatty acids), and 3.6 mg of vitamin E. The high micronutrient treatment was targeted to deliver 100% of the RDA of micronutrients for children aged 7-9 years (25). The low micronutrient treatment provided 15% of the RDA of micronutrients for children aged 7-9 years (25), which was comparable with the micronutrient density of the habitual diet. The nutritional composition of each batch of products was analyzed before and after consumption of the products and, on average, met the target values. RE, retinol equivalents; ALA, $\alpha$-linolenic acid; DHA, docosahexaenoic acid.
[2] Vitamin C in the high micronutrient treatment was provided at 650% of the RDA based on a molar ratio of 4 (vitamin C) to 1 (iron) in order to optimize the bioavailability of iron (27).
[3] Calcium in the high micronutrient treatment was provided at 33% of the RDA to prevent inhibition of iron absorption (28).
[4] Zinc in the high micronutrient treatment was provided at 92% of the RDA based on a molar ratio of 2 (iron) to 1 (zinc) known to minimize the interaction between these nutrients (26).
[5] The high omega-3 fatty acid treatment delivered 50% of the recommendation for ALA for children aged 2-12 years (29). The low omega-3 fatty acid treatment contained 15% of the amount provided by the high omega-3 treatment.
[6] The high omega-3 fatty acid treatment delivered 100 mg DHA which corresponds with 50% of the recommended fish intake for children (30), and the low omega-3 fatty acid treatment did not contain DHA.

## Socio-demographic information

At baseline, information on household composition, parental education, occupation, income, housing, household possessions, languages spoken at home, dietary regimes (e.g. vegetarianism), and use of fortified foods was collected by a structured questionnaire administered to the mothers or the primary caretakers of the study participants.

**Anthropometry**

Anthropometric measurements were recorded in duplicate at baseline and at 6 and 12 months according to standard techniques (31) by three trained research assistants, each of whom was specifically trained to measure weight, height, and mid-upper arm circumference (MUAC). To exclude individual variation, anthropometric measurements were taken by the same research assistant throughout the study. Body weight was recorded to the nearest 0.1 kg using a digital weighing scale (Breuer, Germany) while the subjects were wearing their school uniforms, whereas height was recorded to the nearest 0.1 cm with a locally manufactured stadiometer (Biorad, Mumbai, India). MUAC was recorded to the nearest 0.1 cm with a plastic tape. The anthropometric data, age and gender of the child were used to calculate WAZ and HAZ by using the NCHS/WHO growth reference data (24). Children with WAZ and HAZ < -2 SD of this reference median were classified as underweight and stunted, respectively.

**Cognitive performance**

Cognitive performance was evaluated at baseline and at 6 and 12 months by using age-appropriate, validated psychometric tests that were administered by masters-level psychologists in the local Kannada language. The cognitive test battery consisted of 11 subtests, including six core subtests of the Kaufman Assessment Battery for Children, second edition (KABC-II) for children 3-18 years (pattern reasoning, triangles, rover, number recall, word order, and atlantis) (32); two from the Wechsler Intelligence Scales for Children (WISC-R and WISC-4, picture arrangement, and coding); and three additional tests from Rey Auditory Verbal Learning Test (RAVLT, auditory-verbal learning test), Neuropsychological Assessment Tool (NEPSY, verbal fluency), and number cancellation, which was specifically designed for the study. The 11 subtests covered four cognitive domains as specified in Carroll's model as described in the KABC-II manual (32): 1) fluid reasoning (pattern reasoning, picture arrangement, triangles, and rover), 2) short-term memory (number recall and word order), 3) retrieval ability (auditory-verbal learning test, verbal fluency, and atlantis), and 4) cognitive speediness (number cancellation and coding). See supplementary material at the end of the chapter for details of individual tests.

These domains were chosen because they were previously been shown to be influenced by nutritional interventions (33). Fluid reasoning involves basic processes of reasoning and other mental activities that involve solving novel problems by using reasoning abilities such as induction and deduction but depend only minimally on learning and acculturation. Short-term memory is an ability that requires apprehending and holding information in immediate awareness briefly and then using that information within a few seconds. Retrieval ability comprises

the capacity to store information in memory over the long-term and to retrieve that information fluently and efficiently. Cognitive speediness measures the ability of rapid cognitive processing of information involving attention.

The test battery underwent an extensive adaptation process to ensure its suitability in the local cultural context (34). The qualitative procedure for this adaptation consisted of changing some instructions to ensure understanding, such as replacing some visual items when it required a high familiarity and translation-adaptation of linguistic materials to ensuring their high familiarity. The statistical validation showed that the adapted test battery had good internal consistency and proper external validation with regard to gender and age effects. The normal distributions of the test scores at baseline and at 6 and 12 months indicated that there were no floor and ceiling effects. The conceptual validity of clustering of individual sum scores to form a composite score in four separate cognitive domains against an *a priori* specified model was assessed by a confirmatory factor analysis (structural equation modeling technique (35)) gave a Comparative Fit Index of 0.97, which indicated good conceptual validity.

A team of seven psychologists were trained extensively for three weeks before the study began to ensure standardization in the test administration and scoring procedures; re-training was provided as needed throughout the study period. The training was repeated in the week before the cognitive assessments at 6 and 12 months. The cognitive test battery was administered to each child on a single day over 3 sessions. It consisted of two sessions (30 minutes each) in the forenoon, with a break of 15 minutes between sessions. Care was taken to ensure all children had breakfast before testing began in the morning, because missing breakfast is known to impair cognitive performance (36). The third session took place after the lunch break and lasted ~45 minutes.

For each subtest, a sum score was calculated and converted into a standardized *z*-score. The domain score was composed by taking the average of the standardized *z*-scores for the tests constituting a domain. The average of the domain scores, termed the Mental Processing Index (MPI), was a composite measure of overall cognitive performance based on the KABC-II manual (32). The test scores measured at 6 and 12 months were converted to *z*-scores by using the baseline standardized scores as a reference.

**Biochemical indicators**

At baseline and at 12 months, whole blood (10 mL) was collected from nonfasting children by venipuncture in an EDTA vacutainer. Care was taken to limit the exposure of the samples to light. Hemoglobin concentrations were measured within 4 hours of collection using an AcT Diff$^2$ Counter (Beckman Coulter Inc, Fullerton, CA, USA). One aliquot of whole blood for erythrocyte folate estimation was immediately treated with freshly prepared 1% ascorbic acid. The remaining

blood was immediately centrifuged (3000 rpm, 10 min, 4 ºC), and the plasma was stored in 2 mL eppendorf tubes at –80 ºC until analyzed. One milliliter of erythrocytes was washed with 5 mL saline containing EDTA (1 L normal saline + 0.00324 g disodium EDTA), flushed under nitrogen and stored at –80 ºC until analyzed for fatty acid content. Serum ferritin was measured with an enzyme immunoassay (Access® 2 Beckman Coulter autoanalyser, Beckman, Brea, CA, USA (37) against an external 3-level control material (WHO Standard 80/578; Ramco Laboratories Inc., Houston, TX, USA). Transferrin (TfR) was measured by using an enzyme immunoassay (Ramco Laboratories Inc.) with 2-level control materials provided by the manufacturer. C-reactive protein (CRP) was analyzed by a turbidimetric method (Roche Hitachi 902, Indianapolis, IN, USA (37). Serum vitamin $B_{12}$, and red blood cell folate were analyzed using a Chemiluminescence System (ACS:180, Bayer Diagnostics, Tarrytown, NY, USA (38;39). The fatty acid content of erythrocyte membrane phospholipids was analyzed using gas chromatography with a flame ionization detector (Varian model 3800; Palo Alto, CA, USA). The procedure involved the extraction of total lipids, isolation of phospholipid fraction by thin-layer chromatography, and transmethylation of phospholipids (40-42). The fatty acid methyl esters were separated by chain length and degree of saturation by injection onto a 50 m × 0.2 mm capillary column (Varian) with nitrogen as carrier gas. Anemia was defined as a hemoglobin concentration <11.5 g/dL (43). Iron deficiency was defined as a serum ferritin concentration <15 µg/L (25), or as a TfR concentration >7.6 mg/L (44). Serum ferritin values from subjects with elevated CRP (>5 mg/L) were excluded from the analysis. Folate and vitamin $B_{12}$ deficiency were defined as an erythrocyte folate concentration <305 nmol/L, and serum vitamin $B_{12}$ concentration <148 pmol/L, respectively.

**Data handling and quality checks**

All study forms were checked for inconsistencies and the data double-entered into a structured query language database by two dedicated data entry operators. Cognitive test forms and test scores were double checked by the psychologists before data entry. Discrepancies or mismatches in the data entry were corrected by a senior investigator. In ~20% of the subjects, random data checks between data on paper forms and data entered into the database were conducted.

**Statistical analyses**

Differences in baseline characteristics between the four intervention groups were analyzed by analysis of variance (ANOVA), Kruskal-Wallis H test and Chi-square test. Effect of the high compared with the low micronutrient treatments and the high compared with the low omega-3 treatments, and their interaction were examined by using a 2 factor analysis of covariance (ANCOVA) with baseline cognitive scores, age and gender as covariates. To estimate the expected change

in cognitive performance over 12 months, we calculated the regression coefficient of the baseline cognitive scores with age. The difference between this expected change in cognitive performance scores with age and the observed improvement in cognitive scores at the end of the study was considered to be the effect of the total intervention that included the effect of intervention products together with expected practice effects, placebo effects, and the attention and extra medical care that these participating children received during the course of the study. A similar analysis was conducted for growth outcomes (ANCOVA and regression analysis) and biochemistry (ANCOVA). An $\alpha$ level <0.05 was considered significant. All analyses were performed by using SPSS version 13.0 (SPSS Inc, Chicago, IL) and SAS version 9.1 statistical software package (SAS Institute Inc, Cary, NC).

## Results

Of the 598 children randomly allocated into the study, 550 children completed the study, of whom 548 completed the cognitive assessments. The dropout rate (7-10%) was similar among the four study groups, and the main reason for dropout was relocation out of the study area (see **Figure 1** for other reasons for drop out). Baseline data available for the subjects who were lost to follow-up did not differ from data for those included in the present analysis (data not shown).

The baseline profile of the study participants by treatment group is shown in **Table 2**. There were no significant differences in any of the baseline characteristics between treatment groups. The mean age of the children was 8.7 ± 1.2 years. Mean total family income was 3033 ± 1687 Indian rupees/month (equivalent to

**Table 2**. Baseline characteristics of the study population[1]

| | High micronutrient High omega-3 | High micronutrient Low omega-3 | Low micronutrient High omega-3 | Low micronutrient Low omega-3 |
|---|---|---|---|---|
| N (% girls) | 150 (53) | 148 (49) | 149 (48) | 151 (54) |
| Age (y) | 8.7 ± 1.1[2] | 8.7 ± 1.2 | 8.7 ± 1.2 | 8.7 ± 1.1 |
| Education level of the mother (% no education) | 50 | 45 | 48 | 40 |
| Family income (rupees/month) | 3230 ± 1956 | 3182 ± 1871 | 3000 ± 1528 | 2723 ± 1281 |
| Stunting (% < -2SD HAZ) | 18 | 22 | 22 | 29 |
| Underweight (% < -2SD WAZ) | 27 | 30 | 28 | 39 |
| Anemia (hemoglobin <11.5 g/dL) | 15/150 (10.0)[3] | 15/148 (10.1) | 9/149 (6.0) | 15/151 (9.9) |
| Iron deficiency (serum ferritin <15 mg/L and/or serum transferrin receptor >7.6 mg/L) | 38/139 (27.3) | 50/132 (37.9) | 42/132 (31.8) | 36/134 (26.9) |
| Folate deficiency (erythrocyte folate <305 nmol/L) | 17/137 (12.4) | 25/134 (18.7) | 22/136 (16.2) | 26/132 (19.7) |
| Vitamin $B_{12}$ deficiency (serum cobalamin <148 pmol/L) | 37/138 (26.8) | 31/133 (23.3) | 31/133 (23.3) | 26/129 (20.2) |

[1] There were no significant differences between the 4 groups.
[2] Mean±SD (all such values).
[3] *n* deficient/*n* total (% deficient) (all such values).

**Table 3**. Effects of treatment on biochemical indicators of micronutrient and omega-3 fatty acid status over 12 months[1]

| | Intervention group | | | | Treatment effect after 12 months[2] | |
|---|---|---|---|---|---|---|
| | High micronutrient High omega-3 | High micronutrient Low omega-3 | Low micronutrient High omega-3 | Low micronutrient Low omega-3 | High vs low micronutrient | High vs low omega-3 |
| Blood hemoglobin (g/dL) | | | | | | |
| n | 138 | 134 | 139 | 140 | | |
| Baseline | 12.7 ± 1.0[3] | 12.7 ± 1.0 | 12.9 ± 0.9 | 12.7 ± 1.0 | 0.40 | -0.02 |
| 12 months | 13.6 ± 0.8 | 13.7 ± 0.8 | 13.4 ± 0.9 | 13.2 ± 1.0 | (0.28, 0.52) | (-0.14, 0.10) |
| Serum ferritin (µg/L) | | | | | | |
| n | 127 | 120 | 118 | 123 | | |
| Baseline | 23.9 (15.6, 35.5)[4] | 21.9 (12.9, 31.4) | 25.9 (16.7, 38.7) | 24.1 (14.9, 1.7) | 0.79 (0.71, 0.86) | 0.07 (-0.01, 0.14) |
| 12 months | 48.2 (36.2, 61.2) | 47.5 (35.2, 60.8) | 25.3 (18.5, 35.9) | 21.7 (14.4, 0.6) | | |
| Serum transferrin receptor (mg/L) | | | | | | |
| n | 128 | 116 | 121 | 124 | | |
| Baseline | 6.1 ± 2.1 | 6.2 ± 2.3 | 6.1 ± 1.7 | 6.1 ± 2.1 | -0.74 | 0.02 |
| 12 months | 5.3 ± 1.3 | 5.5 ± 1.4 | 6.2 ± 1.6 | 6.1 ± 2.3 | (-1.00,-0.51) | (-0.22, 0.24) |
| Body iron stores (mg/kg)[5] | | | | | | |
| n | 122 | 108 | 112 | 116 | | |
| Baseline | 3.3 ± 3.2 | 2.7 ± 3.4 | 3.6 ± 2.7 | 3.1 ± 3.2 | 3.25 | 0.15 |
| 12 months | 6.5 ± 1.8 | 6.2 ± 1.7 | 3.6 ± 2.6 | 3.0 ± 3.1 | (2.93, 3.57) | (-0.17, 0.47) |
| Red blood cell folate (nmol/L) | | | | | | |
| n | 127 | 120 | 125 | 121 | | |
| Baseline | 562 ± 261 | 618 ± 395 | 616 ± 351 | 558 ± 335 | 348 | 19 |
| 12 months | 1075 ± 268 | 1058 ± 279 | 732 ± 233 | 697 ± 239 | (310, 386) | (-20, 57) |
| Serum vitamin $B_{12}$ (pmol/L) | | | | | | |
| n | 127 | 119 | 120 | 120 | | |
| Baseline | 229 ± 121 | 217 ± 101 | 234 ± 116 | 213 ± 102 | 116 | 6 |
| 12 months | 389 ± 171 | 378 ± 165 | 283 ± 161 | 253 ± 136 | (94, 138) | (-16, 27) |
| Total omega-3 (% of total fatty acids) | | | | | | |
| n | 125 | 119 | 123 | 124 | | |
| Baseline | 3.6 ± 0.8 | 3.6 ± 0.9 | 3.6 ± 1.0 | 3.9 ± 2.6 | -0.04 | 1.68 |
| 12 months | 5.7 ± 1.3 | 4.1 ± 0.8 | 5.8 ±1.2 | 4.1 ± 1.1 | (-0.23, 0.15) | (1.48, 1.87) |
| ALA (% of total fatty acids) | | | | | | |
| n | 125 | 119 | 123 | 124 | | |
| Baseline | 0.25 ± 0.18 | 0.25 ± 0.16 | 0.26 ± 0.17 | 0.26 ± 0.17 | 0.01 | 0.01 |
| 12 months | 0.23 ± 0.10 | 0.22 ± 0.08 | 0.23 ± 0.09 | 0.21 ± 0.07 | (-0.01, 0.02) | (-0.00, 0.03) |
| EPA (% of total fatty acids) | | | | | | |
| n | 125 | 119 | 123 | 124 | | |
| Baseline | 0.17 ± 0.07 | 0.17 ± 0.08 | 0.18 ± 0.10 | 0.18 ± 0.07 | -0.02 | 0.11 |
| 12 months | 0.35 ± 0.16 | 0.25 ± 0.14 | 0.39 ± 0.19 | 0.26 ± 0.18 | (-0.05, 0.01) | (0.08, 0.14) |
| DHA (% of total fatty acids) | | | | | | |
| n | 125 | 119 | 123 | 124 | | |
| Baseline | 3.2 ± 0.7 | 3.2 ± 0.8 | 3.2 ± 0.9 | 3.3 ± 0.9 | -0.03 | 1.55 |
| 12 months | 5.2 ± 1.2 | 3.6 ± 0.8 | 5.2 ± 1.2 | 3.6 ± 1.0 | (-0.21, 0.15) | (1.36, 1.73) |

[1] Fatty acids were measured in erythrocytes membranes in the phospholipid fraction. ALA, $\alpha$-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. There was no significant interaction between the micronutrients and the omega-3 fatty acids.
[2] Treatment effects estimated by ANCOVA adjusted for baseline values, gender, and age; 95% CIs in parentheses.
[3] Mean ± SD (all such values).
[4] Median; 25$^{th}$, 75$^{th}$ percentile in parentheses; data were log-transformed to perform the ANCOVA, actual values and are presented.
[5] Total body iron stores were calculated based on Cook et al (45).

US$78). Approximately half of the mothers were uneducated. Thirty percent of the children were underweight and 21.8% were stunted. The overall prevalence of anemia, iron, folate and vitamin $B_{12}$ deficiencies were 9.0%, 30.9%, 16.7% and 23.5%, respectively. Compliance with the consumption of intervention products averaged 75% for the biscuits and 70% for the drinks, with no differences between the four treatment groups.

## Biochemical indicators

The concentrations of micronutrients and omega-3 fatty acids at baseline and after 12 months of intervention are presented in **Table 3**. The increases in hemoglobin, ferritin, body iron stores, vitamin $B_{12}$ and folic acid concentrations in the high micronutrient groups were significantly larger than the changes in the low micronutrient treatment groups (all $P<0.001$). Increases in erythrocyte membrane concentrations of ALA, eicosapentaenoic acid (EPA), DHA and total omega-3 fatty acids in the high omega-3 treatment groups were significantly higher than the changes in the low omega-3 treatment groups (all $P<0.001$). Consequently, after 12 months of intervention, the prevalence of anemia, iron, folate and vitamin $B_{12}$ deficiencies dropped significantly more in the high micronutrient groups (data not shown).

## Growth

All the four treatment groups showed similar, significant improvements in weight, height and MUAC after 12 months of intervention, when compared to their baseline values (**Table 4**). After 12 months of intervention, the high micronutrient treatment was more beneficial for linear growth than the low micronutrient treatment (2 mm, $P=0.05$). There were no differences between the two micronutrient treatments for weight and MUAC. Similarly, no differences were found between the high and the low omega-3 fatty acid treatments and there was no interaction of the micronutrient

**Table 4**. Treatment effects on indicators of growth over 12 months[1]

| | Intervention group | | | | Treatment effect after 12 months[2] | |
|---|---|---|---|---|---|---|
| | High micronutrient High omega-3 ($n$=138) | High micronutrient Low omega-3 ($n$=133) | Low micronutrient High omega-3 ($n$=139) | Low micronutrient Low omega-3 ($n$=140) | High vs low micronutrient | High vs low omega-3 |
| Height (cm) | | | | | | |
| Baseline | 123.1 ± 7.7[3] | 122.7 ± 8.1 | 123.0 ± 7.8 | 123.0 ± 8.3 | 0.19 | -0.05 |
| 12 months | 129.3 ± 7.8 | 129.0 ± 8.2 | 129.0 ± 7.8 | 129.0 ± 8.5 | (0.01,0.36) | (-0.22,0.13) |
| Weight (kg) | | | | | | |
| Baseline | 21.5 ± 3.6 | 21.3 ± 3.7 | 21.6 ± 4.0 | 21.4 ± 4.1 | 0.16 | -0.002 |
| 12 months | 25.2 ± 5.1 | 25.2 ± 5.3 | 25.4 ± 5.6 | 24.9 ± 5.4 | (-0.09,0.41) | (-0.25,0.24) |
| MUAC (cm) | | | | | | |
| Baseline | 17.1 ± 1.4 | 17.0 ± 1.3 | 17.1 ± 1.6 | 17.0 ± 1.7 | 0.05 | -0.03 |
| 12 months | 17.7 ± 2.0 | 17.8 ± 1.9 | 17.8 ± 2.1 | 17.6 ± 2.0 | (-0.08,0.19) | (-0.17,0.11) |

[1] There was no significant interaction between the micronutrients and the omega-3 fatty acids.
[2] Treatment effects estimated by ANCOVA adjusted for baseline values, gender, and age; 95% CIs in parentheses.
[3] Mean ± SD (all such values).

and omega-3 fatty acid treatments on any of the anthropometric parameters. For the total group, the prevalence of stunting and underweight decreased significantly from baseline, from 21.8 to 16.0% ($P<0.001$) and from 30.0 to 18.6% ($P<0.001$), respectively, but this decrease did not differ significantly between the four groups.

**Cognitive performance**

All groups showed a significant improvement in short-term memory, retrieval ability, fluid reasoning and cognitive speediness and the MPI compared with baseline (see **Table 5**). The gain in the MPI score was significantly higher than the expected improvement after 12 months (**Figure 2**), which was calculated by the regression coefficient of the baseline cognitive scores with age. Similarly, the scores on the four cognitive domains increased more than expected based on the baseline data (data not shown). The high micronutrient treatment was more beneficial than was the low micronutrient treatment, for short-term memory at 6 months (effect size 0.11, $P=0.025$), but this effect was no longer evident at 12 months. The low micronutrient treatment was more beneficial than the high micronutrient treatment for fluid reasoning at both 6 months (effect size 0.10, $P=0.004$) and 12 months (effect size 0.12, $P=0.004$). There were no significant differences between the high and the low omega-3 fatty acid treatment and no interactions between the micronutrient and omega-3 fatty acid treatments on any of the cognitive measures.

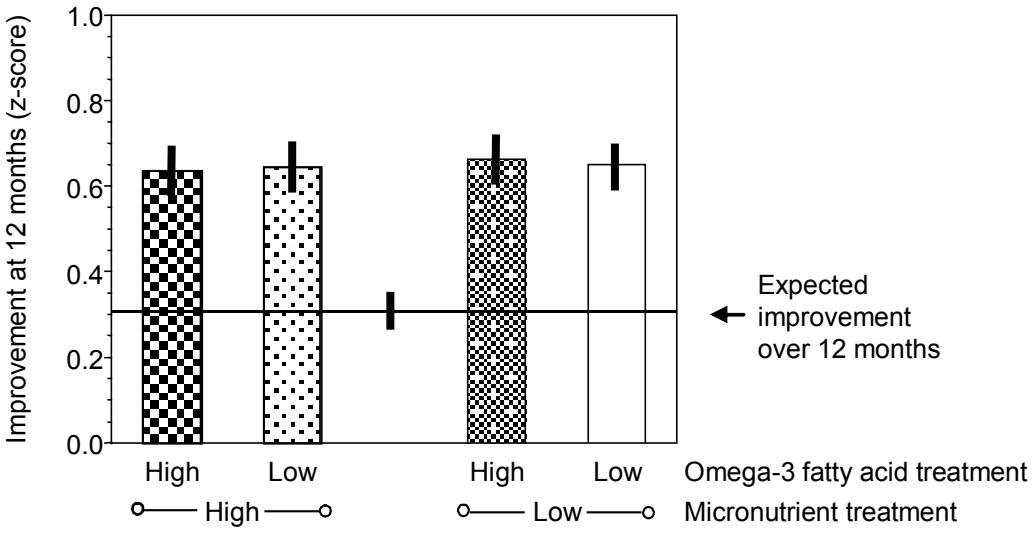


**Figure 2**. Effect of the intervention on Mental Processing Index (MPI) after 12 months compared to the effect expected from a regression model of MPI scores and age at baseline.
Bars represent improvement in MPI score after 12 months from baseline corrected for age, gender and baseline

**Table 5**. Treatment effects on cognitive performance over 6 and 12 months[1]

| | Intervention group | | | | Treatment effect after 6 and 12 months[2] | |
|---|---|---|---|---|---|---|
| | High micronutrient High omega-3 (*n*=137) | High micronutrient Low omega-3 (*n*=133) | Low micronutrient High omega-3 (*n*=139) | Low micronutrient Low omega-3 (*n*=138) | High vs low micronutrient | High vs low omega-3 |
| MPI | | | | | | |
| Baseline | -0.01 ± 0.67[3] | 0.00 ± 0.66 | 0.01 ± 0.63 | 0.02 ± 0.64 | | |
| 6 months | 0.29 ± 0.31 | 0.31 ± 0.29 | 0.32 ± 0.26 | 0.30 ± 0.27 | -0.01 (-0.06, 0.03) | 0.00 (-0.04, 0.05) |
| 12 months | 0.62 ± 0.32 | 0.63 ± 0.32 | 0.65 ± 0.30 | 0.64 ± 0.31 | -0.02 (-0.07, 0.03) | 0.00 (-0.05, 0.05) |
| Short-term memory | | | | | | |
| Baseline | 0.08 ± 1.04 | -0.02 ± 0.85 | -0.02 ± 0.82 | -0.03 ± 0.87 | | |
| 6 months | 0.13 ± 0.66 | 0.21 ± 0.55 | 0.12 ± 0.57 | 0.04 ± 0.58 | 0.11 (0.01, 0.20) | 0.01 (-0.08, 0.11) |
| 12 months | 0.42 ± 0.60 | 0.43 ± 0.54 | 0.37 ± 0.66 | 0.38 ± 0.65 | 0.06 (-0.04, 0.16) | 0.00 (-0.10, 0.10) |
| Fluid reasoning | | | | | | |
| Baseline | -0.06 ± 0.71 | 0.02 ± 0.82 | 0.02 ± 0.81 | 0.04 ± 0.81 | | |
| 6 months | 0.39 ± 0.43 | 0.36 ± 0.40 | 0.48 ± 0.45 | 0.48 ± 0.42 | -0.10 (-0.17, -0.03) | 0.01 (-0.06, 0.08) |
| 12 months | 0.72 ± 0.48 | 0.70 ± 0.45 | 0.84 ± 0.51 | 0.82 ± 0.47 | -0.12 (-0.20, -0.04) | 0.02 (-0.06, 0.10) |
| Retrieval ability | | | | | | |
| Baseline | -0.04 ± 0.79 | 0.04 ± 0.80 | 0.01 ± 0.73 | 0.00 ± 0.74 | | |
| 6 months | 0.25 ± 0.55 | 0.28 ± 0.49 | 0.32 ± 0.47 | 0.29 ± 0.53 | -0.03 (-0.11, 0.04) | 0.00 (-0.08, 0.08) |
| 12 months | 0.55 ± 0.59 | 0.59 ± 0.60 | 0.60 ± 0.51 | 0.58 ± 0.56 | -0.02 (-0.11, 0.06) | -0.02 (-0.10, 0.07) |
| Cognitive speediness | | | | | | |
| Baseline | -0.01 ± 0.88 | -0.05 ± 0.89 | 0.02 ± 0.89 | 0.05 ± 0.87 | | |
| 6 months | 0.37 ± 0.54 | 0.38 ± 0.55 | 0.37 ± 0.61 | 0.39 ± 0.57 | -0.01 (-0.10, 0.07) | -0.01 (-0.09, 0.08) |
| 12 months | 0.80 ± 0.61 | 0.81 ± 0.57 | 0.79 ± 0.56 | 0.76 ± 0.59 | 0.02 (-0.06, 0.11) | 0.02 (-0.06, 0.11) |

[1] MPI, Mental Processing Index. There was no significant interaction between the micronutrients and the omega-3 fatty acids.
[2] Treatment effects estimated by ANCOVA adjusted for baseline values, gender, and age; 95% CIs in parentheses.
[3] Mean ± SD (all such values).

MPI score with 95% CI. Horizontal line with 95% CI represents the expected improvement in MPI score calculated by the regression coefficient of the baseline cognitive scores with age.

## Discussion

Our study was designed to investigate the effect of two different concentrations of a combination of micronutrients and omega-3 fatty acids on growth and cognitive performance of marginally nourished school-age children. We showed that the high micronutrient treatment was more beneficial for linear growth at 12 months and for short-term memory at 6 months, but that the low micronutrient treatment was more beneficial for fluid reasoning at 6 and 12 months. The high and low micronutrient treatments had similar effects on changes in body weight, MUAC

and cognitive functions such as, retrieval ability, cognitive speediness, and the MPI. No significant differences between the omega-3 treatments were observed for growth and cognitive performance. The effects observed did not depend on maternal education and degree of compliance with consumption of the intervention products.

The strengths of our study were the low attrition rate, adequate consumption of the intervention products and significant increases in biochemical indicators for micronutrients and omega-3 fatty acids in the high micronutrient and high omega-3 groups, respectively. The study was sufficiently powered to detect very small but significant effects of the intervention on growth (2 mm) and cognitive performance (0.10-0.12 SD).

One of the limitations of the study design was the lack of a real placebo group in the study. It was, however, considered unethical to provide substantial amounts of energy and protein foods devoid of essential micronutrients over a prolonged period. Inclusion of a placebo group would have enabled us to disentangle the effect of micronutrients from that of omega-3 fatty acids on study outcomes. On the basis of the records kept on the diets of the study children, it was clear that the children maintained their regular intake and that the study products did not replace their meals (data not shown). This resulted in two design disadvantages: 1) all children in all four treatment groups received substantial amounts of energy and protein in addition to their habitual diet, which may have contributed to the improvements in growth and cognitive performance (46), and 2) children in the low micronutrient groups received 15% of the RDA of micronutrients, which, for some micronutrients, was 50% more than the amount consumed in their poor habitual diets, which may have resulted in a larger than expected effect on growth and cognitive outcomes in the low micronutrient groups.

We showed a small but significant effect of the high micronutrient treatment on height, but not weight and MUAC, which is likely to be attributed to synergetic effects of the micronutrients, and the presence of zinc (47). Only one placebo-controlled study that provided micronutrients at 35% of the RDA demonstrated significant positive effects on linear growth in schoolchildren (4), whereas others providing micronutrients at 30-100% of the RDA showed trends towards a positive effect (3;5-7). At 12 months, all intervention groups showed significant increases in body weight (3.7 kg) and height (6.1 cm) compared with baseline, which exceeded the increase expected from Indian growth norms (2.4 kg and 5.5 cm, respectively) (48). This may have been partly attributed to the additional 40% energy and 50% protein in the intervention products, because deficiencies of energy and protein have long been known to impair linear growth (49;50). The finding that all children grew more than expected is in line with the findings of the placebo-controlled trials that showed positive effects of micronutrients on growth (3-7). However, we cannot conclude whether the improvements in growth in all four groups were due

to the extra protein and energy or to the micronutrients.

The significantly higher increases in cognitive scores than expected by growing one year older could have been the effect of extra energy, protein and micronutrients; however our study design does not allow us to distinguish the micronutrient effect from the protein and energy effect. Also extra attention and (medical) care by the study staff may have had an effect on the children's cognitive performance as care-giving and adequate environmental stimulation are known to be important for optimal intellectual development (51). In addition, training effects of performing cognitive tests repeatedly at 6 and 12 months may have had an influence on cognitive performance, but the size of these effects are mostly unknown, except for short-term memory where no practice effects are expected (52).

Whereas the high micronutrient treatment was more beneficial for short-term memory than was the low micronutrient treatment, it was less beneficial for fluid reasoning. The effect sizes were extremely small, and we have no satisfactory explanation for the findings on fluid reasoning. We speculate that a type I error or an imbalance in the micronutrient status occurred in the high micronutrient treatment group, as high doses of iron and zinc may influence the absorption of other minerals such as copper (53). However, an analysis of covariance with the biochemical status of micronutrients at the end of the intervention as covariate confirmed that the micronutrient status had no influence on fluid reasoning. We therefore conclude that, overall, there was no difference in cognitive performance between the high and low micronutrient treatment groups. This is in agreement with a previous study in US children that investigated the effect of multiple micronutrient tablets supplying 50%, 100% and 200% of the RDA on intelligence which showed no difference between the three doses (14;15). Regardless of the amount of micronutrients, there is evidence from six studies that food fortification with multiple micronutrients benefits cognitive performance in marginally nourished schoolchildren, which suggests that a lower dose (30-50% of the RDA) (7;11) may be as effective as a higher dose (100% of the RDA) (8-10;12).

We found no differences between the low omega-3 treatment and the high omega-3 treatment on any of the growth and cognitive performance outcomes, despite significant increases in biochemical indicators. Our results agree with an earlier study in 7-10 year-old children in Indonesia and Australia, which failed to show any significant effect of 110 mg EPA + DHA on cognitive performance (12). In contrast, in a study involving only children with neurodevelopmental disorders, positive effects on attention and learning were observed after an intake of 250-500 mg EPA + DHA (54-57). Possibly, the cognitive domains assessed in our study may not be affected by DHA supplementation, or the concentrations used in our study may have been too low. It is currently unknown whether additional dietary DHA may improve cognition in children at all, because humans are able to synthesize DHA from ALA and there is currently no evidence whether low intakes of DHA will

impact physical functioning in healthy children (30). On the basis of all evidence combined, it is currently too premature to either accept or dismiss a benefit of DHA on cognitive performance in schoolchildren. More research is required to clarify such.

In conclusion, compared with a lower concentration of micronutrients (15% of the RDA), our study showed that a higher concentration of micronutrients (100% of the RDA) was more beneficial for linear growth and had differential effects on two of the four cognitive domains, whereas there were no differences on ponderal growth and overall cognitive performance. These findings suggest that lower concentrations of micronutrients might be as effective as higher concentrations for some, but not all, health outcomes in school-age children living under deprived conditions. Further research is recommended to identify the optimal amount of micronutrients for growth and cognitive performance and to identify the specific cognitive domains sensitive to nutrient interventions.

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## References

1. Diaz JR, de las Cagigas A, Rodriguez R. Micronutrient deficiencies in developing and affluent countries. European Journal of Clinical Nutrition 2003;57:S70-S72.
2. Lotfi M, Mannar MGV, Merx RJHM, Naber-Van den Heuvel P. Micronutrient fortification of foods. Current practices, research, and opportunities. The Micronutrient Initiative / International Development Research Centre / International Agricultural Centre, 1996.
3. Abrams SA, Mushi A, Hilmers DC, Griffin IJ, Davila P, Allen L. A multinutrient-fortified beverage enhances the nutritional status of children in Botswana. J Nutr 2003;133:1834-40.
4. Ash DM, Tatala SR, Frongillo EA, Jr., Ndossi GD, Latham MC. Randomized efficacy trial of a micronutrient-fortified beverage in primary school children in Tanzania. Am J Clin Nutr 2003;77:891-8.
5. Kumar MV, Rajagopalan S. Impact of a multiple-micronutrient food supplement on the nutritional status of schoolchildren. Food Nutr Bull 2006;27:203-10.
6. Sarma KV, Udaykumar P, Balakrishna N, Vijayaraghavan K, Sivakumar B. Effect of micronutrient supplementation on health and nutritional status of schoolchildren: growth and morbidity. Nutrition 2006;22:S8-14.
7. Manger MS, McKenzie JE, Winichagoon P et al. A micronutrient-fortified seasoning powder reduces morbidity and improves short-term cognitive function, but has no effect on anthropometric measures in primary school children in northeast Thailand: a randomized controlled trial. Am J Clin Nutr 2008;87:1715-22.
8. Solon FS, Sarol JN, Bernardo AB, I et al. Effect of a multiple-micronutrient-fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of schoolchildren in the Philippines. Food and Nutrition Bulletin 2003;24:S129-S140.
9. Kumar MV, Rajagopalan S. Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children. Asia Pac J Clin Nutr 2007;16:505-11.

10. Vazir S, Nagalla B, Thangiah V, Kamasamudram V, Bhattiprolu S. Effect of micronutrient supplement on health and nutritional status of schoolchildren: mental function. Nutrition 2006;22:S26-S32.
11. van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benade AJS. Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary school children: a randomized controlled trial. (vol 69, pg 497, 1999). Am J Clin Nutr 1999;69:1294.
12. Osendarp SJ, Baghurst KI, Bryan J et al. Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia. Am J Clin Nutr 2007;86:1082-93.
13. Jinabhai CC, Taylor M, Coutsoudis A, Coovadia HM, Tomkins AM, Sullivan KR. A randomized controlled trial of the effect of antihelminthic treatment and micronutrient fortification on health status and school performance of rural primary school children. Ann Trop Paediatr 2001;21:319-33.
14. Schoenthaler SJ, Amos SP, Eysenck HJ, Peritz E, Yudkin J. Controlled trial of vitamin-mineral supplementation: effects on intelligence and performance. Personal Individ Diff 1991;12:351-62.
15. Peritz E. The Turlock Vitamin Mineral Supplementation Trial - A Statistical Reanalysis. Journal of Biosocial Science 1994;26:155-64.
16. Allen L, de Benoist B, Hurrell RF, Dary O. Guidelines on food fortification with micronutrients. Geneva: World Health Organization and Food and Agriculture Organization of the United Nations, 2006.
17. U.S.Institute of Medicine, Food and Nutrition Board, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Energy, Carbohydrate, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. Washington, D.C.: National Academy Press, 2005.
18. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129-S138.
19. Yehuda S. Omega-6/omega-3 ratio and brain-related functions. World Rev Nutr Diet 2003;92:37-56.
20. McCann JC, Ames BN. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. Am J Clin Nutr 2005;82:281-95.
21. Eilander A, Hundscheid DC, Osendarp SJ, Transler C, Zock PL. Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: a review of human studies. Prostaglandins Leukot Essent Fatty Acids 2007;76:189-203.
22. Narasinga Rao BS. Anaemia and micronutrient deficiency. Natl Med J India 2003;16:46-50.
23. Muthayya S, Dwarkanath P, Thomas T et al. The effect of fish and omega-3 LCPUFA intake on low birth weight in Indian pregnant women. Eur J Clin Nutr 2007.
24. World Health Organization Expert Committee on Physical Status. Physical Status: The Use and Interpretation of Anthropometry. WHO Technical Report Series no. 854. Geneva: World Health Organization, 1995.
25. World Health Organization/Food and Agricultural Organization. Vitamin and mineral requirements in human nutrition 2nd ed. Geneva: World Health Organization, 2004.
26. Christian P, Khatry SK, LeClerq SC, Shrestha SR, Kimbrough-Pradhan E, West KP. Iron and zinc interactions among pregnant Nepali women. Nutr Res 2001;12:141-8.
27. Lynch SR, Stoltzfus RJ. Iron and ascorbic acid: proposed fortification levels and recommended iron compounds. J Nutr 2003;133:2978S-84S.
28. Lynch SR. The effect of calcium on iron absorption. Nutr Res Rev 2000;13:141-58.
29. Uauy R, Castillo C. Lipid requirements of infants: implications for nutrient composition of fortified complementary foods. J Nutr 2003;133:2962S-72S.
30. Kris-Etherton PM, Innis S, American DA, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc 2007;107:1599-611.
31. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Publishers, 1988.
32. Kaufman AS, Kaufman LN. Kaufman Assessment Battery for Children, Second Edition: Manual. Circle Pines, MN: AGS Publishing, 2004.
33. Hughes D, Bryan J. The assessment of cognitive performance in children: considerations for detecting nutritional influences. Nutr Rev 2003;61:413-22.
34. Malda M, van de Vijver FJR, Srinivasan K, Transler C, Sukumar P, Rao K. Adapting a cognitive test for a different culture: An illustration of qualitative procedures. Psychol Sci Q 2008;50:451-68.
35. Arbuckle JL. Amos (Version 6.0) [Computer Program]. Chicago: SPSS, 2005.
36. Grantham-McGregor S. Can the provision of breakfast benefit school performance? Food Nutr Bull 2005;26:S144-S158.
37. Burtis CA, Ashwood ER. Fundamentals of clinical chemistry. Philadelphia, PA: WB Saunders Company, 1996.
38. Miale JB. Laboratory Medicine: Hematology. St. Louis: CV Mosby, 1982.
39. Chen W, Sperling MI, Heminger LA. Vitamin B12. In: Pesce AJ, Kaplan LA, eds. Methods in Clinical Chemistry. St. Louis: CV Mosby 1987:569-73.
40. Rose HG, Oaklander M. Improved procedure for the extraction of lipids from human erythrocytes. Journal of Lipids Research 1965;6:428-31.
41. Gentner PR, Bauer M, Dieterich I. Separation of major phospholipids classes of milk without previous isolation from total lipid extracts. Journal of Chromatography 1981;13:200-4.
42. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipids Research 1964;5:600-8.
43. World Health Organization, Centers for Disease Conrol and Prevention. Assessing the iron status of populations. Report ot a joint technical consultation on the assessment of iron status at the population level. Geneva,

Switzerland, 6-8 April 2004. Geneva: World Health Organization, 2004.
44. Zimmermann MB, Molinari L, Staubli-Asobayire F. Serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children. Am J Clin Nutr 2005;81:615-23.
45. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359-64.
46. Grantham-McGregor S, Baker-Henningham H. Review of the evidence linking protein and energy to mental development. Public Health Nutr 2005;8:1191-201.
47. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2002;75:1062-71.
48. Khadilkar VV, Khadilkar AV, Choudhury P, Agarwal KN, Ugra D, Shah NK. IAP Growth Monitoring Guidelines for Children from Birth to 18 Years. Indian Pediatrics 2007;44:187-97.
49. Mora JO, Herrera MG, Suescun J, de Navarro L, Wagner M. The effects of nutritional supplementation on physical growth of children at risk of malnutrition. Am J Clin Nutr 1981;34:1885-92.
50. Gopalan C, Swaminathan MC, Kumari VK, Rao DH, Vijayaraghavan K. Effect of calorie supplementation on growth of undernourished children. Am J Clin Nutr 1973;26:563-5.
51. Walker SP, Wachs TD, Gardner JM et al. Child development in developing countries 2 - Child development: risk factors for adverse outcomes in developing countries. Lancet 2007;369:145-57.
52. Tuma JM, Appelbaum AS. Reliability and practice effects of WISC-R IQ estimates in a normal population. Educational and Psychological Measurement 1980;40:671-8.
53. Sandstrom B. Micronutrient interactions: effects on absorption and bioavailability. Br J Nutr 2001;85 Suppl 2:S181-S185.
54. Richardson AJ, Puri BK. A randomized double-blind, placebo-controlled study of the effects of supplementation with highly unsaturated fatty acids on ADHD-related symptoms in children with specific learning difficulties. Prog Neuro-Psychopharmacol Biol Psychiatry 2002;26:233-9.
55. Richardson AJ, Montgomery P. The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. Pediatrics 2005;115:1360-6.
56. Stevens L, Zhang W, Peck L et al. EFA supplementation in children with inattention, hyperactivity, and other disruptive behaviors. Lipids 2003;38:1007-21.
57. Vaisman N, Kaysar N, Zaruk-Adasha Y et al. Correlation between changes in blood fatty acid composition and visual sustained attention performance in children with inattention: effect of dietary n-3 fatty acids containing phospholipids. Am J Clin Nutr 2008;87:1170-80.

# Supplementary material

Description of cognitive test battery

| Cognitive domain | Indivivual test | Description of test |
|---|---|---|
| Fluid reasoning | Picture arrangement | This test presents four pictures to be arranged in an order predicted by social conventions. The pictures are presented in a random order; the child has to put them back in the correct order. This test evaluates some aspects of the inductive reasoning. It requires the child to analyze the cues provided in complex pictures and induce the issues, the development and the solution to a problem or the outcome of a scenario. |
| | Pattern reasoning | The principle of this test is to present a series of drawings (objects, animals, plants…) organized according to a certain pattern (AAAA; ABAB;…). One drawing is missing in the series, symbolized by a "?". The series is followed by 4 drawings among which the child must choose one to make the first series complete. This subtest measures inductive reasoning. It requires that the child induces the underlying rules organizing the series. This process requires also planning abilities because the child has to 'try' (visualize mentally) one answer, check what would be the series if the answer was a certain one; then conclude whether his answer is correct or not; and if not try another solution, etc. |
| | Triangles | The child is given simple and abstract geometric shapes of primary colors (red, blue, yellow). The child is required to produce new shapes based on models provided by the assessor. The complexity of the models augments progressively. The accuracy and the production time are recorded. This test evaluates visual processing (or simultaneous processing). It requires planning abilities and is fairly correlated with general intelligence. |
| | Rover | "Rover" is the name of a dog that is placed on a small 'chess board' with 'obstacles' represented on it. The dog has to go to his "bone" by jumping on a minimum amount of squares to jump in. A short training takes place to explain to the child how to move the dog and how to count the points it takes for him to reach his bone. New rules are progressively introduced during the test. It is therefore a complex test requiring planning abilities and counting abilities. The test is a measure of simultaneous processing abilities because the information is provided simultaneously. |
| Short-term memory | Word order | In the subtest word order, a series of frequent and concrete words are read aloud by the assessor. The child is presented with a series of picture and has to point out the pictures in the same order in which they were mentioned to him. This test measures short-term memory: it provides a measure of the memory span of the child. This concerns verbal memory with meaningful content; the fact that the answers are given by pointing instead of verbally (repetition) should minimize possible effects of language production difficulties. |
| | Number recall | The principle is to present a series of digits which the child has to repeat in the same order ("1-6-4" => "1-6-4") immediately after hearing the last digit of the series. The second part of the test consists of asking the child to repeat the digits in the reverse order ("9-2-4" = "4-2-9"); this is "number backward" condition. Digit span in general measures auditory short-term memory, sequencing skills, attention and concentration. The first part of the test, the forward recall, evaluates short-term memory. The digit Span Backward in particular involves working memory, transformation of information and mental manipulation, and according to some authors, visual-spatial imaging. |
| Retrieval ability | Atlantis | The principle of the test is to present pseudo words to children and ask them to remember them in a recognition task. Children are presented with an imaginary sea animal and are given its name ("Tra"); then a series of sea animals are presented and the child has to find out "Tra". More and more pseudo words are introduced progressively and the child has to point out several of them. The test evaluates the learning abilities of the children in the long-term on verbal non-meaningful (new) information. This is a recognition test and no verbal production is expected from the child. |
| | Verbal fluency | In verbal fluency tests, the child is required to retrieve as many words as possible with specific properties. We asked them to retrieve as many names of animals as possible ("cow, goat, dog…"). We repeated the test with different instruction, replacing the animal names by first names. The scores are the number of names given by the child for both tests. This test measures the ability to retrieve information. |
| | RAVLT | The administration of the RAVLT consists into reading a list of 15 unrelated, concrete nouns aloud and ask for a free recall ('free" means here that the order has no importance). There were three recall trials given in the short-term, and one after 20 min for the delayed recall. Three lists of words were created for repeated measures at baseline, 6 and 12 months. The score on the delayed recall was taken into statistical analyses. This test evaluates long-term memory and retrieval ability of the child. |
| Cognitive speediness | Number cancellation | Long series of digits ranging from 1 to 5 were presented in random order. Children had to cross systematically 2 digits (3 and 5 for example). This task activates sustained attention as well as selective attention. |
| | Coding | In this test, the child copies symbols that are paired with numbers. Using a key, the child draws each symbol in its corresponding shape or box within a specified time limit. This test involves visual-motor processing speed, but also short-term memory, learning ability, visual perception, visual-motor coordination, visual scanning ability, cognitive flexibility, attention and motivation. |

# Chapter 6

## Diet in relation to anthropometric measurements and cognitive performance in Indian schoolchildren

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## Abstract

**Objective**: This study aimed to investigate the associations between diet, including energy, protein and polyunsaturated fatty acid (PUFA) intake and micronutrient adequacy, and indicators of body size and cognitive performance in children.
**Subjects/Methods**: Baseline data of a clinical study in 598 children aged 6-10 years living in the urban slums of Bangalore, India were used to explore relationships between energy and nutrient intake, anthropometry and cognitive performance using analyses of variance adjusted for several confounding factors. Dietary intake was measured by a 24-h recall administered to both mother and child. The Kaufman Assessment Battery for Children II was used to measure mental performance.
**Results**: Most children had a diet inadequate with regard to energy (98%), total fat (100%), PUFA (91%) and micronutrients (85%). Energy intake was positively associated with body size and cognitive performance, with (borderline) significant linear trends for height ($P$=0.03), weight ($P$=0.01), mid-upper arm circumference ($P$=0.10) and cognition ($P$=0.09). Total PUFA intake was positively related to cognitive performance ($P$=0.06), but not to body size. Intakes of protein and $\alpha$-linolenic acid and adequacy of micronutrients were not significantly linked to anthropometric and cognitive measurements.
**Conclusions**: Our findings indicate that diets of Indian children from a poor socio-economic background were monotonous and dramatically low in energy, fat, PUFA and micronutrients. Introduction and/or expansion of school feeding programs and micronutrient interventions are urgently required in order to increase the energy and nutrient intakes to support children's growth and development.

## Introduction

Nutritional deficiencies are a major problem among children in developing countries, in particular among those living in Africa and Asia (1). While the problem of undernutrition is most severe in children <5 years of age, also a significant number of older children are affected by micronutrient deficiencies. In India, approximately 40% of the schoolchildren suffer from iron deficiency (1), 23% from vitamin A deficiency (2) and 4% of 6-12 year-old children have goiter (3). Poverty and consumption of monotonous diets appear to be underlying causes of inadequate intakes of energy and micronutrients and subsequent nutritional deficiencies (4). Data on dietary intake patterns of families living in urban India are limited, and specific data for school-age children are lacking. The last national survey, conducted in 1993-1994 in families living in slums areas in India, showed that intakes of energy, iron, calcium, vitamin A and B-vitamins were below the Indian Recommended Dietary Allowances (RDA) (5).

Malnutrition has important consequences on children's physical growth and cognitive development. Studies have shown that deficits in energy, protein, fat, zinc and other micronutrient intake increase the risk of growth faltering and result in stunting (6-9). Protein-energy malnutrition (10) and micronutrient deficiencies (11) may also lead to impaired mental development. In school-age children, there is evidence from placebo-controlled intervention studies for an essential role of iodine and iron in intellectual development, while research on B-vitamins, zinc and omega-3 fatty acids and cognition is limited and inconclusive (11). Very few and relatively small studies have linked dietary intake of energy, protein, fat and micronutrients to cognitive performance in school-age children (12;13).

The objective of this paper was to investigate the associations between diet, including energy, protein and polyunsaturated fatty acid (PUFA) intake and micronutrient adequacy, and indicators of body size and cognitive performance in Indian schoolchildren. We hypothesized that intakes of energy, protein, PUFA and micronutrients would be positively related to anthropometric measurements and cognition.

## Subjects and Methods

Baseline data collected in the period of November 2005 - February 2006 of the Children's Health And Mental Performance Influenced by Optimal Nutrition (CHAMPION) study were used to investigate energy and nutrient intake and their association with indicators of body size and cognitive performance. The study was designed to investigate the efficacy of foods fortified with omega-3 fatty acids and micronutrients on improving intellectual performance and growth in Indian children (14). The study protocol was approved by the ethics committees at St John's National Academy of Health Sciences, Bangalore, India and Wageningen University, the Netherlands.

**Subjects**

The study was conducted at two primary schools serving children from a poor socio-economic background in Bangalore city, India. All children aged 6-10 years, attending grades 2-5, were selected for participation in the study. Informed, written consent from the parents and verbal assent from their children was obtained from 645 parent-child pairs. In- and exclusion criteria have been described elsewhere (14). A total of 598 children were enrolled in the study.

**Socio-demographic information**

Socio-demographic information on household composition, parental education, income, and use of fortified foods was collected by a structured questionnaire which was administered to the mother or primary caretaker of the subjects.

**Dietary intake**

Dietary intake of each child was assessed using the 24-h dietary recall method by interviewing both the child and the child's mother or caretaker, individually, on separate occasions within the same week. Standard household measures of known volume were used during the recall to quantify the portion size of food items. The dietary recall by the mother or caretaker was taken as the basis for the child's dietary intake and completed with additional food items reported by the child (which were mostly foods consumed out of home). Nutrient intakes were associated with biochemical indices (methods for biochemistry have been described elsewhere (14)). Correlation coefficients found were: between folate intake and erythrocyte folate $r$=0.08 ($P$=0.056); between vitamin $B_{12}$ intake and plasma vitamin $B_{12}$ $r$=0.11 ($P$=0.009); between iron intake and blood hemoglobin $r$=0.03 ($P$=0.49), serum ferritin $r$=-0.01 ($P$=0.74), and serum transferrin receptor $r$=0.04 ($P$=0.38); between iodine intake and urinary iodine concentrations $r$=-0.05 ($P$=0.27); and between intake of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) and erythrocyte DHA fatty acid concentrations $r$=0.02 ($P$=0.70).

Energy and nutrient intakes were calculated using a nutrient database which was specifically composed for poor socio-economic populations in Bangalore. For development of the database, raw ingredients for each recipe were weighed, and volume to weight conversions measured for each cooked food item was obtained. Nutrient composition of the food items were calculated using standard food conversion tables for Indian foods (15) and USDA nutrient database (16). Ingredients of different foods items were categorized into different food groups. Energy and macronutrient intakes were compared to WHO/FAO recommendations for energy (moderate activity) (17), protein (per kg body weight) (18) and total fat and fatty acids (19).

**Probability of adequacy for micronutrients**

The probability of adequacy (PA) for intake of micronutrients with a normal distribution was calculated by using the PROBNORM function in SAS on the difference of the reported micronutrient intake and Estimated Average Requirement (EAR) divided by the standard deviation (SD) of the EAR (20). EARs were calculated from RDA for vitamins and minerals by WHO/FAO (21). SDs of EARs were computed based on coefficients of variation for intakes of micronutrients by the Institute of Medicine's Dietary Reference Intake reports (22;23). For zinc, the recommendation for a low bioavailability diet was used.

For iron intake, which has a skewed distribution, the PA was computed according to methods described by Allen et al (1), using published tables for a 5% bioavailability of iron. For calcium intake, no EAR was available and the Adequate Intake (AI) has been used to calculate the PA as described by Foote et al (24). The Mean Probability of Adequacy (MPA) of micronutrients was defined as the average of the PA's for 13 micronutrients in which PA's ≥100% were set at 100%.

**Anthropometry**

Anthropometric measurements were conducted in duplicate according to standard techniques (25). Height was recorded to the nearest 0.1 cm using a locally made stadiometer (Biorad, India) that was fixed to a wall. Body weight was recorded to the nearest 0.1 kg using a digital weighing scale (Breuer, Germany). Mid-upper arm circumference (MUAC) was recorded to the nearest 0.1 cm using a plastic tape.

**Cognitive performance**

Cognitive performance was evaluated using age-appropriate, validated psychometric tests that were administered by seven masters-level psychologists in the local Kannada language. The cognitive test battery consisted of 11 subtests, including six subtests from the Kaufman Assessment Battery for Children (KABC-II) for children 3-18 years. The test battery was adapted and validated to ensure its suitability in the local cultural context (26;27). For each subtest, a sum score was calculated and converted into a standardized *z*-score. The domain score was composed by taking the average of standardized *z*-scores for the tests constituting a domain. The average of the domain scores named the Mental Processing Index (MPI) was a composite measure of overall cognitive performance based on the KABC-II manual (28).

**Statistical analyses**

Energy and nutrient intakes were divided in tertiles. Mean and 95% CI for height, weight, MUAC and cognitive outcomes were calculated for each tertile. The middle

and highest tertile were compared to the lowest; and the middle tertile to the highest. Analysis of variance (SAS General Linear Modeling procedure) was applied to investigate the relationships between energy and nutrient intake and body size indicators, using a model adjusting for age; and for nutrient intakes, the energy intake was added as a covariate to the model. To assess the associations between energy and nutrient intake and cognitive performance, the model also included the covariates gender, school, maternal education and assessor of cognitive tests. A *P*-value for linear trend was calculated with the energy or nutrient intake as continuous variable. A transformation with natural logarithm was applied to variables that did not have a normal distribution. All analyses were performed using SAS version 9.1 statistical software package (SAS Institute Inc, Cary, NC).

## Results

A total of 598 children completed the baseline measurements on dietary intake, anthropometry and cognitive performance. The baseline characteristics of the study population are presented in **Table 1**. Mean age of the children was 8.7 ± 1.2 years and 49% of them were boys. Less than 4% of the children were vegetarian and none consumed micronutrient fortified foods. Iodized salt was used by 23.5% of the families.

**Table 1**. Characteristics of the study population

| Characteristic | N | |
|---|---|---|
| Gender (% males) | 598 | 49 |
| Age (y) | 598 | 8.7 ± 1.2[1] |
| Family income (Indian rupees/month) | 598 | 2700 (2000, 3800)[2] |
| Uneducated mothers (%) | 571 | 45.5 |
| Vegetarian (%) | 598 | 3.6 |
| Use of iodized salt in household (%) | 598 | 23.5 |
| Use of fortified foods[3] (%) | 598 | 0 |
| Height (m) | 598 | 122.9 ± 7.8 |
| Weight (kg) | 598 | 21.4 ± 3.8 |

[1] Mean ± SD (all such values).
[2] Median ($25^{th}$, $75^{th}$ percentiles).
[3] Including fortified biscuits, wheat flour or bread.

### Food groups

In general, the diet of the children consisted of three main meals of rice with sambar (lentil and vegetable curry), and a glass of milk or tea with milk as an early morning drink. The general diet was largely cereal-based, and in addition, consisting of some legumes, vegetables, roots, nuts, fats and oils (see **Table 2**). Of the animal foods, milk and milk products were part of the diet of 94% of the subjects, while egg (7%), meat (2%) and fish (0%) were not regularly consumed.

**Table 2**. Intake by different food groups

| | Intake (g/d) | Children consuming food group (%) |
|---|---|---|
| Cereals | 179.3 (156.9, 212.8)[1] | 100 |
| Legumes | 24.6 (18.5, 34.6) | 100 |
| Vegetables | 77.1 (55.2, 93.8) | 100 |
| Roots | 25 (19.7, 33.4) | 100 |
| Nuts | 7.7 (5.6, 11.6) | 99 |
| Fats and oils | 8.0 (5.1, 13.1) | 100 |
| Sugars | 9.7 (5.6, 14.3) | 92 |
| Fruit | 1.7 (0, 5.8) | 72 |
| Egg | 0 (0, 0) | 7 |
| Milk and milk products | 106 (65.9, 132.9) | 94 |
| Fish | 0 (0, 0) | 0 |
| Meat | 0 (0, 0) | 2 |

[1] Median ($25^{th}$, $75^{th}$ percentiles) (all such values).

## Energy and nutrient intake

An overview of median energy and nutrient intakes of the children can be found in **Table 3**. The age-specific energy intake was below the RDA in 98% of the children, and on average, only 61% of the energy requirements were met. When correcting the energy intake for body weight, 88% of the children still had intakes below the RDA. Most of the energy (72% of total energy (en%)) was provided by carbohydrates. Protein requirements (per kg body weight) were met by 91% of the children, and on average the amount of protein consumed (27.0 g/d) exceeded the requirements with almost 50%.

Intakes of total fat and polyunsaturated fatty acids (PUFA) were below the recommendations of 20-30% and 5-10 en% for 100% and 91% of the children, respectively. Saturated fatty acid (SAFA) intake was too high (>10 en%) in 20% of the subjects. None of the children met the recommendations for $\alpha$-linolenic acid (ALA) of 1-2 en%. Similarly, none of the children consumed >200 mg/d of EPA+DHA, which corresponds with the recommendation of 2 portions of fatty fish per week for prevention of chronic diseases (29), and the majority of children (92%) did not consume these fatty acids at all.

The MPA was 14.5%, meaning that less than 15% of the children had an adequate intake of micronutrients. Especially for niacin, folate, vitamin $B_{12}$, iron and zinc PA's were low (0-5%), whereas approximately 30% had adequate intakes for vitamin $B_6$ and calcium. According to the calculations nearly 70% had adequate vitamin C intakes, mainly from vegetables sources. However, it must be noted that these calculations did not take into account the losses of vitamin C by cooking processes.

## Associations of energy and nutrient intake with anthropometric measurements and cognitive performance

An overview of the associations of dietary intake with indicators of body size and cognitive performance can be found in **Table 4**. Energy intake adjusted for age was positively associated with anthropometric measurements and cognitive

**Table 3**. Energy and nutrient intake of 598 Indian children

| | Daily intake | % of total energy | PA (%) |
|---|---|---|---|
| Energy (MJ) | 4.33 (3.78, 5.04)[1] | | |
| Energy (kcal) | 1034 (902, 1203) | | |
| | | | |
| *Macronutrients* | | | |
| Protein (g) | 27.0 (23.0, 31.6) | 10.2 | |
| Total fat (g) | 19.7 (15.1, 25.9) | 17.8 | |
| SAFA (g) | 9.0 (7.3, 11.5) | 8 | |
| MUFA (g) | 5.6 (4.1, 7.8) | 5 | |
| PUFA (g) | 2.7 (1.8, 4.2) | 3 | |
| Linoleic acid (g) | 2.4 (1.5, 3.9) | 2.7 | |
| α-Linolenic acid (g) | 0.19 (0.16, 0.23) | 0.2 | |
| EPA+DHA (mg) | 0 (0, 0) | 0 | |
| Carbohydrates (g) | 185 (163, 215) | 72.0 | |
| | | | |
| *Micronutrients* | | | |
| Retinol (µg RE) | 170 (143, 201) | | 7.6 |
| Thiamin (mg) | 0.51 (0.42, 0.64) | | 12.6 |
| Riboflavin (mg) | 0.51 (0.41, 0.63) | | 12.3 |
| Niacin (mg) | 5.8 (5.0, 7.1) | | 4.4 |
| Pyridoxin (µg) | 0.71 (0.60, 0.89) | | 30.1 |
| Folate (µg) | 108 (90, 140) | | 3.0 |
| Vitamin $B_{12}$ (µg) | 0.40 (0.25, 0.51) | | 0.6 |
| Vitamin C (mg) | 34.5 (28.3, 40.5) | | 68.6 |
| Vitamin E (mg) | 2.56 (1.87, 4.01) | | 9.7 |
| Calcium (mg) | 283 (225, 366) | | 28 |
| Iodine (µg) | 44. 0 (37.7, 54.2) | | 8.7 |
| Iron (mg) | 4.9 (4.1, 6.2) | | 2.8 |
| Zinc (mg) | 4.19 (3.63, 4.94) | | 0 |

PA, probability of adequacy; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid RE, retinol equivalents.
[1] Median ($25^{th}$, $75^{th}$ percentiles) (all such values).

performance (see **Figure 1**), with significant linear trends for height ($P$=0.03) and weight ($P$=0.01), and borderline significant linear trends for MUAC ($P$=0.10) and MPI ($P$=0.09). PUFA intake was positively related to cognitive performance, with a borderline significant linear trend for MPI ($P$=0.06), whereas no significant associations were found between PUFA intake and body size. No significant relationships were found between the indicators of body size and cognition and intake of protein and ALA, and micronutrient adequacy (MPA).

## Discussion

This study showed that the diet of 598 children from a poor socio-economic background in Bangalore city was inadequate with regard to energy, protein, fat and micronutrients. Energy intake was positively related to height and weight, and possibly to MUAC of the children, whereas energy and PUFA intake may be positively associated with their cognitive performance. Intakes of protein and ALA and adequacy of micronutrients in the diet were not significantly linked to indicators of body size and cognitive performance.

**Table 4**. Energy and nutrient intakes in relation to growth and cognitive performance of Indian children

| | Height[1] N=598 | | Weight[1] N=598 | | MUAC[1] N=598 | | Mental Processing Index[2] N=571 | |
|---|---|---|---|---|---|---|---|---|
| | Mean (95% CI) | *P* for trend | Mean (95% CI) | *P* for trend | Mean (95% CI) | *P* for trend | Mean (95% CI) | *P* for trend |
| Energy (kcal) | | | | | | | | |
| <948 | 122.3 (121.5-123.0) | 0.03 | 21.1 (20.6-21.4) | 0.01 | 17.0 (16.8-17.2) | 0.10 | 0.00 (-0.12, 0.11) | 0.09 |
| 948-1142 | 122.9 (122.2-123.7) | | 21.4 (21.0-21.8) | | 17.0 (16.8-17.2) | | 0.07 (-0.04, 0.18) | |
| >1142 | 123.4 (122.7-124.2 | | 21.8 (21.4-22.2) | | 17.2 (17.0-17.4) | | 0.06 (-0.06, 0.17) | |
| | | | | | | | | |
| Protein (g) | | | | | | | | |
| <24.2 | 121.8 (120.8-122.8) | 0.95 | 21.3 (20.7-21.9) | 0.62 | 17.0 (16.8-17.2) | 0.37 | 0.11 (-0.02, 0.23) | 0.10 |
| 24.2-29.9 | 123.5 (122.8-124.2) | | 21.7 (21.2-22.1) | | 17.1 (16.9-17.3) | | 0.03 (-0.08, 0.14) | |
| >29.9 | 123.3 (122.3-124.4) | | 21.3 (20.7-21.9) | | 17.1 (16.9-17.3) | | -0.03 (-0.18, 0.11) | |
| | | | | | | | | |
| PUFA (g) | | | | | | | | |
| <2.0 | 122.9 (122.1-123.7) | 0.19 | 21.6 (21.1-22.0) | 0.14 | 17.1 (16.9-17.3) | 0.94 | 0.01 (-0.11, 0.12) | 0.06 |
| 2.0-3.6 | 123.1 (122.4-123.9) | | 21.5 (21.0-21.9) | | 17.0 (16.9-17.2) | | 0.03 (-0.08, 0.15) | |
| >3.6 | 122.6 (121.9-123.4) | | 21.3 (20.8-21.7) | | 17.0 (16.8-17.2) | | 0.08 (-0.04, 0.19) | |
| | | | | | | | | |
| $\alpha$-Linolenic acid (g) | | | | | | | | |
| <0.170 | 122.6 (121.8-123.4) | 0.12 | 21.5 (21.0-22.0) | 0.87 | 17.1 (16.9-17.2) | 0.54 | 0.08 (-0.05, 0.20) | 0.74 |
| 0.170-0.216 | 123.0 (122.2-123.7) | | 21.4 (21.0-21.8) | | 17.0 (16.8-17.2) | | 0.02 (-0.09, 0.13) | |
| >0.216 | 123.1 (122.2-123.9) | | 21.4 (20.9-21.9) | | 17.1 (16.9-17.3) | | 0.03 (-0.09, 0.15) | |
| | | | | | | | | |
| MPA (%) | | | | | | | | |
| <8.4 | 122.9 (122.0-123.7) | 0.15 | 21.7 (21.2-22.1) | 0.07 | 17.1 (16.9-17.2) | 0.92 | 0.03 (-0.09, 0.15) | 0.36 |
| 8.4-15.8 | 123.6 (122.9-124.4) | | 21.8 (21.3-22.2) | | 17.0 (16.8-17.2) | | 0.03 (-0.09, 0.15) | |
| >15.8 | 122.2 (121.3-123.0) | | 20.9 (20.4-21.4) | | 17.1 (16.9-17.3) | | 0.06 (-0.06, 0.18) | |

PUFA, polyunsaturated fatty acids; MPA, Mean Probability of Adequacy of micronutrients; MUAC, mid-upper arm circumference.
[1] For energy intake a model adjusting for age was applied, for other intake variables a model adjusting for age and energy intake was used.
[2] For energy intake a model adjusting for age, gender, school, maternal education, assessor of cognitive tests was applied, for other intake variables a model adjusting for age, gender, school, maternal education, assessor of cognitive tests and energy intake was used.

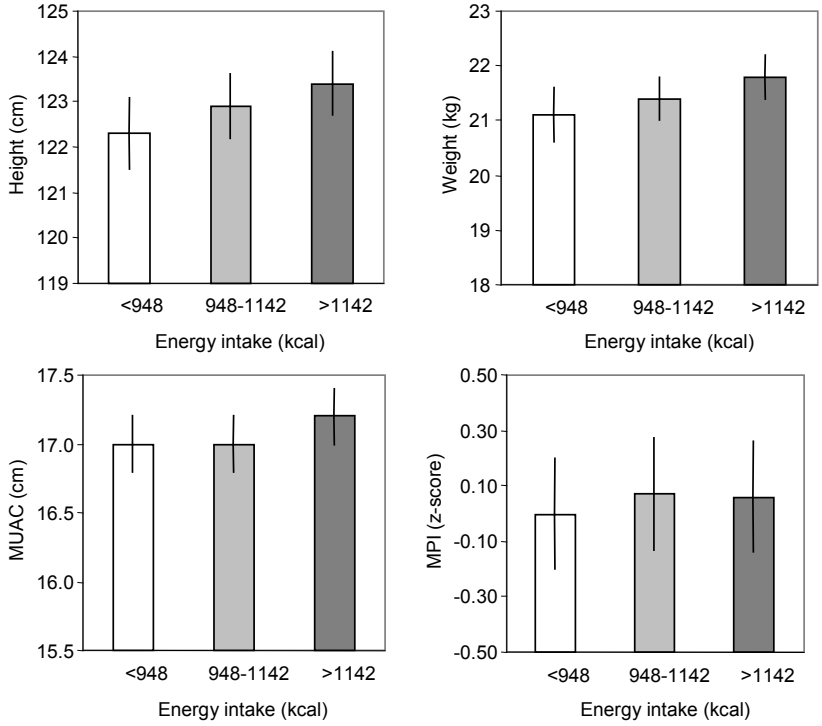


**Figure 1**. Associations between energy intake and anthropometric measurements and cognitive performance of Indian children. Bars and error bars represent means and 95% confidence intervals in height, weight, mid-upper arm circumference (MUAC) and Mental Processing Index (MPI) of three groups of children with ascending energy intakes.

Based on comparable prevalences of anemia in the current study with those found in other studies conducted in surrounding areas in Bangalore (30), we assume that the current study population is representative for school-age children of low socio-economic backgrounds in Bangalore city. Though a 24-h dietary recall is not the most accurate method to estimate energy and nutrient intakes, data is considered to be sufficient to provide insights in usual nutrient and food intake patterns on a group level (31). Compared to actual intakes, a 24-h recall has been shown to have good validity for estimating energy, protein, calcium and zinc intakes, but not for B-vitamins and iron (31). Correlation analyses of micronutrient intake with biochemical indices showed weak significant correlation coefficients for folate and vitamin $B_{12}$, while iron, iodine and fatty acid intakes were not related to their corresponding biochemical parameters. The findings did not alter after correcting for possible confounders and may be attributed to a low variation in intake of the nutrients.

One of the strengths of the study concerns the robust measurements of anthropometry and cognitive performance in a relatively large group of children. The cognitive test battery was thoroughly adapted to local language and culture and showed good internal and external validity, which is essential to detect any associations between cognitive functioning and nutritional status (32). Furthermore, we adjusted our findings for several confounding factors, such as age, gender and

maternal education. However, the cross-sectional design of the current study does not allow us to assert causal relations.

Our findings on energy and protein intakes correspond with the results of the country wide survey in urban slums in India, which showed that Indian diets were short of energy but adequate in protein (5). However, as most protein in the diet came from rice, the protein quality may have been suboptimal, and in fact, 35% of the children in our study did not meet the requirements for lysine (18). Total fat intakes of the children were too low (18 en%), which has also been shown in adults in South India with average intakes of 22 en% (33). The low fat intake in children is of concern as fat intakes below 25 en% may increase the risk of poor growth and inadequate intake of vitamins and minerals (34). Requirements for PUFA were not met, while SAFA intake exceeded the recommendations in 20% of the subjects. Furthermore, none of the children consumed fish, which is in line with observations in pregnant women from urban slums in Bangalore (35). Our findings indicate a possible public health threat because low PUFA intakes may increase the risk of later chronic diseases (36), which is of particular concern for societies in transition (37).

Only 15% of the children had an adequate intake of micronutrients, and even if the diet would be sufficient in energy, the requirements for iron, zinc, and vitamin $B_{12}$ in particular would not be met by most of the children. This may be explained by the low intake of animal products, such as eggs, fish and meat, and the low bioavailability of iron and zinc in the present diet.

Our findings of a positive association of energy intake with body size and cognitive performance, but not for intakes of other nutrients after controlling for energy intake, may be explained by the low variation in intakes of the nutrients because of the monotonous diets of the children. However, intervention studies have shown that adequate energy, protein and micronutrient intakes are essential for development of children <2 years of age (10) and may also benefit growth and cognitive performance in older children (38). Furthermore, in a cross-sectional study conducted in 83 Spanish 6 year-old children, iron and folate intake were positively related to intelligence scores after adjusting for gender, body mass index, maternal education, and vitamin $B_{12}$ and C intakes (13). In contrast to the absence of an association between micronutrient intake and cognition observed in our study, data from these other studies suggest that enhancing the intake of micronutrients, such as iron, zinc, folate and vitamin $B_{12}$, may benefit children's intellectual development, especially when intakes are low.

In spite of low PUFA intakes, our data suggest a positive association of PUFA intake with cognition, which may have been stronger and significant when there was more variation in the intakes. Our finding has not been shown before in similar populations in developing countries, but is in line with a cross-sectional study in 6-16 year-old US children, in which a higher PUFA intake at the expense of

SAFA or carbohydrate intake was more beneficial for short-term memory (39). For ALA, no evidence exists for positive effects of higher ALA intakes on cognition in children, which is in line with our findings. In contrast, higher intakes of fish during pregnancy may benefit infant development (40-42). However, no such evidence is available for fish intake during childhood and in absence of fish consumption in our study population, we failed to investigate whether higher intakes of EPA+DHA would improve cognitive performance at school age.

In conclusion, our findings imply that current diets of poor Indian schoolchildren are dramatically low in energy and essential nutrients and therefore, there is a need for increasing intakes to support growth and development of these deprived children. This could be achieved, for instance, through dietary diversification, food fortification or school feeding.

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## References

1. Allen L, de Benoist B, Hurrell RF, Dary O. Guidelines on food fortification with micronutrients. Geneva: World Health Organization and Food and Agriculture Organization of the United Nations, 2006.
2. Singh V, West KP, Jr. Vitamin A deficiency and xerophthalmia among school-aged children in Southeastern Asia. Eur J Clin Nutr 2004;58:1342-9.
3. National Nutrition Monitoring Bureau. Prevalence of micronutrient deficiencies. NNMB Technical Report No. 22. Hyderabad: 2003.
4. Narasinga Rao BS. Anaemia and micronutrient deficiency. Natl Med J India 2003;16:46-50.
5. National Nutrition Monitoring Bureau. Interim Report of Urban Survey - Slums (1993-94). NNMB Technical Report No. 15. Hyderabad: 1994.
6. Martorell R, Mendoza F, Castillo R. Poverty and stature in children. In: Waterlow JC, ed. Linear growth retardation in less developed countries. New York: Raven Press 1988:57-73.
7. Butte NF. Fat intake of children in relation to energy requirements. Am J Clin Nutr 2000;72:1246S-52S.
8. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2002;75:1062-71.
9. Ramakrishnan U, Aburto N, McCabe G, Martorell R. Multimicronutrient interventions but not vitamin a or iron interventions alone improve child growth: results of 3 meta-analyses. J Nutr 2004;134:2592-602.
10. Grantham-McGregor S, Baker-Henningham H. Review of the evidence linking protein and energy to mental development. Public Health Nutr 2005;8:1191-201.
11. Bryan J, Osendarp S, Hughes D, Calvaresi E, Baghurst K, van Klinken JW. Nutrients for cognitive development in school-aged children. Nutr Rev 2004;62:295-306.
12. Sigman M, Neumann C, Jansen AA, Bwibo N. Cognitive abilities of Kenyan children in relation to nutrition, family characteristics, and education. Child Dev 1989;60:1463-74.
13. Arija V, Esparo G, Fernandez-Ballart J, Murphy MM, Biarnes E, Canals J. Nutritional status and performance in test of verbal and non-verbal intelligence in 6 year-old children. Intelligence 2006;34:141-9.
14. Muthayya S, Eilander A, Transler C et al. Effect of fortification with multiple micronutrients and n-3 fatty acids on growth and cognitive performance in Indian schoolchildren: the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. Am J Clin Nutr 2009;89:1766-75.
15. Gopalan C, Rama Sastri BV, Balasubramanian SC, Narasinga Rao BS, Deosthale YG, Pant KC. Nutritive value of Indian foods. Hyderabad: National Institute of Nutrition / Indian Council of Medical Research, 1996.
16. USDA/ARS. USDA National Nutrient Database for Standard Reference. http://www.nal.usda.gov/fnic/foodcomp/search/2009.
17. FAO/WHO/UNU. Human energy requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. FAO Food and Nutrition Technical Report Series No. 1. Rome: Food and Agriculture Organization, 2004.
18. WHO/FAO/UNU. Protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/UNU

expert consultation. WHO Technical report series 935. Geneva: World Health Organization, 2007.
19. WHO/FAO. Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation. WHO technical report series No. 916. Geneva: World Health Organization, 2003.
20. Murphy SP. Impact of the new Dietary Reference Intakes on nutrition calculation programs. J Food Comp Anal 2003;16:365-72.
21. WHO/FAO. Vitamin and mineral requirements in human nutrition 2nd ed. Report of a joint WHO/FAO expert consultation. WHO/FAO non-series publication. Geneva: World Health Organization, 2004.
22. Institute of Medicine (IOM). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Managenes, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Food and Nutrition Board, Institute of Medicine. Washington DC: National Academy Press 2001.
23. Institute of Medicine (IOM). Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press, 1998.
24. Foote JA, Murphy SP, Wilkens LR, Basiotis PP, Carlson A. Dietary variety increases the probability of nutrient adequacy among adults. J Nutr 2004;134:1779-85.
25. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Publishers, 1988.
26. Malda M, van de Vijver FJR, Srinivasan K, Transler C, Sukumar P, Rao K. Adapting a cognitive test for a different culture: An illustration of qualitative procedures. Psychol Sci Q 2008;50:451-68.
27. Malda M, van de Vijver FJR, Srinivasan K, Transler C, Sukumar P. Traveling with cognitive tests: Testing the validity of a KABC-II adaptation in India. Assessment 2009.
28. Kaufman AS, Kaufman LN. Kaufman Assessment Battery for Children, Second Edition: Manual. Circle Pines, MN: AGS Publishing, 2004.
29. Kris-Etherton PM, Innis S, American DA, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc 2007;107:1599-611.
30. Muthayya S, Thankachan P, Zimmermann MB et al. Low anemia prevalence in school-aged children in Bangalore, South India: possible effect of school health initiatives. Eur J Clin Nutr 2007;61:865-9.
31. Gibson RS. Principles of nutritional assessment. Oxford: Oxford University Press, 1990.
32. Isaacs E, Oates J. Nutrition and cognition: assessing cognitive abilities in children and young people. Eur J Nutr 2008;47 Suppl 3:4-24.
33. Sudha V, Radhika G, Sathya RM, Ganesan A, Mohan V. Reproducibility and validity of an interviewer-administered semi-quantitative food frequency questionnaire to assess dietary intake of urban adults in southern India. Int J Food Sci Nutr 2006;57:481-93.
34. Uauy R, Castillo C. Lipid requirements of infants: implications for nutrient composition of fortified complementary foods. J Nutr 2003;133:2962S-72S.
35. Muthayya S, Dwarkanath P, Thomas T et al. The effect of fish and omega-3 LCPUFA intake on low birth weight in Indian pregnant women. Eur J Clin Nutr 2009;63:340-6.
36. Rasanen M, Lehtinen JC, Niinikoski H et al. Dietary patterns and nutrient intakes of 7-year-old children taking part in an atherosclerosis prevention project in Finland. J Am Diet Assoc 2002;102:518-24.
37. Stein AD, Thompson AM, Waters A. Childhood growth and chronic disease: evidence from countries undergoing the nutrition transition. Matern Child Nutr 2005;1:177-84.
38. Neumann CG, Bwibo NO, Murphy SP et al. Animal source foods improve dietary quality, micronutrient status, growth and cognitive function in Kenyan school children: Background, study design and baseline findings. Journal of Nutrition 2003;133:3941S-9S.
39. Zhang J, Hebert JR, Muldoon MF. Dietary fat intake is associated with psychosocial and cognitive functioning of school-aged children in the United States. J Nutr 2005;135:1967-73.
40. Daniels JL, Longnecker MP, Rowland AS, Golding J. Fish intake during pregnancy and early cognitive development of offspring. Epidemiol 2004;15:394-402.
41. Oken E, Wright RO, Kleinman KP et al. Maternal fish consumption, hair mercury, and infant cognition in a U.S. Cohort. Environ Health Perspect 2005;113:1376-80.
42. Hibbeln JR, Davis JM, Steer C et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 2007;369:578-85.

# Chapter 7

## General Discussion

## Main findings

The aim of this thesis was to investigate the role of multiple micronutrients and omega-3 fatty acids on cognitive performance in school-age children. Three major research questions were addressed and the answers to them are presented in **Table 1** and will be summarized here:

*1. To what extent do multiple micronutrient interventions influence different cognitive domains in children?*

Initially, we hypothesized that the size of the effect of multiple micronutrients on cognitive outcomes would be small to moderate, and that the domains of fluid intelligence, memory and learning, visual perception, retrieval ability, and cognitive speediness could be positively influenced. We found that the cognitive domain of fluid intelligence and academic performance may be improved by multiple micronutrient supplementation with small effects of 0.15-0.30 SD. Crystallized intelligence seems not affected and for the other domains the number of studies is currently too limited to draw conclusions (chapter 2).

*2. What is the role of omega-3 fatty acids on children's cognitive development?*

Because DHA is a structural component of the brain and involved in neurotransmitter functioning, we expected that omega-3 fatty acid supplementation would be beneficial for cognitive performance in children. However, we did not find evidence that additional intake of omega-3 fatty acids, and that of DHA in particular, improves intellectual development at school age (chapter 3-6).

*3. What is the effect of an intervention with different doses of micronutrients and omega-3 fatty acids on cognitive performance in schoolchildren in India? Is there an interaction between micronutrients and omega-3 fatty acids?*

We postulated that higher doses of multiple micronutrients and omega-3 fatty acids would be more effective in improving intellectual development in children than lower doses, and that omega-3 fatty acids would have additional effects to micronutrients on cognitive outcomes. Our randomized controlled trial showed no differences between the high (100% of the Recommended Dietary Allowance (RDA)) and low (15% of the RDA) dose of micronutrients on overall cognitive performance in Indian schoolchildren. Neither there were any differences between the high and low dose of omega-3 fatty acids (chapter 5), nor any interactions between micronutrients and omega-3 fatty acids on cognitive parameters.

Besides answering these three research questions, a number of issues in the field of nutrition and cognition in children remain to be addressed. In the previous chapters, we elaborated on specific points for discussion referring to the study presented in each of these chapters. Here, we will focus on general aspects that

arose from the totality of research described in this thesis, including the study population, cognitive assessment methodology, impact of nutritional status on cognitive outcomes, optimal composition of multiple micronutrient supplement, and a reflection on findings of omega-3 fatty acids on cognition. The chapter will end with implications for public health and directions for future research.

**Table 1**. Overview of main findings to research questions addressed in this thesis

| **1. To what extent do multiple micronutrient interventions influence different cognitive domains in children?** | | |
|---|---|---|
| Design | Main outcomes | Conclusions |
| Meta-analysis (chapter 2) | Pooled effect sizes of micronutrient supplementation:<br>Fluid intelligence 0.14 SD (95% CI: -0.02, 0.29); *P*=0.083<br>Crystallized intelligence -0.03 SD (95% CI: -0.21, 0.15); *P*=0.74<br>Academic performance 0.30 SD (95% CI: 0.01, 0.58); *P*=0.04. | Effects of micronutrients tend to be small. Domains of fluid intelligence and academic performance may positively be influenced, while crystallized intelligence seems not affected. For other domains, data is too limited to draw any conclusions. |
| Cross-sectional study (chapter 4) | Blood hemoglobin concentrations were positively associated with fluid reasoning, short term memory, retrieval ability and cognitive speediness. Plasma vitamin $B_{12}$ concentrations were inversely related to short term memory and retrieval ability. Indicators of iron, iodine and folate status were not significantly linked to cognitive domains. | |
| Randomized controlled trial (chapter 5) | Compared to the low dose, the high dose of micronutrients was less beneficial for fluid reasoning -0.12 SD (95% CI: -0.20, -0.04) and more beneficial for short term memory 0.06 SD (95% CI: -0.04, 0.16). No significant effects were found on retrieval ability and cognitive speediness. | |
| **2. What is the role of omega-3 fatty acids on children's cognitive development?** | | |
| Design | Main outcomes | Conclusions |
| Systematic review (chapter 3) | Only one cohort study in children aged 7 years was reported in literature, which did not find significant associations between plasma DHA concentrations and cognitive performance. | Studies did not show benefits of omega-3 fatty acids on cognitive development in healthy children older than two years of age, and evidence is too limited to draw a conclusion. |
| Cross-sectional study (chapter 4) | No significant associations between erythrocyte DHA concentrations and cognition were found. | |
| Randomized controlled trial (chapter 5) | Effect sizes of high versus low dose of omega-3 fatty acids after 12 months on Mental Processing Index: 0.00 SD (95% CI: -0.05, 0.05). | |
| Cross-sectional study (chapter 6) | PUFA intake was positively related to cognitive performance (*P* for linear trend=0.06), but ALA intake was not. | |
| **3. What is the effect of an intervention with different doses of micronutrients and omega-3 fatty acids on cognitive performance in schoolchildren in India? Is there an interaction between micronutrients and omega-3 fatty acids?** | | |
| Design | Main outcomes | Conclusions |
| Randomized controlled trial (chapter 5) | Effects of high vs low dose of micronutrients after 12 months on Mental Processing Index: -0.02 SD (95% CI: -0.07, 0.03).<br>Effects of high vs low dose of omega-3 fatty acids after 12 months on Mental Processing Index: 0.00 SD (95% CI: -0.05, 0.05).<br>No significant interactions between micronutrients and omega-3 fatty acids were detected. | On overall cognitive performance, there were no differences between high and low doses of micronutrients and between high and low doses of omega-3 fatty acids. No interactions between micronutrients and omega-3 fatty acids were found. |

## Study population

### Methodological issues

The subjects of research described in this thesis were healthy school-age children, without clinically diagnosed developmental disorders. In this section, we will address two methodological issues with regard to the selection of our study population which may have influenced the study outcomes, including selection bias and age.

Selection bias occurs when the characteristics of the subjects selected for the study differ systematically from subjects who are not. For the trials included in the meta-analysis (chapter 2), we were unable to assess this type of bias due to lack of information in the publications. For our randomized controlled trial (chapter 4, 5 and 6), we do not have evidence that selection bias has occurred, because children who completed the study were not different with regard to socio-demographic and nutritional factors at baseline compared to children who were lost to follow-up. Furthermore, the prevalence of anemia in our study population was similar to that measured in other studies conducted in Bangalore city and its surroundings (1), indicating that the children in our study were representative for children from a poor socio-economic background in Bangalore.

Secondly, age may have had an influence on the study outcomes, since the largest brain growth and major events in cognitive development take place before the age of 5 years. Therefore, effects of nutrition on cognitive performance may have been larger in young children than in children of school age. However, brain development is continuing into early adulthood, in particular development of frontal lobes, which are involved in executive functioning, such as reasoning and planning (2). Moreover, by the age of 5 years, cognitive functions have matured sufficiently and therefore it is possible to measure all cognitive domains in older children. In younger children, psychological assessments are more general and may be less sensitive to effects of nutrition interventions (3). Thus, by studying children older than 5 years of age, we were able to assess to what extent micronutrients and omega-3 fatty acids influenced the different cognitive domains.

### Interpretation of findings

Our meta-analysis showed that effects of multiple micronutrients on cognitive performance in schoolchildren 5-16 years of age were small. We failed to compare studies conducted in children <5 years with those performed in children aged 5-16 years, due to the limited number of studies in younger children (chapter 2). However, two (4;5) out of the three (4-6) studies in children <5 years showed positive effects on gross motor development, implying that micronutrients may also improve development at younger ages.

In contrast to our findings, two systematic reviews on interventions with iron only have demonstrated convincing evidence for beneficial effects on mental

development in children >2 years but not in children <2 years (7;8). However, there is no adequate explanation that younger children would not benefit from iron supplementation. Possibly, psychological assessment methods may have been too general to detect effects, or other factors influencing mental development, such as lack of social stimulation, may have overruled the effects of iron supplementation (8).

Furthermore, since development of the brain structures and cognitive functions takes place at different times and build on each other (2), small disturbances in these processes early in life –for example through undernutrition– can have consequences for later cognitive performance (9). Therefore, the timing of nutrition interventions is important and experts have recently recommended that public health programs targeted at younger children are most effective to improving mental development (10).

### Conclusion

The limited number of studies in children <5 years gives no clear indication that effects of multiple micronutrients on mental development are different from those in children >5 years of age. Because of the rapid development of the brain and cognitive functions in the early years of life, it is expected that the impact of nutrition interventions is larger and therefore easier to detect at this age. However, research in older children has the advantage that specific cognitive functions have more matured and are therefore better measurable, which increases the chances of detecting possible effects in efficacy trials. On the other hand, for policy-making, more research in younger children would be of interest to conclude at what age supplementation with micronutrients would be most efficacious for improving cognitive development on both short and longer term.

## Cognitive assessment

### Methodological issues

In this section, we will focus on a number of aspects that were important for the methodology used to assess cognitive performance in children in our studies, including the application of the general model of intelligence to non-western populations and validity and reliability of cognitive tests.

#### *General model of intelligence*

In our meta-analysis (chapter 2), we categorized the cognitive tests used in the trials into cognitive domains proposed in Carroll's model of general intelligence (11) and added sustained attention and academic performance. Although the model is based on analysis of 460 datasets reported in psychometric literature, covering about six decades of intelligence testing in various populations, there may be some uncertainty regarding its validity in non-western populations. However,

Carroll indicated that there was little evidence that the model would differ in any systematic way across genders and different cultures, but differentiations may occur due to variations in education or other environmental factors (11). Moreover, similar models of general intelligence have shown to be valid in other cultures (12-14). Therefore, it is justified to apply Carroll's model to trials in non-western populations.

Also the Kaufman Assessment Battery for Children, second edition (KABC-II), used in our intervention study (chapter 4, 5 and 6), measures several cognitive domains that can be interpreted in the context of the model by Carroll (15). This test battery has been carefully developed to minimize the influence of language and cultural knowledge on test results, indicating that the designers of the test battery also felt that the structure of cognitive domains can be used in other cultures. After the cross-cultural adaptation of the test battery used in our intervention, we could confirm that the test measured the same psychological concepts as in the culture of test origin (16), indicating a good validity.

***Validity and reliability of cognitive tests***

A limitation of the meta-analysis was that we were unable to assess the validity and reliability of the cognitive tests used in the different studies. Tests with a low quality may have been less sensitive to detect effects of the intervention. Indeed, the effects of micronutrients on fluid intelligence were less visible in developing countries compared to developed countries, which may be attributed to use of less robust tests in the trials in developing countries. However, other factors that differ between developed and developing countries, such as environmental factors (e.g. poverty) may also have influenced the results.

The cognitive tests used in our intervention study (chapter 4, 5 and 6) were thoroughly adapted for use in the local setting by a process of translation and back-translation, followed by a pilot study during which the tests were fine-tuned based on observations and feedback from the local psychologist administering the tests to children in a non-standard way (17). As already indicated in the previous section, the test battery showed good validity and reliability.

**Interpretation of findings**

In this section, we will discuss the interpretation of findings for the meta-analysis and the randomized controlled trial separately.

***Meta-analysis (chapter 2):***

The meta-analysis suggested that multiple micronutrients were beneficial for fluid intelligence, which is the cognitive domain with the highest correlation with general intelligence. No effects were observed for crystallized intelligence and evidence was inconclusive for the other cognitive domains because of limited data

(**Figure 1**). Furthermore, academic performance assessed by school exams may also be influenced by micronutrients.

It has been hypothesized that during cognitive development, not all domains would mature at a similar rate. Development of some of the domains could be stimulated by nutritional interventions while at the same time development of other domains may stand still or even reverse (18). The finding that crystallized intelligence may not be influenced by multiple micronutrients could possibly be attributed to the relatively short duration of studies preventing them from showing significant differences in acquired skills and knowledge. In fact, studies on the effect of protein-energy supplements provided in the first two years of life, have shown to benefit children's verbal and reading skills at school age (19), suggesting that nutrition interventions may also benefit this domain but with a longer time-lag.

Furthermore, environmental factors may be more important determinants of certain cognitive domains, such as crystallized intelligence and therefore effects of micronutrient interventions may not become apparent. This hypothesis has also been suggested as explanation for the lack of effects of iron supplementation on development of young children (8).

Finally, the cognitive tests assessing various cognitive domains may differ in sensitivity. Therefore, it would be of interest to make an inventory of individual cognitive tests used in trials investigating micronutrients that are most responsive. For instance, the digit span test that measures short-term memory was sensitive to micronutrient interventions in a number of trials (20-22).

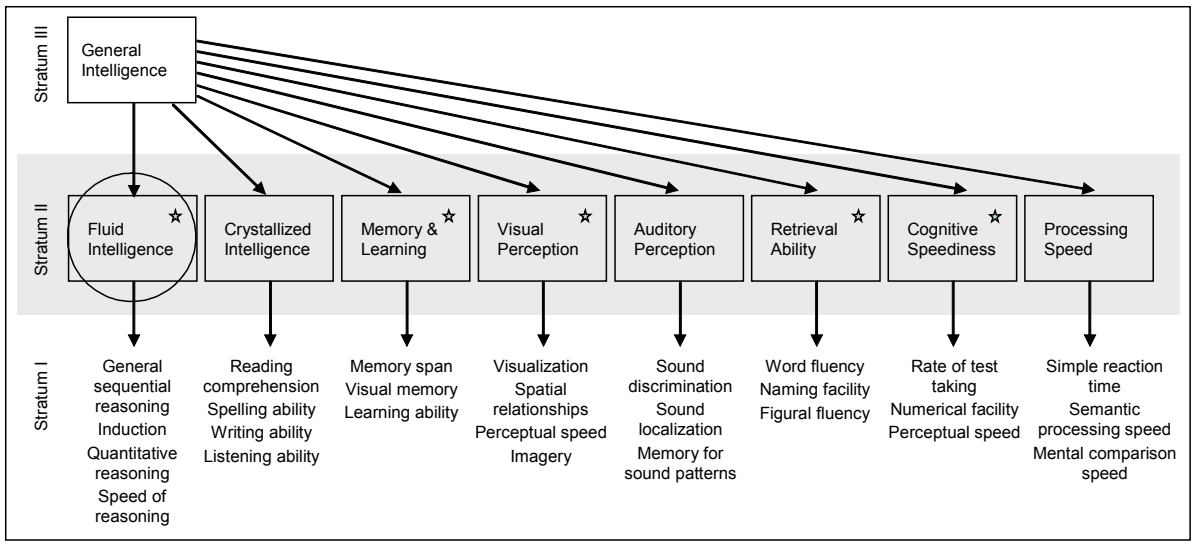


**Figure 1**. Model of intelligence adapted from Carroll (23).
The cognitive domains in stratum II marked with an asterisk were expected to be influenced by micronutrient interventions (24), while the cognitive domain marked with a circle was positively affected by multiple micronutrient supplementation according findings of the meta-analysis.

***Intervention study (chapter 5):***

Compared to the low dose of micronutrients, we found a small positive effect of the high dose of micronutrients on short-term memory and a small negative effect on fluid reasoning (including tests of both fluid intelligence and visual perception domains). We do not have a good explanation why these domains were affected whereas the other domains were not. Because micronutrient status at baseline and compliance to consumption of the study products did not influence the cognitive outcomes, we concluded that the effects found for short-term memory and for fluid reasoning may have been due to chance.

**Conclusion**

We showed that micronutrients seem to have small effects on fluid intelligence and therefore, it is recommended to include tests assessing this domain in future studies. Tests measuring crystallized intelligence may only be included in studies with a long duration of more than a year, as no effects are expected on shorter term. For a robust assessment of a particular cognitive domain, 2-3 different tests per domain are required. In addition, for future studies in developing countries in particular, the cognitive tests need a thorough adaptation which is essential to ensure their validity in a local setting.

## Impact of nutritional status on cognitive outcomes

**Methodological issues**

An important question, which could not be adequately answered by the research in this thesis, is whether nutritional status at baseline has a significant impact on the effect of micronutrient supplementation on cognition. Findings from the meta-analysis indicated that the development status of the country was a significant factor explaining the heterogeneity between studies, which could be attributed to differences in nutritional status of subjects between developed and developing countries (chapter 2). Unfortunately, data on prevalence of stunting and hemoglobin concentration of the study population were lacking. Therefore, it was not possible to explore whether baseline nutritional status would be a significant predictor of cognitive outcomes.

For the intervention study, we chose to conduct the study in a population of children from a poor socio-economic background because a high prevalence of nutritional deficiencies was expected (chapter 4, 5 and 6). However, the prevalence of anemia appeared to be 9% only, which was similar to that found in other studies in deprived children in Bangalore and its surroundings conducted at the same time (1). Despite inadequate intakes of micronutrients in ~85% of the children, only 20-30% of the children in our study suffered from other iron, folate or vitamin $B_{12}$ deficiencies. The lower prevalence of micronutrient deficiencies may be attributed to introduction of sanitation programs in slums resulting in reduced morbidity and

prevalence of worm infestations. In addition, vitamin A supplementation twice per year (a program which was stopped during our intervention) and use of iodized salt in subsidized school meals or at home may have contributed to decreasing the prevalence of micronutrient deficiencies. Consequently, our intervention may have been less efficacious in improving cognitive performance of the children compared to a population of children who all have micronutrient deficiencies. However, our findings did not alter when we adjusted our analyses for initial nutritional status. In contrast, in cross-sectional analyses (chapter 4), we showed that baseline body size indicators and hemoglobin concentrations were positively associated with cognitive performance across all domains, indicating that nutritional status may influence intelligence in children.

**Interpretation of findings**

We postulated that children in developing countries who often have a higher risk of micronutrient deficiencies would benefit more from micronutrient supplementation than their peers in developed countries. Contrary to our expectations, the overall effect size of multiple micronutrient supplementation on fluid intelligence among the studies conducted in developed countries using micronutrient tablets was significantly larger compared to studies in developing countries using fortified foods (chapter 2). As indicated above, it might be that cognitive test batteries used in developing countries were less sensitive due to problems with validity. Other explanations may be that the diet of children living in developing countries is usually inadequate in energy and protein, which is reflected in high prevalence of stunting and wasting. Possibly, the beneficial effect of micronutrients may become more apparent when energy and protein intakes are adequate, which is usually the case in children in developed countries. This has also been observed in a trial conducted in Australia and Indonesia, which showed that a micronutrient fortified beverage improved learning and memory in Australian children, while these effects were only seen in Indonesian girls (23). Thus, the extra energy provided by the fortified foods used in studies in malnourished children in developing countries may overrule the effect of micronutrients on cognitive performance.

Whereas we did not find evidence for differences in effects of multiple micronutrient interventions on the cognitive outcomes with a poorer micronutrient status at baseline (chapter 5), some studies have found that nutritional status at baseline did modify the effect of the intervention. In a study in schoolchildren in the Philippines, effects of a multiple micronutrient beverage on non-verbal abilities were significant in iron and iodine deficient children, while overall no effects were found (24). In addition, the effect seemed larger in children with more severe deficiencies. Similarly, a study in South African schoolchildren showed a larger effect of micronutrient fortified biscuits on cognitive function in children who had a poorer iron status or goiter at baseline (20). Also, a meta-analysis

on iron supplementation on mental development in children demonstrated that supplementation was more effective in iron deficient and anemic children (7). Taken together, these studies indicate the iron and iodine status at baseline influences the outcomes of micronutrient interventions on intellectual performance.

### Conclusion

Although evidence is limited, children who suffer from iron and iodine deficiency in particular may gain more from multiple micronutrient interventions compared to children with adequate iron and iodine status. Therefore, we keep our assumption that children in developing countries would experience greater cognitive benefits of multiple micronutrient interventions than children in developed countries; although this was not confirmed in our meta-analysis (chapter 2). In order to increase the chances of detecting effects of micronutrient interventions on cognition in developing countries, trials should preferably use designs enabling them to disentangle effects of energy versus effects from micronutrients.

## Composition of multiple micronutrient supplements

### Methodological issues

The term 'multiple micronutrients' was defined as ≥3 vitamins and minerals and this definition has been used in other meta-analyses (25;26). This definition is broad and covered a wide variation of micronutrient interventions used in different studies that were included in the meta-analysis (chapter 2). Unfortunately, the number of studies in the meta-analysis was too small for adequate investigation on type and dose of vitamins and minerals in relation to cognitive performance. However, in most studies, iron and iodine were included in the micronutrient mix and more than 10 different micronutrients were provided.

In the intervention study, a high dose (100% of the RDA) of micronutrients was compared to a low dose (15% of the RDA) (chapter 5). A limitation of the study design was the lack of a group not receiving any micronutrients and/or a group receiving a dose in between 15 and 100% of the RDA, in order to investigate a dose response effect on cognition. The low dose of micronutrients of 15% of the RDA increased the intake of micronutrients considerably (with 25-50%) compared to the habitual intakes of vitamins and minerals. Furthermore, the fact that all children received substantial amounts of energy and protein of 40-50% in addition to their habitual diet may have blurred the effects of the micronutrients.

### Interpretation of findings

Data on effects of different doses of multiple micronutrients on cognition are limited to one trial conducted in US children aged 12-16 years. In this study, no significant differences were found among doses of 50, 100 and 200% of the RDA

(27). These findings are in line with those of our intervention study (chapter 5).

It has been questioned whether additional micronutrient supplementation in well-nourished populations, with micronutrient status within the normal range, would be potentially harmful (28). Data at the end of our intervention study showed that none of the children in the high micronutrient group had concentrations of hemoglobin or body iron stores above the higher cut-off levels (chapter 5); indicating that iron stores were filled and plateau levels were reached. Furthermore, the fact that extra micronutrients were beneficial for fluid intelligence in children in developed countries (chapter 2), suggests that even well-nourished children may have a suboptimal micronutrient status with regard to cognitive function. However, more research is required to assess the optimal micronutrient status for cognitive performance. In addition, dose-response studies should be conducted to identify a cost-effective and safe dose of micronutrients for optimizing children's intellectual development.

Since iron and iodine have the strongest link to mental performance in schoolchildren (29), it is clear that ensuring adequate intake of these micronutrients is a pre-requisite for optimizing cognitive performance. Moreover, most of the studies in the meta-analysis included folate, vitamin $B_{12}$ and zinc in addition to iron and iodine in the multiple micronutrient intervention, suggesting that these micronutrients are considered to be important for cognitive development (chapter 2). Furthermore, results from the meta-analysis indicated that a higher number of micronutrients yielded larger effects on fluid intelligence (chapter 2). Therefore, also other vitamins and minerals that are not directly related to cognitive function may be required to optimize cognitive performance. Since investigation of all possible combinations of micronutrients is inefficient, selection of the type of micronutrients to be included in future studies may be based on the prevalence of individual micronutrient deficiencies and/or the role of vitamin or mineral in brain function.

**Conclusion**

Our study and one other trial (27) suggest that lower doses of micronutrients (<50% of the RDA) are as effective as higher doses (≥100% of the RDA) for improving cognitive function. Our meta-analysis indicates that a higher number of micronutrients would be more effective, and because of the strong evidence with cognition in children, iron and iodine should be included in the supplements. However, the number of studies is currently too limited to provide exact guidance on dose and composition of micronutrients that would be required for optimizing cognitive development in children and therefore more studies are needed.

## Reflection on findings of omega-3 fatty acids

### Methodological issues

Since no effects of omega-3 fatty acids were found on cognitive performance in children, we may question whether our intervention study in Indian children was adequately designed. The children had low intakes of ALA and very low intakes of DHA, because of limited presence of meat and eggs and absence of fish in the habitual diet. This was reflected in a study population with less variation in erythrocyte fatty acid concentrations and dietary intake of omega-3 fatty acids, which may have been too homogenous to investigate associations with cognitive function (chapter 4 and 6). In contrast, for the intervention study (chapter 5), the poor omega-3 fatty acid intake should have maximized the likelihood of finding effects of additional omega-3 fatty acid supplementation on cognitive performance. However, the amounts of ALA and DHA in the high dose may have been too low to improve cognitive test scores in spite of a significant increase in erythrocyte DHA concentrations.

### Interpretation of findings

#### *Evidence for role of omega-3 fatty acids in schoolchildren*

Overall, a limited number of studies have investigated the role of omega-3 fatty acids on cognition in healthy children. The findings of the intervention study (chapter 5) are in agreement with two other studies in children in which no effects of doses 100-1000 mg DHA were found on tests of intelligence (23;30). Limitations of these and our studies was that either the dose may have been too low (23) or duration may have been too short (30) to detect effects. However, also for other age groups, evidence for benefits of DHA on cognitive function is inconclusive (infants) or not existing (preschoolers and elderly) (31-34).

Another explanation for not finding effects of omega-3 fatty acids on cognitive outcomes may be that the amount of ALA in the diet is sufficient and that the conversion of ALA to DHA is adequate to supply the brain. In fact, the median ALA intake in our study population (190 mg ALA/d) corresponds with the recommendation of a minimum of 0.2% of total energy for ALA required to prevent deficiency set by the British Nutrition Foundation (35). Moreover, animal studies have shown that synthesis of DHA in the liver is enhanced and the turnover of DHA in the brain is reduced when diets were low in $\alpha$-linolenic acid and free of DHA (36). This explanation is supported by the absence of a positive association between erythrocyte or plasma fatty acid status and cognitive performance in children as observed in the current (chapter 4) and other studies in schoolchildren (37).

#### *Biomarkers of omega-3 fatty acid status in relation to cognitive function*

As no significant associations were found between plasma or erythrocyte DHA status and cognitive function, it may be questioned whether these biomarkers

are representative for brain DHA status. A recent study in adults postulated that approximately 3.8 mg of DHA is daily transported from the unesterified DHA plasma pool into the brain (38). Since the amount of DHA consumption by the brain seems relatively small and the observations that DHA turnover is decreased when dietary intake of omega-3 fatty acids is low (36), it may be speculated that erythrocyte and plasma fatty acids status may not be suitable indicators for assessments of DHA sufficiency in the brain.

Another gap in knowledge concerns the question to what extent brain DHA status is related to cognitive function in humans. Therefore, studies involving positron emission tomography (PET) to image the incorporation of DHA in the brain (38), may be helpful to further study the role of omega-3 fatty acids in different brain regions and related cognitive functioning.

***Effects of omega-3 fatty acids versus fish consumption on cognition***

Although there is limited evidence for effects of DHA supplementation on cognitive development (chapter 3), a number of observational studies indicate a significant positive association of fish consumption during pregnancy and cognitive development of infants (39-41). These cross-sectional findings do not proof causality between fish consumption and cognition, and may be due to other differences in dietary habits and lifestyle. However, a randomized controlled trial has demonstrated significant beneficial effects of fish flour, rich in EPA and DHA, versus a bread flour on verbal learning of South-African children aged 6-9 year old (42). The positive effects on cognition found following fish consumption but not with EPA and DHA supplementation, may be attributed to other components in fish that may benefit mental development of children, such as iodine (43) or phospholipids (44). These nutrients may act synergistically with the fish fatty acids on improving cognitive performance.

***Interactions of omega-3 fatty acids and multiple micronutrients***

No significant interactions between the micronutrient and omega-3 fatty acid treatments were found on neither cognitive nor biochemical outcomes of the children (chapter 5), which is in line with findings of another study in children of similar age (23). Also, no interactions of PUFA and micronutrient supplementation on behavioral problems in children with attention-deficit hyperactivity disorders (45). Since evidence for positive effects of omega-3 fatty acids on cognitive development of children is currently lacking, it is not surprising that the addition of DHA to micronutrients has also no effect. Furthermore, interactions may be expected only from interventions with ALA and not DHA, since micronutrients may enhance the conversion of ALA to DHA (46). In addition, other factors influencing the conversion may be more important such as single nucleotide polymorphisms in the gene encoding δ6-desaturase (*FADS2*) or its regulatory elements, which

result in different conversion rates of ALA to EPA and DHA (47;48). Subjects with the genetic variations resulting in lower conversion rates may benefit from extra dietary EPA and DHA intake and subsequently improve their cognitive performance.

### Conclusion

The research in this thesis added to a number of studies showing no evidence for an effect of additional dietary intake of omega-3 fatty acids on cognitive performance in healthy children; or in populations with low intakes of ALA and DHA, such as in the Indian schoolchildren. Since the current studies were either too short of duration or too low in doses of DHA, a trial using a high dose (>200 mg DHA per day) and sufficiently long duration (>4 months) is needed to conclude whether DHA supplementation would benefit cognition at school age. Lastly, it may be of interest to conduct studies in populations with variation in δ6-desaturase encoding genes to conclude whether additional EPA and DHA intake in specific subgroups with low conversion rates of ALA to DHA will benefit cognitive function.

## Implications for public health

### Current public health strategies to optimize children's cognitive development

Based on strong scientific evidence of the detrimental effects of iodine and iron deficiency on brain development and cognitive function, public health authorities of the United Nations declared in the 1990s to make all efforts to eliminate these deficiencies globally. To combat iodine deficiency, programs to promote consumption of iodized salt and monitor iodine status have been implemented world wide (49). To eradicate iron deficiency, multiple strategies to improve iron intake including dietary improvement, food fortification and supplementation have been introduced (50). Although schoolchildren also benefit from these programs, they are not specifically targeted to them.

With regard to omega-3 fatty acids and possible benefits on cognition there are no specific public health programs to improve the intakes of ALA and DHA. Official international recommendations for children on intake of fatty acids are currently lacking. However, consumption of fish as part of a healthy diet is also considered important for children (51).

Public health nutrition programs, targeted at schoolchildren, focus mostly on improving general health and well-being of children, thereby indirectly improving cognitive development (52). For instance, school feeding programs have been introduced in children from deprived backgrounds to improve energy and micronutrient intakes by providing a breakfast, mid-morning snack or lunch at school, thereby also increasing school attendance rates (53). Our findings of the dramatically low intakes of energy, essential fatty acids and micronutrients in Indian children (chapter 6) support the need for such programs.

**Implications for public health resulting from research described in this thesis**

In this section, we will focus on the consequences of our research for public health on the basis of the three main research questions that were addressed in this thesis.

With regard to the first research question, we showed that there is suggestive evidence for a beneficial effect of multiple micronutrient supplementation on fluid intelligence in schoolchildren. Since evidence is in particular strong for children living in developed countries, we can not exclude the possibility that the intake of micronutrients in apparently well-nourished children could be improved for optimal cognitive development. However, at present it is too early to recommend massive public health campaigns to increase micronutrient intake in children to improve their intellectual performance. Therefore, further research will be needed to conclude whether a higher micronutrient status above the current cut-off values for deficiencies would be related to better cognitive development of children; thereby including measurements of cognitive performance in addition to regular functional parameters of growth and health.

For malnourished children in developing countries, such as the Indian children studied in this thesis, it is currently unclear whether multiple micronutrients would improve their cognitive development. This may be attributed to an overall lack of energy and protein in the habitual diet and other factors associated with poverty. Therefore, our findings underline the need for current public health initiatives to increase the intakes of energy and essential nutrients to improve general health and development of children living in deprived environments.

The answers to our second research question indicate that there is no evidence to substantiate recommendations aiming to enhance intellectual development by increasing intakes of omega-3 fatty acids, and of DHA in particular. In developing countries, where diets tend to be low in ALA and absent of DHA, no benefits of additional DHA intakes were found on cognitive performance. However, higher PUFA intakes may be associated with better cognitive performance, suggesting that public health authorities may consider strategies to improve the intake of omega-3 and omega-6 fatty acids. In addition, for prevention of chronic disease (54;55), and possibly cognitive functioning (56) in later life, children may benefit from higher intakes of the long chain omega-3 fatty acids. An evaluation of the current evidence on this topic is needed to develop recommendations on fatty acid intake for children’s future health.

For the third research question, we attempted to elucidate on doses of

micronutrients needed to improve cognitive performance in children. Our study showed that lower doses may be as adequate as higher doses (chapter 5) and therefore policy-makers may consider the use of lower doses in public health programs for reasons of safety and cost-effectiveness. However, further research is required to confirm our observations in other countries, and to investigate the effect of different doses on other functional outcomes, such as growth. In addition, besides the importance iron and iodine for cognitive development in children, more guidance is needed on the composition of multiple micronutrient supplements for use in public health strategies aimed at improving cognition.

## Recommendations for future research

As indicated above, a number of gaps in knowledge on the role of micronutrients, omega-3 fatty acids and cognitive performance in children are remaining and need to be addressed in future studies.

With regard to effect of multiple micronutrients on cognitive performance in schoolchildren, further research is needed to determine the impact on cognitive domains other than fluid intelligence, and to identify a cost-effective micronutrient supplement to optimize cognitive performance in children. Given the limited number of studies conducted in developing countries, more research should be conducted in malnourished populations to determine whether effects of micronutrients on cognition depend on nutritional status.

More specifically, future trials are recommended to investigate:

- The effects of multiple micronutrients on different cognitive domains, e.g. fluid intelligence, short-term memory, visual perception, retrieval ability, cognitive processing speed, sustained attention and academic performance.
- Different doses of micronutrients, e.g. 0, 30, 50 and 100% of the RDA.
- The effect of single (i.e. iron or iodine) versus multiple micronutrients.
- The influence of baseline micronutrient status on the effect of micronutrients on cognitive outcomes.
- The effect of energy and protein versus multiple micronutrients on cognition in malnourished children.

To end the current debate whether DHA may improve cognitive performance in children of school age, a trial using a high dose of DHA (>200 mg/d) and adequate duration (>4 months) will be required. In addition, future research may clarify whether populations with lower conversion rates of ALA to DHA due to genetic variation may benefit from additional dietary DHA with regard to cognitive function.

Finally, methods to assess cognitive performance in children need to be sensitive to detect small effects of nutritional interventions. Therefore, it is important to use well-adapted valid cognitive tests with adequate psychometric properties. Besides tests assessing different cognitive domains, more advanced measures such as

structural and functional brain imaging techniques may be added to investigate the effects of nutrition in the brain. Combining brain imaging techniques with assessments of cognitive performance in future trials is recommended to more precisely understand the role of nutrients in the brain and relate this to functional outcomes.

## References


1. Muthayya S, Thankachan P, Zimmermann MB et al. Low anemia prevalence in school-aged children in Bangalore, South India: possible effect of school health initiatives. Eur J Clin Nutr 2007;61:865-9.
2. Thompson RA, Nelson CA. Developmental science and the media. Early brain development. Am Psychol 2001;56:5-15.
3. Flanagan DP, Harrison PL. Contemporary Intellectual Assessment, Theories, Tests and Issues. New York: The Guilford Press, 2005.
4. Faber M, Kvalsvig JD, Lombard CJ, Benade AJS. Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. Am J Clin Nutr 2005;82:1032-9.
5. Olney DK, Pollitt E, Kariger PK et al. Combined iron and folic acid supplementation with or without zinc reduces time to walking unassisted among Zanzibari infants 5- to 11-mo old. J Nutr 2006;136:2427-34.
6. Dhingra P, Menon VP, Sazawal S et al. Effect of fortification of milk with zinc and iron along with vitamins C, E, A and selenium on growth, iron status and development in preschool children - A community based double-masked randomized trial. Pediatr Gastroenterol 2004;53-7.
7. Sachdev HPS, Gera T, Nestel P. Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. Public Health Nutr 2005;8:117-32.
8. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 2001;131:649S-66S.
9. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B. Child development in developing countries 1 - Developmental potential in the first 5 years for children in developing countries. Lancet 2007;369:60-70.
10. Engle PL, Black MM, Behrman JR et al. Child development in developing countries 3 - Strategies to avoid the loss of developmental potential in more than 200 million children in the developing world. Lancet 2007;369:229-42.
11. Isaacs E, Oates J. Nutrition and cognition: assessing cognitive abilities in children and young people. Eur J Nutr 2008;47 Suppl 3:4-24.
12. van de Vijver F. Inductive reasoning in Zambia, Turkey, and the Netherlands: Establishing cross-cultural equivalence. Intelligence 2002;30:313-51.
13. Irvine SH. Factor analysis of African abilities and attainments: Constructs across cultures. Psychological Bulletin 1969;71:20-32.
14. Irvine SH. The place of factor analysis in cross-cultural methodology and its contribution to cognitive theory. In: Eckensberger LH, Lonner WJ, Poortinga YH, eds. Cross-cultural contributions to psychology: selected papers from the fourth International Congress of the International Association for Cross-Cultural Psychology, held at Munich, Federal Republic of Germany, July 28-August 5, 1978. Lisse: Swets & Zeitlinger 1979:300-41.
15. Kaufman AS, Kaufman LN. Kaufman Assessment Battery for Children, Second Edition: Manual. Circle Pines, MN: AGS Publishing, 2004.
16. van de Vijver FJR, Leung K. Personality in cultural context: methodological issues. J Pers 2001;69:1007-31.
17. van de Vijver F, Tanzer NK. Bias and equivalence in cross-cultural assessment: An overview. European Review of Applied Psychology-Revue Europeenne de Psychologie Appliquee 1997;47:263-80.
18. Wainwright PE, Colombo J. Nutrition and the development of cognitive functions: interpretation of behavioral studies in animals and human infants. Am J Clin Nutr 2006;84:961-70.
19. Walker SP, Chang SM, Powell CA, Grantham-McGregor SM. Effects of early childhood psychosocial stimulation and nutritional supplementation on cognition and education in growth-stunted Jamaican children: prospective cohort study. Lancet 2005;366:1804-7.
20. van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benade AJS. Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary school children: a randomized controlled trial. (vol 69, pg 497, 1999). Am J Clin Nutr 1999;69:1294.
21. Seshadri S, Gopaldas T. Impact of Iron Supplementation on Cognitive Functions in Preschool and School-Aged Children - the Indian Experience. Am J Clin Nutr 1989;50:675-86.
22. Benton D, Roberts G. Effect of vitamin and mineral supplementation on intelligence of a sample of schoolchildren. Lancet 1988;1:140-3.
23. Osendarp SJ, Baghurst KI, Bryan J et al. Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia. Am J Clin Nutr 2007;86:1082-93.
24. Solon FS, Sarol JN, Bernardo ABI et al. Effect of a multiple-micronutrients-fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of schoolchildren in the Philippines. Food Nutr Bull 2003;24:S129-S140.
25. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. Cochrane

Database Syst Rev 2006;CD004905.
26. Allen LH, Peerson JM, Olney DK. Provision of multiple rather than two or fewer micronutrients more effectively improves growth and other outcomes in micronutrient-deficient children and adults. J Nutr 2009;139:1022-30.
27. Schoenthaler SJ, Amos SP, Eysenck HJ, Peritz E, Yudkin J. Controlled trial of vitamin-mineral supplementation: effects on intelligence and performance. Personal Individ Diff 1991;12:351-62.
28. Grantham-McGregor SM, Ani CC. The role of micronutrients in psychomotor and cognitive development. Br Med Bull 1999;55:511-27.
29. Bryan J, Osendarp S, Hughes D, Calvaresi E, Baghurst K, van Klinken JW. Nutrients for cognitive development in school-aged children. Nutr Rev 2004;62:295-306.
30. Kennedy DO, Jackson PA, Elliott JM et al. Cognitive and mood effects of 8 weeks' supplementation with 400 mg or 1000 mg of the omega-3 essential fatty acid docosahexaenoic acid (DHA) in healthy children aged 10-12 years. Nutr Neurosci 2009;12:48-56.
31. Simmer K, Schulzke SM, Patole S. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev 2008;CD000375.
32. Simmer K, Patole SK, Rao SC. Longchain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database Syst Rev 2008;CD000376.
33. Ryan AS, Nelson EB. Assessing the effect of docosahexaenoic acid on cognitive functions in healthy, preschool children: a randomized, placebo-controlled, double-blind study. Clin Pediatr (Phila) 2008;47:355-62.
34. van de RO, Geleijnse JM, Kok FJ et al. Effect of fish oil on cognitive performance in older subjects: a randomized, controlled trial. Neurology 2008;71:430-8.
35. Department of Health. Dietary reference values for food energy and nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO, 1991.
36. Rapoport SI, Rao JS, Igarashi M. Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. Prostaglandins Leukot Essent Fatty Acids 2007;77:251-61.
37. Bakker EC, Ghys AJA, Kester ADM et al. Long-chain polyunsaturated fatty acids at birth and cognitive function at 7y of age. Eur J Clin Nutr 2003;57:89-95.
38. Umhau JC, Zhou W, Carson RE et al. Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. J Lipid Res 2009;50:1259-68.
39. Daniels JL, Longnecker MP, Rowland AS, Golding J. Fish intake during pregnancy and early cognitive development of offspring. Epidemiol 2004;15:394-402.
40. Oken E, Wright RO, Kleinman KP et al. Maternal fish consumption, hair mercury, and infant cognition in a U.S. Cohort. Environ Health Perspect 2005;113:1376-80.
41. Hibbeln JR, Davis JM, Steer C et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 2007;369:578-85.
42. Dalton A, Wolmarans P, Witthuhn RC, van Stuijvenberg ME, Swanevelder SA, Smuts CM. A randomised control trial in schoolchildren showed improvement in cognitive function after consuming a bread spread, containing fish flour from a marine source. Prostaglandins Leukot Essent Fatty Acids 2009;80:143-9.
43. Bleichrodt N, Born MP. A meta-analysis of research on iodine and its relationship to cognitive development. In: Stanbury JB, ed. The damaged brain of iodine deficiency. New York, NY: Cognizant Communication Corporation 1994:195-200.
44. Vaisman N, Kaysar N, Zaruk-Adasha Y et al. Correlation between changes in blood fatty acid composition and visual sustained attention performance in children with inattention: effect of dietary n-3 fatty acids containing phospholipids. Am J Clin Nutr 2008;87:1170-80.
45. Sinn N, Bryan J. Effect of supplementation with polyunsaturated fatty acids and micronutrients on learning and behavior problems associated with child ADHD. J Dev Behav Pediatr 2007;28:82-91.
46. Smit EN, Muskiet FA, Boersma ER. The possible role of essential fatty acids in the pathophysiology of malnutrition: a review. Prostaglandins Leukot Essent Fatty Acids 2004;71:241-50.
47. Schaeffer L, Gohlke H, Muller M et al. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Hum Mol Genet 2006;15:1745-56.
48. Rzehak P, Heinrich J, Klopp N et al. Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 ( FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. Br J Nutr 2009;101:20-6.
49. World Health Organization, United Nations Children's Fund, International Council for Control of Iodine Deficiency Disorders. Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. Geneva: World Health Organization, 2001.
50. World Health Organization, United Nations Children's Fund, United Nations University. Iron deficiency anemia. Assessment, prevention and control. A guide for programme managers. Geneva: World Health Organization, 2001.
51. Kris-Etherton PM, Innis S, American DA, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc 2007;107:1599-611.
52. United Nations System Standing Committee on Nutrition. School-age children their health and nutrition. SCN News. A periodic review of developments in international nutrition. Number 25. Geneva: SCN Secretariat, 2002.
53. World Food Program. School meals. http://www.wfp.org/school-meals. Accessed 15-7-2009.
54. Rasanen M, Lehtinen JC, Niinikoski H et al. Dietary patterns and nutrient intakes of 7-year-old children taking part in an atherosclerosis prevention project in Finland. J Am Diet Assoc 2002;102:518-24.

55. Stein AD, Thompson AM, Waters A. Childhood growth and chronic disease: evidence from countries undergoing the nutrition transition. Matern Child Nutr 2005;1:177-84.
56. Whalley LJ, Fox HC, Wahle KW, Starr JM, Deary IJ. Cognitive aging, childhood intelligence, and the use of food supplements: possible involvement of n-3 fatty acids. Am J Clin Nutr 2004;80:1650-7.

# Summary

In spite of decades of efforts by public health organizations to eliminate the problem of undernutrition, millions of children are not reaching their full developmental potential because of nutritional deficiencies. Poverty and monotonous diets are underlying causes of inadequate intakes of micronutrients and omega-3 fatty acids, which may have severe consequences for intellectual development of children. Whereas single micronutrient interventions with iron and iodine have been proven to enhance cognitive performance, there are indications from a relatively large number of studies that multiple micronutrient interventions improve mental development of children, but a systematic approach to evaluate the evidence is currently lacking. Omega-3 fatty acids are important for normal development of children. While some studies have shown that supplementation with docosahexaenoic acid (DHA) may benefit mental development of infants, it is presently unclear whether additional intake of omega-3 fatty acids improves cognitive performance in children of school age.

This thesis aims to investigate the role of multiple micronutrients and omega-3 fatty acids on cognitive performance in school-age children living in deprived environments (**chapter 1**), thereby addressing three main research questions:

1. How large is the impact of multiple micronutrient interventions on different cognitive domains in children?
2. What is the role of omega-3 fatty acids on children's cognitive development?
3. What is the effect of an intervention with different doses of multiple micronutrients and omega-3 fatty acids on cognitive performance in schoolchildren in India? Is there an interaction between multiple micronutrients and omega-3 fatty acids?

The current literature on the effect of multiple micronutrients on different cognitive domains in schoolchildren was systematically reviewed in **chapter 2**. Randomized controlled trials investigating the effect of ≥3 micronutrients versus placebo on cognition in healthy children aged 0-18 years were included. The cognitive tests used in the trials were grouped into several cognitive domains, including fluid intelligence (i.e. reasoning abilities), crystallized intelligence (i.e. acquired knowledge), short-term memory, visual perception, retrieval ability, cognitive processing speed, attention and academic performance. Pooled random effect sizes were calculated per domain. Seventeen trials were retrieved in children aged 5-16 years. Findings indicated a small positive effect of multiple micronutrient supplementation on fluid intelligence of 0.14 SD (95% CI: -0.02, 0.29; *P*=0.083), while crystallize intelligence seemed unaffected with a pooled effect of -0.03 SD (95% CI: -0.21, 0.15; *P*=0.74). Limited data from four trials yielded a significant positive effect on academic performance (0.30 SD; 95% CI: 0.01, 0.58; *P*=0.044). For the other domains, there were too few studies to draw a clear conclusion.

Investigation of heterogeneity indicated that the positive effects on fluid intelligence were more pronounced in the studies conducted in developed countries, in which micronutrient tablets (instead of fortified foods) were provided. Furthermore, effects were larger in studies with a higher number of different micronutrients.

**Chapter 3** comprises an evaluation of the most recent randomized controlled trials assessing the efficacy of the omega-3 long chain polyunsaturated fatty acid (LCPUFA) supplementation (i.e. eicosapentaenoic acid (EPA) and DHA) during pregnancy, lactation, infancy and childhood on cognitive development. Available evidence suggested a beneficial effect of maternal omega-3 LCPUFA supplementation during pregnancy and lactation on mental development of infants and children, while evidence for an effect supplementation during infancy was inconclusive. For healthy children older than two years of age, data were too limited to conclude whether supplementation with EPA and DHA would benefit cognitive performance. After the review was performed, two additional trials in schoolchildren and one study in preschoolchildren have not showed effects of DHA supplementation on cognitive outcomes.

A randomized controlled nutrition intervention study was conducted to investigate the effect of two different concentrations of a combination of micronutrients and omega-3 fatty acids on indicators of growth and cognitive performance in 598 marginally nourished Indian schoolchildren aged 6-10 years. The Kaufman Assessment Battery for Children II was used to measure four cognitive domains, including fluid reasoning, short-term memory, retrieval ability and cognitive speediness. Scores were combined into an overall measure, named Mental Processing Index (MPI). Baseline data of this trial were used to explore associations between indicators of body size, fatty acid and micronutrient status on cognitive performance using analyses of variance adjusting for age, gender, school, maternal education and cognitive tester (**chapter 4**). Results showed that body size indicators and hemoglobin concentrations were significantly positively related to all cognitive domains and MPI, while vitamin $B_{12}$ concentrations were significantly inversely associated with short-term memory, retrieval ability and MPI. Other indicators of iron, iodine, folate and fatty acid status were not significantly related to cognition. The findings for body size, fatty acids and micronutrients were in agreement with previous observational studies, but the inverse association of vitamin $B_{12}$ was unexpected and needs further study.

For the intervention study described in chapter 5, children were randomly allocated to one of four intervention groups to receive foods fortified with either 100% or 15% of the Recommended Dietary Allowance (RDA) of micronutrients in combination with either 900 mg $\alpha$-linolenic acid (ALA) plus 100 mg DHA or 140 mg ALA for 12 months duration. Children receiving the high micronutrient treatment

(100% of the RDA) significantly improved on linear growth at 12 months (0.19 cm; 95% CI: 0.01, 0.36) and short-term memory at 6 months (0.11 SD; 95% CI: 0.01, 0.20), while they performed less well on fluid reasoning at 6 (-0.10 SD; 95% CI: -0.17, -0.03) and 12 (-0.12 SD; 95% CI: -0.20, -0.04) months compared to children receiving the low micronutrient treatment (15% of the RDA); while there were no significant differences for weight and other cognitive parameters. Neither significant differences between the n-3 treatments nor an interaction between omega-3 fatty acids and micronutrients were found on cognitive outcomes. It was concluded that with some small differential effects, higher micronutrient doses were as effective as lower doses for improving cognitive performance.

In **chapter 6**, baseline data of the trial in Indian schoolchildren were used to investigate relationships between energy and nutrient intake, anthropometry and cognitive performance using analyses of variance, adjusting for confounding factors. The diets of the children were monotonous and dramatically low in energy, fat, PUFA and micronutrients. Energy intake was positively related to height, weight, mid-upper arm circumference and cognitive performance. After correction for energy, PUFA intake was positively associated with cognitive performance, while no such relationships could be detected for protein, ALA and micronutrient intake. The findings indicate the need for public health programs aiming to increase dietary intake of energy and nutrients through dietary diversification, food fortification or school feeding.

In conclusion, this thesis showed that multiple micronutrient supplementation may be associated with a small increase in the cognitive domain of fluid intelligence in children, and possibly with academic performance. However, it is currently too early to recommend massive public health campaigns to increase micronutrient intake in children to improve their intellectual performance (**chapter 7**). Since effects were particularly strong for children in developed countries, research is required to assess whether a higher micronutrient status, above the current cut-off values for deficiencies, is related to better cognitive development.

For children in developing countries, public health programs are needed to improve the intake of energy and essential nutrients for general health and well-being, which will indirectly improve cognitive performance. Our findings of diets dramatically low in energy, fatty acids and micronutrients in Indian children strongly support the need for such programs.

Based on our results that lower doses of micronutrients were as effective doses, policy-makers may consider the use of lower doses in public health programs. However, more research is required to confirm our observations. In addition, guidance is needed on the composition of multiple micronutrient supplements.

Regarding the role of omega-3 fatty acids on mental development in children, findings of the research described in this thesis add to those of existing studies

that additional intake of omega-3 fatty acids, and of DHA in particular, does not improve cognitive function. Therefore, there is currently no evidence to substantiate recommendations for enhancing intellectual development in school-age children through increasing intakes of omega-3 fatty acids.

Further research is required 1) to determine the impact of multiple micronutrients on cognitive domains other than fluid intelligence; 2) to identify the optimal composition of a multiple micronutrient supplement; and 3) to assess the impact of nutritional status on cognitive outcomes of micronutrient interventions. To end the current debate whether DHA may improve cognitive performance in children of school age, a trial using an adequately high dose of DHA and sufficiently long duration will be needed. Finally, combining cognitive tests assessing different cognitive domains with more advanced measures such as structural and functional brain imaging techniques is recommended for a better understanding of the role of nutrients in the brain and related cognitive function.

# Samenvatting

In de afgelopen decennia hebben publieke gezondheidsorganisaties getracht het probleem van ondervoeding op te lossen. Toch zijn er nog steeds miljoenen kinderen in de wereld die lijden aan ondervoeding. Door armoede en eenzijdige voeding hebben deze kinderen een te lage inname van micronutriënten en omega-3 vetzuren, dat ernstige gevolgen kan hebben voor hun intellectuele ontwikkeling

Interventiestudies met één enkel micronutriënt, zoals bijvoorbeeld ijzer of jodium, hebben overtuigend aangetoond dat het cognitief functioneren van kinderen kan worden verbeterd. Tevens zijn er aanwijzingen dat suppletie met meerdere micronutriënten de mentale ontwikkeling van kinderen ook kan bevorderen. Echter, tot op heden ontbreekt een systematische evaluatie van deze onderzoeken.

Omega-3 vetzuren zijn belangrijk voor normale groei en ontwikkeling van kinderen. Sommige studies hebben aangetoond dat suppletie met het omega-3 vetzuur docosahexeenzuur (DHA) de mentale ontwikkeling van zeer jonge kinderen kan bevorderen, maar het is onduidelijk of extra inname van omega-3 vetzuren ook bij schoolkinderen het cognitief functioneren kan verbeteren.

In dit proefschrift wordt de rol van multivitaminen en mineralen en omega-3 vetzuren op het cognitief functioneren van in armoede opgroeiende schoolkinderen onderzocht (**hoofdstuk 1**). Hierbij zullen drie hoofdvragen beantwoord worden:

1. Hoe groot is het effect van multivitaminen en mineralen op de verschillende cognitieve domeinen bij kinderen?
2. Wat is de rol van omega-3 vetzuren op de cognitieve ontwikkeling van kinderen?
3. Wat is het effect van een interventiestudie met verschillende doseringen multivitaminen en mineralen en omega-3 vetzuren op het cognitief functioneren van schoolkinderen in India? Is er een interactie tussen multivitaminen en mineralen en omega-3 vetzuren?

De huidige literatuur over het effect van interventies met multivitaminen en mineralen op de verschillende cognitieve domeinen in kinderen is systematisch geëvalueerd in **hoofdstuk 2**. Hierbij werden alleen gerandomiseerde, gecontroleerde interventie studies bekeken die het effect van drie of meer micronutriënten versus een placebo op cognitie in gezonde kinderen van 0-18 jaar onderzochten. De cognitieve testen die gebruikt werden in deze studies werden gegroepeerd in verschillende cognitieve domeinen, waaronder logisch redeneren, verworven kennis, het korte termijn geheugen, visuele perceptie, het lange termijn geheugen, cognitieve snelheid, concentratie en schoolprestaties. Samengestelde random effecten werden per cognitief domein berekend. In totaal werden 17 studies gevonden bij kinderen in de leeftijd van 5-16 jaar. Er werd een klein positief effect gevonden op domein "logisch redeneren" van 0.14 standaarddeviatie (SD) (95% betrouwbaarheidsinterval (btbh-i): -0.02. 0.29; *P*=0.083), terwijl er geen effect was op het domein "verworven kennis" -0.03 SD (95% btbh-i: -0.21. 0.15; *P*=0.74). Vier studies vonden een significant positief effect van multivitaminen en mineralen

op schoolprestaties (0.30 SD; 95% btbh-i: 0.01, 0.58; *P*=0.044). Er waren echter te weinig studies beschikbaar om een goede conclusie te trekken voor de andere cognitieve domeinen. Onderzoek naar de heterogeniteit tussen de verschillende studies liet zien dat de positieve effecten op het domein "logisch redeneren" meer naar voren kwamen in de studies die uitgevoerd werden in westerse landen, waarin de micronutriënten in de vorm van supplementen in plaats van verrijkte voedingsmiddelen werden gegeven. Daarnaast waren de effecten groter in studies waarin meerdere verschillende micronutriënten werden verstrekt.

**Hoofdstuk 3** omvat een evaluatie van de meest recente gerandomiseerde, gecontroleerde interventie studies naar de effectiviteit van suppletie met langketige omega-3 vetzuren (namelijk eicosapenteenzuur (EPA) en DHA) gedurende de zwangerschap, lactatie, het eerste levensjaar en kindertijd. Tevens werden effecten op de cognitieve ontwikkeling van het kind geëvalueerd. De beschikbare literatuur suggereerde dat suppletie met langketige omega-3 vetzuren bij de moeder de mentale ontwikkeling van haar kind zou kunnen bevorderen, terwijl het effect van suppletie gedurende het eerste levensjaar nog onduidelijk is. Wegens onvoldoende studies in kinderen ouder dan 2 jaar, was het niet mogelijk te concluderen of suppletie met DHA de cognitieve prestaties van kinderen zouden kunnen verbeteren. Nadat deze evaluatie had plaatsgevonden, hebben 2 studies in schoolkinderen en 1 studie in kleuters geen effect kunnen aantonen van suppletie met DHA op cognitieve uitkomsten. Hiermee is er momenteel geen bewijs dat extra inname van omega-3 vetzuren een positief effect kan hebben op de cognitieve ontwikkeling van kinderen ouder dan 2 jaar.

Vervolgens werd een gerandomiseerde, gecontroleerde voedingsinterventie studie uitgevoerd waarbinnen het effect van twee verschillende doseringen van een combinatie van micronutriënten en omega-3 vetzuren werd onderzocht op groei en cognitie bij 598 marginaal gevoede Indiase schoolkinderen van 6-10 jaar. Verschillende cognitieve domeinen, waaronder logisch redeneren, het korte en lange termijn geheugen en cognitieve snelheid, werden gemeten met testen van de Kaufman Assement Battery for Children II. Scores werden gecombineerd tot een totaal score, genaamd de Mental Processing Index (MPI). Gegevens verzameld aan het begin van het onderzoek van deze studie werden gebruikt om het verband tussen indicatoren van lichaamsgrootte, vetzuur- en micronutriëntstatus en cognitie te onderzoeken (**hoofdstuk 4**). Dit werd gedaan door middel van variantie analyse, waarbij gecorrigeerd werd voor leeftijd, geslacht, school, opleidingsniveau van de moeder en de afneemster van de cognitieve testen. Het onderzoek toonde significant positieve associaties aan van indicatoren van lichaamsgrootte en hemoglobine concentraties met alle cognitieve domeinen en de MPI. In tegenstelling werden vitamine $B_{12}$ concentraties in plasma significant negatief geassocieerd met het korte en lange termijn geheugen en de MPI. Overige indicatoren van

ijzer-, jodium-, folaat- en vetzuurstatus hielden geen verband met cognitie. De bevindingen voor lichaamsgrootte, vetzuren en micronutriënten kwamen overeen met eerdere observationele studies. Vervolgonderzoek is aanbevolen om het omgekeerde verband van vitamine $B_{12}$ met cognitie verder te bestuderen.

In de voedingsinterventie studie (**hoofdstuk 5**) werden kinderen willekeurig verdeeld over vier interventiegroepen en kregen gedurende 12 maanden voedingsmiddelen verrijkt met 1) 100% van de dagelijkse aanbevolen hoeveelheid (ADH) van micronutriënten met 900 mg $\alpha$-linoleenzuur (ALA) en 100 mg DHA; 2) 100% van de ADH van micronutriënten met alleen 140 mg ALA; 3) 15% van de ADH van micronutriënten met 900 mg ALA en 100 mg DHA; of 4) 15% van de ADH van micronutriënten met alleen 140 mg ALA. De kinderen die de hoge dosering micronutriënten (100% van de ADH) kregen, waren na 12 maanden significant meer gegroeid (0.19 cm; 95% btbh-i: 0.01, 0.36) vergeleken met kinderen die de lage dosering micronutriënten (15% van de ADH) kregen. Tevens presteerden kinderen die de hoge dosering micronutriënten kregen na 6 maanden significant beter op testen van het korte termijn geheugen (0.11 SD; 95% btbh-i: 0.01, 0.20), maar presteerden zij minder goed op testen van het domein "logisch redeneren" na 6 (-0.10 SD; 95% btbh-i: -0.17, -0.03) en 12 maanden (-0.12 SD; 95% btbh-i: -0.20, -0.04). Er werden geen verschillen waargenomen tussen de interventiegroepen met betrekking tot gewicht en andere cognitieve domeinen. Ook werden er geen verschillen waargenomen tussen groepen met hoge en lage omega-3 vetzuur doseringen, en was er geen interactie tussen micronutriënten en omega-3 vetzuren op groei en cognitieve domeinen. Er is geconcludeerd dat de hoge en lage dosering, met enige kleine verschillen op sommige cognitieve domeinen, even effectief waren voor het verbeteren van de cognitieve prestaties.

In **hoofdstuk 6** werd het verband onderzocht tussen inname van energie en nutriënten en met anthropometrie en cognitie. Voor dit onderzoek werden gegevens gebruikt die verzameld zijn aan het begin van de interventie studie bij Indiase schoolkinderen. De kinderen hadden een eenzijdig voedingspatroon, en een dramatisch lage inname van energie, vet, meervoudig onverzadigde vetzuren en micronutriënten. Variantie analyse met correctie voor mogelijke confounders liet zien dat energie inname positief geassocieerd was met lengte, gewicht, bovenarmomtrek en cognitieve prestaties. Na correctie voor energie, was de inname van meervoudig onverzadigde vetzuren positief geassocieerd met cognitie, terwijl er geen verbanden werden gevonden met inname van eiwit, ALA en micronutriënten. Deze resultaten suggereren dat er dringend publieke gezondheidsprogramma's nodig zijn om de inname van energie en nutriënten te verhogen door middel van een meer gevarieerde voeding, verrijking van voedingsmiddelen of het verstrekken van maaltijden op scholen.

Samenvattend laat het onderzoek in dit proefschrift zien dat suppletie met multivitaminen en mineralen mogelijk geassocieerd is met een kleine verbetering in het cognitieve domein "logisch redeneren" en schoolprestaties van kinderen. Toch is het momenteel te vroeg om aanbevelingen te doen voor grootschalige publieke gezondheidsprogramma's om de inname van micronutriënten te verhogen om de cognitieve ontwikkeling te bevorderen (**hoofdstuk 7**). Aangezien de effecten vooral zichtbaar waren bij kinderen in westerse landen is er meer onderzoek nodig om vast te stellen of een betere micronutriënten status, boven de officiële grenswaarden voor micronutriëntentekorten, gerelateerd is aan een betere cognitieve ontwikkeling.

Voor kinderen in ontwikkelingslanden zijn gezondheidsprogramma's nodig die zich richten op een adequate inname van energie en essentiële nutriënten om de algehele gezondheid te verbeteren en hiermee indirect de cognitieve prestaties te verhogen. Onze bevinding, dat de voeding van Indiase kinderen zeer laag in energie, vetzuren en micronutriënten was, onderstreept de noodzaak voor zulke programma's.

Onze conclusie dat de lage dosering micronutriënten net zo effectief was als de hoge dosering, duidt er op dat beleidsmakers mogelijk een lage dosering zouden kunnen gebruiken voor het samenstellen van publieke gezondheidsstrategieën om de cognitieve ontwikkeling te verbeteren. Er is echter meer onderzoek nodig om onze resultaten te bevestigen.

Wat de rol van omega-3 vetzuren in de mentale ontwikkeling van kinderen betreft, sluiten de bevindingen in dit proefschrift aan bij de resultaten van andere studies die geen verbetering in het cognitief functioneren zien na extra inname van omega-3 vetzuren, en van DHA in het bijzonder. Er is daarom momenteel geen wetenschappelijk bewijs om de inname van omega-3 vetzuren te verhogen ten bate van het intellectuele vermogen van schoolkinderen.

Meer onderzoek zal nodig zijn om vast te stellen 1) wat het effect is van multivitaminen en mineralen op andere cognitieve domeinen dan logisch redeneren; 2) wat de optimale samenstelling van een multivitaminen en mineralen supplement is; en 3) wat het effect is van de micronutriënten status op de cognitieve uitkomsten van interventiestudies met micronutriënten. Om een einde te maken aan de huidige discussie of DHA de cognitieve prestaties van schoolkinderen zou kunnen beïnvloeden, zou een studie nodig zijn met een voldoende hoge dosering van DHA en voldoende lange duur van de suppletieperiode. Tot slot is het aanbevolen om in toekomstige studies gebruik te maken van een combinatie van cognitieve testen met meer geavanceerde technieken die de structuur en het functioneren van de hersenen in beeld brengen. Met deze combinatie kan de rol van nutriënten in de hersenen en het cognitief functioneren beter worden begrepen.

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# About the author

## About the author

Ans Eilander was born on 22 August 1975 in Zwolle, the Netherlands. From 1992-1994, she studied Nutrition and Dietetics at the Hanzehogeschool Groningen, the Netherlands. Because she wanted to extend her knowledge of nutrition, she switched studies and started her MSc in Nutrition and Health at Wageningen University, the Netherlands in 1995. During her study she focused on nutritional epidemiology and nutrition in developing countries, and collaborated with the Nutrition and Development Centre in Bogor, Indonesia and Royal Tropical Institute, Amsterdam, the Netherlands.

After her graduation in 2000, she went to Cambodia to work at Helen Keller International, where she evaluated the performance of the national vitamin A capsule distribution program for children aged 6-59 months. A year later, she returned to Wageningen University where she worked as a researcher on the bioefficacy of carotenoids and applied for research grants. In 2002, Ans was appointed as epidemiologist at the municipal Health Service Utrecht.

Since 2003, Ans has been employed at Unilever Research & Development Vlaardingen, the Netherlands as scientist international nutrition. She conducted the overall management of the Children's Health And Mental Performance Influenced by Optimized Nutrition (CHAMPION) study which lay the foundation of this thesis. In November 2007, she formally registered at Wageningen University as external PhD fellow. During the October 2008 meeting of the Dutch organization for scientific research (NWO), she won the young investigators award – Foppe ten Hoor for a presentation on the CHAMPION study. Currently, she will continue her work on micronutrients, fatty acids and children's health and development at Unilever.

## List of publications

**Eilander A**, Muthayya S, van der Knaap HCM, Srinivasan K, Thomas T, Kok FJ, Kurpad AV, Osendarp SJM. Undernutrition, fatty acid and micronutrient status in relation to cognitive performance in Indian school children: a cross-sectional study. Br J Nutr 2009. doi:10.1017/S000711450999273X.

**Eilander A**, Gera T, Sachdev, HS, Transler C, van der Knaap HCM, Kok FJ, Osendarp SJM. Multiple micronutrient supplementation for improving cognitive performance in children: systematic review of randomized controlled trials. Am J Clin Nutr 2009. doi: 10.3945/ajcn.2009.28376.

Koletzko B, Uauy R, Palou A, Kok FJ, Hornstra G, **Eilander A**, Moretti D, Osendarp SJM, Zock PL, Innis SM. Dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in children - a workshop report. Br J Nutr 2009 (in press).

Transler C, **Eilander A**, Mitchell S, van der Meer N. The Impact of Polyunsaturated Fatty Acids in Reducing Child Attention Deficit and Hyperactivity Disorders. J Attention Disorders 2009. doi: 10.1177/1087054709347250.

**Eilander A**, Osendarp SJM, Tiwari J. Functional Foods for the Brain. In: Smith J & Charter E. Functional Food Product Development. Oxford: Whiley Blackwell, 2009 (in press).

Muthayya S, **Eilander A**, Transler C, Thomas T, van der Knaap HCM, Srinivasan K, Klinken BJW, Osendarp SJM, Kurpad AV. Effect of fortification with multiple micronutrients and n-3 fatty acids on growth and cognitive performance in Indian schoolchildren: the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. Am J Clin Nutr 2009;89:1766-75.

Srihari G, **Eilander A**, Muthayya S, Kurpad AV, Seshadri S. Nutritional status of affluent Indian school children: what and how much do we know? Indian Pediatr. 2007;44: 204-13.

**Eilander A**, Hundscheid DC, Osendarp SJ, Transler C, Zock PL. Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: a review of human studies. Prostaglandins Leukot Essent Fatty Acids. 2007;76:189-203.

Muthayya S, Thankachan P, Zimmermann MB, Andersson M, **Eilander A**, Misquith D, Hurrell RF, Kurpad AV. Low anemia prevalence in school-aged children in Bangalore, South India: possible effect of school health initiatives. Eur J Clin Nutr. 2007;61:865-9.

Fernandes TM, Schout C, De Roda Husman AM, **Eilander A**, Vennema H, van Duynhoven YT. Gastroenteritis associated with accidental contamination of drinking water with partially treated water. Epidemiol Infect. 2007;135:818-26.

West CE, **Eilander A**, van Lieshout M. Consequences of Revised Estimates of Carotenoid Bioefficacy for Dietary Control of Vitamin A Deficiency in Developing Countries. J Nutr 2002;132:2920-6.

**Eilander A**, Schmidt M, Verhoef H. Verslag van een WOTRO workshop: Voeding en gezondheid in ontwikkelingslanden. Voeding Nu 2002;1: 19-21 (publication in Dutch).

West CE & **Eilander A**. Bioefficacy of carotenoids. Sight & Life Newsletter 2/2001.

**Eilander A**, de Pee S, Panagides D. Ways to improve vitamin A capsule distribution in Cambodia. Cambodia Helen Keller Nutrition Bulletin 2001; Vol 2 (5).

Van Liere MJ, Kusin JA, **Eilander A**. Annotated bibliography on household food and nutrition security. Amsterdam: Royal Tropical Institute, 2001.

# VLAG graduate school activities

## Discipline-specific activities

### *Courses*

- Advanced Course in Nutrition and Lifestyle Epidemiology, VLAG, 2005
- N-3 Fatty Acids and Mental Health Symposium, VLAG, 2007
- Introduction Neuroscience, Unilever, 2007
- Advanced topics in Cognitive Neuroscience, Unilever, 2009

### *Congresses*

- NWO dagen, Papendal, the Netherlands, 2004
- ASO Workshop "Measurement Physical Activity" Cambridge, UK, 2005
- ISSFAL Cairns, Australia, 2006
- Forum "Lancet Series Child Development in Developing Countries" (UCL Centre for International Health and Development and UNICEF), London, UK, 2007
- Micronutrient Forum, Istanbul, Turkey, 2007 (poster and oral presentation)
- Workshop "Towards a recommendation on DHA/EPA", Paris, France, 2007
- Special WUR seminar "Iron deficiency in malaria endemic areas: Is fortification the solution?" Wageningen, the Netherlands, 2008
- Brain Lipids Congress, Oslo, Norway, 2008
- Workshop "Maternal and Child undernutrition: challenges and opportunities" Utrecht, he Netherlands, 2008
- NWO dagen, Deurne, the Netherlands, 2008 (oral presentation)
- Wageningen Nutritional Sciences Forum: Too Much - Too Little, Arnhem, the Netherlands, 2009
- EASO Childhood Obesity Task Force Workshop, Amsterdam, the Netherlands, 2009
- Micronutrient Forum, Beijing, China, 2009 (poster)

## General courses

- Make Science Make Sense – communication using targeted messages, Unilever, 2003
- Clinical Trials, Erasmus Summer Programme, 2004
- Statistics for Industry – refresher course, Unilever, 2004
- Patent course, Unilever, 2004
- Project Management Techniques Training, Unilever, 2005
- Effective Intercultural Communication India, B van Houten, 2005
- Strategic Influencing, Boertien Training, 2006
- R&D Foundation course, Unilever, 2006
- Recruitment Training, Unilever, 2006
- Career Framework Workshop, Unilever, 2008
- Introduction Advanced Statistics, Unilever, 2008
- Claims Foundation course, Unilever, 2008
- Advanced Statistics, Unilever, 2009

## Optional courses and activities

- Preparation PhD research proposal
- Skillbase meetings, project team meetings, science forum, Unilever, 2003-2009
- Literature study programmes, Unilever, 2003-2009
- Organization ISSFAL satellite symposium, Cairns, Australia 2005
- Organization workshop "Towards a recommendation on DHA/EPA", Paris, France, 2007

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