The role of maternal nutrition in growth and health of Indonesian infants:
a focus on vitamin A and iron

Marjanka K. Schmidt

PROMOTOREN:
Prof. Dr. J. G. A. J. Hautvast, MD
Hoogleraar in de voeding en gezondheid, Wageningen Universiteit

Prof. Dr. C. E. West, PhD Dsc FRACI
Universitair hoofddocent, Divisie Humane Voeding en Epidemiologie,
Wageningen Universiteit/Bijzonder hoogleraar Voeding in relatie tot gezondheid en ziekte, Faculteit der Medische wetenschappen, Katholieke Universiteit Nijmegen

CO-PROMOTOR:
Dr. J. W. Schultink, PhD
Voormalig wetenschappelijk consultant SEAMEO TROPMED, Indonesia/UNICEF, Micronutrient Unit, New York, USA

PROMOTIECOMMISSIE:
Prof. Dr. A. Niehof, Wageningen Universiteit

Dr. M.J.R. Nout, Wageningen Universiteit

Dr. J. J. M. Tolboom, Universitair Medisch Centrum St. Radboud, Nijmegen

Prof. Dr. W. A. van Staveren, Wageningen Universiteit

The role of maternal nutrition in growth and health of Indonesian infants: a focus on vitamin A and iron

Marjanka Kathelina Schmidt

Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de Rector Magnificus
van Wageningen Universiteit,
Prof. dr. ir. L. Speelman,
in het openbaar te verdedigen
op maandag 17 december 2001
des namiddags te twee uur in de Aula.

Schmidt, Marjanka K.

The role of maternal nutrition in growth and health of Indonesian infants:
a focus on vitamin A and iron
Thesis Wageningen University, The Netherlands – With references – With summaries in Dutch and Indonesian

ISBN 90-5808-547-3

Propositions

1. Vitamin A supplementation of pregnant women leads to increased serum retinol concentrations of their newborn infants (this thesis).

2. Further research should be directed towards improving growth during infancy rather than increasing birth weight of Indonesian infants. However, pregnancy should still be considered as a possible period for intervention (this thesis).

3. The concern of possible toxicity of vitamin A during pregnancy is out of proportion to the lack of concern of the world-wide burden of vitamin A deficiency.

4. The problem of stress perceived by women during pregnancy and thereafter needs more attention because of possible negative consequences for their infant's development.

5. More recognition of the interaction of psychological and physiological problems by general practitioners would enhance human well-being and reduce the costs of health care.

6. Research projects aimed at solving malnutrition in developing countries should be enriched with a national PhD fellow. Such a policy would lead to capacity building and more sustainable application of results.

7. The perceived lack of culture in the Netherlands derives from the definition of culture applied.

8. Als je oude koeien uit de sloot haalt, kunnen de kalfjes vers water drinken (after a Dutch saying).

Propositions pertaining to the thesis 'The role of maternal nutrition in growth and health of Indonesian infants: a focus on vitamin A and iron'

Marjanka K. Schmidt
Wageningen, 17 December 2001

Abstract

The role of maternal nutrition in growth and health of Indonesian infants:
a focus on vitamin A and iron.

PhD thesis of Marjanka K. Schmidt, Division of Human Nutrition and Epidemiology, Wageningen University, The Netherlands, 17 December 2001.

Nutrition during pregnancy is important for women's health, pregnancy outcome, and infant growth and health. The aim of the present study was to investigate whether weekly supplementation with iron and vitamin A of pregnant women improves growth and health, as indicated by reduced morbidity and improved mental and psychomotor development, of their infants. At ~18 weeks of pregnancy, women from 5 villages were randomly assigned on an individual basis to supervised, double-masked supplementation once weekly from enrolment until delivery. Supplementation comprised 120 mg iron as Fe_2SO_4 and 500 μg folic acid with (n=121) or without (n=122) 4800 RE vitamin A. Pregnant women participating in the ongoing national iron supplementation programme and receiving iron/folic acid tablets through medical services were recruited at the same time from 4 neighbouring villages ('daily' group, n=123). Compliance with iron tablet intake in this group was expected to be very low. Newborn infants were followed up at least until 1 year of age.

At ~4 months of age, infants in the weekly vitamin A plus iron group had significantly higher serum retinol concentrations than infants in the weekly iron group. However, in all groups >70% of the infants had serum retinol concentrations <0.70 μmol/L. Higher serum retinol levels were associated with better growth and nutritional status during the first 6 months of life. Iron status did not differ among groups, while 29% of the infants had a haemoglobin concentration <100 g/L and only 3 infants had a serum ferritin concentration <12 μg/L. During the first year of life, anthropometric parameters, morbidity and mental and psychomotor development of infants, whose mothers were supplemented with vitamin A plus iron during pregnancy, did not differ from those whose mothers had received iron alone. In addition, none of the investigated parameters differed between the infants in the weekly iron supplementation group and infants in the 'daily' group. Growth faltering of infants started at 6 months of age and led to high prevalence of stunting (24%) and underweight (32%) at 12 months of age. Almost all infants were breast-fed during the whole follow up period. Neonatal weight and length, reflecting prenatal factors, were the strongest predictors of growth and nutritional status of infants at 12 months of age. In addition, maternal weight and height at ~18 weeks of pregnancy, housing, and postnatal variables such as intake of certain food groups and morbidity were predictors of growth and nutritional status of infants at 12 months of age. In conclusion, vitamin A supplementation in concurrence of iron supplementation of pregnant women does not improve growth or health of their infants. However, it improved vitamin A status of their infants at ~4 months of age. Considering the association between growth and vitamin A status, and the high prevalence of serum retinol levels <0.70 μmol/L, vitamin A intake during infancy may need to be increased. However, in order to optimise nutritional status and growth of infants the whole life cycle should be considered.

Contents

Article 25 of the Universal Declaration of Human Rights

1. Everyone has the right to a standard of living adequate for the health and well-being of himself and of his family, including food, clothing, housing and medical care and necessary social services, and the right to security in the event of unemployment, sickness, disability, widowhood, old age or other lack of livelihood in circumstances beyond his control.
2. Motherhood and childhood are entitled to special care and assistance. All children, whether born in or out of wedlock, shall enjoy the same social protection.

1

General Introduction

1 Infant nutrition and health

1.1 The importance of pre- and postnatal nutrition

Nutrition during pregnancy is important for women's health, pregnancy outcome, and infant growth and health [1,2]. Iron and marginal vitamin A deficiency during pregnancy and lactation is a common problem world-wide and specifically in Indonesia [3-8]. In South East Asia, including Indonesia, 40% of infants and children are stunted (linear growth retarded) and suffer from deficiencies of iron and vitamin A [3,4,9]. Stunting is defined as linear growth retardation as indicated by a height-for-age z-score less than –2 SD below the median of a reference population of children exposed relatively little to malnutrition or infection [10].

1.2 Infant growth and nutritional status

Most growth faltering, as indicated by being underweight or stunted, occurs from birth until about 2 years of age and may already start during the gestation period [11,12]. Intra-uterine growth retardation (being small for gestational age) is estimated to occur in 24% of newborn infants worldwide [13]. Because gestational age is difficult to assess accurately in field settings, low birth weight is often used as a proxy for intra-uterine growth retardation. Although some catch-up growth is possible, infants who are born too small or with low birth weight are at high risk of becoming stunted during subsequent phases of childhood [11,14,15]. Stunting indicates an important public health problem because of its association with impaired cognitive development [16,17], increased susceptibility to infection [18] and increased risk of mortality [19,20]. The long-term outcome of stunting includes increased risk for chronic disease [21] and elevated risk of poor reproductive outcomes (**Figure 1.1**). Thus infant growth retardation indicates a loss of health for the next generation.

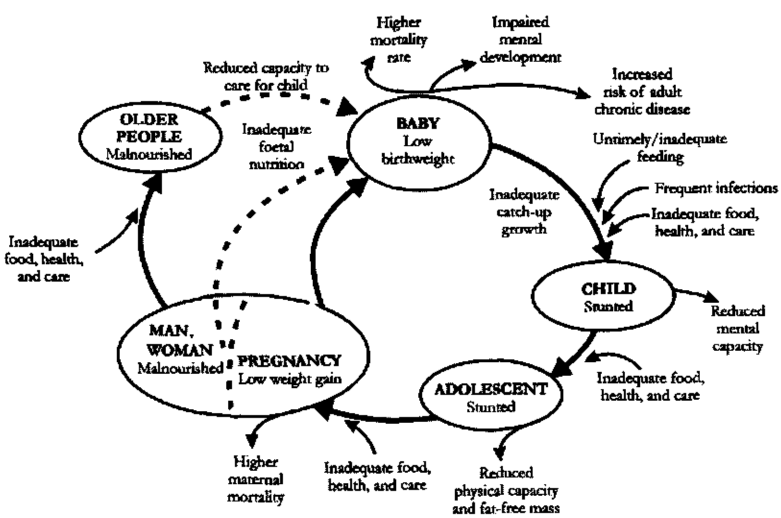

Figure 1.1 Nutrition throughout the life cycle (adapted from reference 4).

1.3 Aetiology of infant growth retardation

Infant linear growth retardation is determined by factors during the gestation period as well as during the postnatal period. However the causes and the underlying biological explanations of infant stunting are poorly understood [22,23]. Genetic background plays a limited role in the prevalence of stunting [24]. This is illustrated in a study in Indonesian families of high socio-economic class, which shows that growth of pre-school children is equal to that of the American reference population [25]. Inadequate maternal nutrition during gestation may contribute to growth retardation of the foetus. In addition, infants may be born with low nutrient stores [12,26-28]. During the postnatal period, insufficient food intake, inadequate feeding practices and continued or repeated infections contribute to growth retardation [24,29,30]. In countries with a high prevalence of micronutrient deficiencies such as Indonesia, it may be that specific deficiencies such as those of vitamin A and iron contribute to growth retardation during the prenatal and postnatal period [11].

2 Prenatal nutrition

2.1 The impact of maternal nutrient intake on pregnancy outcome

Maternal weight before pregnancy and weight gain during pregnancy has been related to infant birth weight [1]. Reviews of studies directed at increasing energy and protein intake during pregnancy concluded that a balanced increase of energy and protein intake results in modest increases in maternal weight gain and foetal growth [26,31]. 'Balanced' was defined as a supplement in which protein provided <25% of the energy content. However protein supplementation during pregnancy did not show any benefit on infant birth weight or length [31]. Other trials that supplemented pregnant women with food together or not with micronutrients were not effective in preventing intra-uterine growth retardation [26]. One trial in Gambian women showed that supplementation with groundnut based biscuit and fortified tea during pregnancy increased birth weight, but only in the wet season [32]. Another trial in Colombian women showed that supplementation with food and micronutrients (mostly vitamin A and iron) during the third trimester of pregnancy improved weight gain of women but did not significantly improve infant birth weight [33]. A trial in Indonesian women showed that energy supplementation during the last trimester of pregnancy increased length and weight of their infants significantly from the third month of age and height throughout the first 5 years of age, although birth weight was not increased [27]. Thus increasing energy or protein intake of pregnant women, especially in community settings where logistics, targeting and leakage are a problem, will have a small chance of increasing birth weight or reduce stunting in infants.

2.2 Vitamin A

2.2.1 The impact of maternal vitamin A status and intake on pregnancy outcome

Vitamin A is an essential nutrient throughout the life cycle but most importantly during pregnancy and early childhood. Vitamin A is needed for proliferation and differentiation of cells and plays a role in the immune system. Vitamin A deficiency may result from inadequate dietary intake or from losses or increased requirements during disease [34]. Most dietary vitamin A is obtained from animal products or by conversion in the body from provitamin A from green leafy vegetables and orange/yellow fruits. However bioconversion of provitamin A to retinol is much less than previously assumed and when needs are increased, it is impossible to acquire sufficient vitamin A through plant foods [35].

The role of retinoic acid, a metabolite of vitamin A, in embryonic growth and development has been demonstrated in animal studies [36-38]. A vitamin A-deficient diet during pregnancy has been shown to affect organ growth and development of rats [39]. In cross-sectional studies in humans, vitamin A deficiency during pregnancy has been associated with low birth weight [28]. Serum retinol concentrations in cord blood of newborn infants have been reported to be associated with birth weight, while infants with intra-uterine growth retardation have been shown to be at almost three times greater risk of having retinol concentrations <0.70 μmol/L [40,41]. However, these studies cannot provide causal evidence for an impact of vitamin A on birth weight of infants. In 2 intervention studies, supplementation with vitamin A during pregnancy has not been shown to improve birth weight [42,43]. However, one of these 2 studies had insufficient statistical power to detect differences in birth weight while the other study started supplementation only at 28 weeks of pregnancy, which may have been too late. A well-designed trial in Nepal did not show an impact of vitamin A supplementation on infant mortality [44] while effects on infant birth weight or growth have not been reported.

Women with low vitamin A status and intake during pregnancy have low retinol levels in breast milk, which may be insufficient for infants to build up their stores in the postnatal period [45,46]. Vitamin A supplementation during pregnancy reduces night blindness [47] and has been shown to reduce maternal mortality by 40% [48]. Night blindness during pregnancy has been found to be associated with infant mortality [49]. In conclusion, from animal studies it is clear that vitamin A has an important role in pregnancy outcome, however so far this has not been confirmed in human studies. It may be that improving vitamin A status of pregnant women will have less impact on birth weight than on vitamin A stores in the foetus and vitamin A concentrations in breast milk, which may affect growth and immunity during infancy.

2.2.2 The impact of maternal vitamin A status and intake on vitamin A stores of the infant

During pregnancy, vitamin A is transferred from mother to foetus across the placenta. This process involves retinol binding protein, but the exact mechanism is not yet known [36]. Vitamin A concentrations in serum of newborn infants are low, about half of those found in their mothers [37]. According to Underwood [50], vitamin A status of newborn infants is not correlated with that of their mothers, except at the extremes of deficiency or excess. However, in vitamin A sufficient and as well as in vitamin A deficient women, maternal vitamin A status during pregnancy has been shown to be associated with foetal liver and infants cord blood concentrations of vitamin A [51-53]. In addition, aborted foetuses of Indian mothers from a high-income group were found to have higher liver retinol concentrations than those from a low-income group [51]. It also has been reported that, serum retinol binding protein concentrations and saturation (serum retinol concentrations/retinol binding protein concentrations) in cord blood of newborn, fullterm infants are positively correlated with these parameters in their mothers [54]. Two intervention studies assessed the impact of vitamin A supplementation on vitamin A levels in cord blood of infants. In one study, supplementation with 6000 IU Vitamin A together with iron for more than 12 weeks during pregnancy increased cord levels of vitamin A [42]. In another study, daily supplementation with 8000 IU vitamin A from 30 weeks of gestation until term increased plasma retinol concentrations in the mother and in cord blood, although the increase in the latter was not significant [43]. A major constraint is that most studies, except for 2 [43,54], did not use HPLC for determination of retinol concentrations. Vitamin A supplementation of vitamin A-deficient pregnant women may lead to an increase of placental transfer of retinol to the foetus. However, whether vitamin A stores of the infant are increased through vitamin A supplementation during gestation cannot be concluded from the available evidence.

2.3 Iron

2.3.1 The impact of maternal iron status and intake on pregnancy outcome

Although iron absorption increases with advancement of gestation [55,56], this is often insufficient to meet the demands for iron. As a result, pregnant women often become anaemic. In addition, iron deficiency may result from consumption of diets with insufficient iron, reduced dietary iron availability, and losses due to intestinal worm infestation [57]. Metabolism and bioavailability of iron has been reviewed extensively [58,59]. Daily iron supplementation is usually prescribed for iron deficient anaemic women and has been implemented in health care programmes in many countries including Indonesia. Unfortunately, these programmes have not been shown to be successful in reducing iron deficiency anaemia [60]. Folic acid is commonly included because folate deficiency is a common cause of megaloblastic anaemia [61]. Anaemia during pregnancy has been shown to be associated with low birth weight, premature delivery, and with perinatal and foetal death [62-65]. One study has shown that inadequate iron status during the first trimester of pregnancy is associated with low birth weight and preterm delivery [66]. Many other trials have also investigated the consequences of iron deficiency anaemia during pregnancy on pregnancy outcome but no firm conclusions can be drawn [28,67]. This is because many of the studies were poorly designed and because of the difficulty in interpreting haemoglobin concentrations during pregnancy.

2.3.2 Relationship with infant iron status

It is generally thought that maternal iron status during pregnancy has little influence on iron status of infants as indicated by haemoglobin concentrations or serum indicators of iron stores [68]. However there are reports that iron levels in cord blood and iron stores in infants of mothers with iron deficiency anaemia are reduced [69,70]. Thus, in iron-deficient anaemic women, iron stores of infants may be affected by those of their mothers during pregnancy [71]. In a placebo-controlled study in Niger, iron supplementation during pregnancy improved infant length at birth and serum ferritin concentration at 3 months after birth [72]. Sufficient iron status of infants at birth is very important as infants will deplete their iron stores during the first half year of life when rapid growth occurs. Iron intake from breast milk is low and may not be sufficient to support growth and building of iron stores [73]. Considering the evidence from the trial in Niger and associations found in other studies, it may well be that iron supplementatation of pregnant women can improve iron status of their infants at birth.

3 Postnatal nutrition

3.1 Nutrient intake

Adequate energy and protein intakes of infants are important for their growth. Breast-feeding is considered the optimal nutrition for infants up to 4-6 months of age [74] and decreases the risk of morbidity and mortality in infants in developing countries [75,76]. Moderate maternal malnutrition does not diminish the capacity to produce sufficient milk to sustain infant growth [77-79]. However low levels of specific nutrients in breast milk might contribute to infant growth faltering [77]. After 6 months, growth of breast-fed infants may be limited by energy, protein or micronutrient content of complementary foods [74,80,81]. The highest impact of supplementation on growth of infants has been shown in periods of weaning or high incidence of diarrhoea [82]. However, intervention studies with supplements late in infancy have not shown sustainable effects on growth into childhood [83,84].

3.2 Vitamin A

3.2.1 Vitamin A status and intake during infancy

Colostrum and breast milk in the first week after delivery, are rich sources of vitamin A exceeding 3.5 µmol/L [37]. Feeding colostrum and this 'transitional' breast milk can therefore increase the neonatal body stores of vitamin A significantly [50]. In breast-fed infants, breast milk continues to be an importance source of vitamin A. However, if their mothers have poor vitamin A status, vitamin A levels in breast milk may be insufficient to build up their stores [46]. Breast milk concentrations of vitamin A have been found to be lower in developing than in developed countries [74], with the values from a study in West Java being among the lowest concentrations reported: 0.58-0.76 µmol/L [85]. Therefore, vitamin A supplementation during lactation is recommended for improving maternal and infant vitamin A status in populations with vitamin A deficiency [86]. Indonesian women supplemented with 300000 IU vitamin A within 2 weeks after delivery had higher vitamin A concentrations in their milk up to 8 months after delivery compared with those who received placebo [6]. In addition, the proportion of infants with low serum retinol concentrations (<0.52 µmol/L) and low vitamin A stores at 6 months of age was lower in infants whose mothers were supplemented with vitamin A than in their counterparts whose mothers received placebo [6].

3.2.2 Consequences of vitamin A deficiency

Vitamin A plays an important role in immune function and vision, as severe deficiency inevitable leads to blindness [87,88]. Mild vitamin A deficiency in infants and children is associated with diarrhoea, respiratory infections and measles [89,90]. In populations where vitamin A deficiency is prevalent, vitamin A supplementation can reduce mortality and morbidity in children aged 6 months until 5 years of age [91]. Vitamin A supplementation to mothers during lactation has been shown to reduce morbidity of infants through increased intake of vitamin A via breast milk [6,92,93]. It should be noted that such effects have not been shown in infants younger than 6 months of age [94-96]. As reviewed before [83], vitamin A supplementation has not been shown to improve growth in many studies. However vitamin A supplementation has been shown to improve growth of children with vitamin A deficiency as indicated by low serum retinol concentrations or xerophthalmia [97-100]. Hence, vitamin A clearly plays an important role in growth and morbidity of infants, although the impact on young infants is not yet clear.

3.3 Iron

3.3.1 Iron status and intake during infancy

Circumstances around the time of delivery, e.g. early or late clamping of the cord, and foetal blood loss into the placenta or mother, may affect neonatal iron status [73,101]. Haemoglobin concentrations are particularly high at birth, reflecting the oxygen-poor environment in the uterus, and decrease thereafter before stabilising at about 3 months of age. During that same period serum ferritin levels increase, reflecting increased iron stores in the liver cells [73]. Birth weight, the rate of growth and iron stores at birth are major influences on iron status during infancy [68,73,102]. Bioavailability of iron in human breast milk is high, but concentrations are low and fall during lactation from about 0.6 mg/L at 2 weeks to 0.4 mg/L at 6-8 weeks and 0.3 mg/L at 3-5 months [73]. Iron levels in breast milk seem to be relatively unaffected by food intake, iron supplements or iron status [103,104]. Full term, breast-fed infants normally do not require additional sources of iron in the diet until 6 months of age [104,105]. However after 6 months of age, if iron from complementary foods is insufficient, infants may develop iron deficiency anaemia [73,105].

3.3.2 Consequences of iron deficiency

Iron is needed for a wide range of metabolic activities and is essential for growth. Iron deficiency has been found to have a negative impact on immunity [106] and is associated with decreased growth rate and increased morbidity in children [83,107-110]. Iron supplementation has been shown to improve growth of anaemic pre-school and school children in 2 studies in Indonesia [107,108]. During infancy and childhood, iron deficiency anaemia leads to impaired mental and motor development with possible long term consequences [111-113].

4 Other factors in infant nutrition and health

Many other factors, including sanitation, clean water, time for child care, access to health services and dietary factors, can play a role in pregnancy outcome and growth of infants [28,114]. Feeding practices, i.e. timely introduction of complementary food, the duration of breast feeding and type of food, but also feeding behaviour and feeding style may affect infants growth [74,115]. Infection can affect nutrition through anorexia, impaired nutrient absorption, losses of micronutrients in urine, increased metabolism or impaired transport to target tissues [116,117]. The severity of the impact of morbidity on growth differs among age groups and populations [83,116]. Studies in The Gambia and Bangladesh, for example, have shown that lower respiratory tract infections, diarrhoea and gastro-enteritis were associated with a reduction in growth [118-120]. Thus infections may limit growth in infants and children, however breast-feeding may play a protective role [24,120].

5 Interaction between vitamin A and iron

Vitamin A is known to play a role in iron metabolism and it has been suggested that vitamin A deficiency impairs erythropoiesis and mobilisation of iron from body stores into the circulation [121]. In marginally vitamin A deficient rats, supplementation with iron and vitamin A was more effective in restoring normal iron status than was iron supplementation alone [122]. An observational study in children showed a positive association of serum retinol concentrations with those of serum iron, ferritin and transferrin [123]. Supplementation with vitamin A in children increased haemoglobin concentrations, serum iron concentrations and of transferrin saturation but did not affect serum ferritin concentrations [124,125]. In pregnant Indonesian women who were anaemic, daily supplementation with vitamin A and iron increased haemoglobin concentrations levels about 12 g/L of which one third could be attributed to vitamin A [126].

6 Study rationale

A large proportion of Indonesian infants suffer from nutritional deficiencies and growth retardation while deficiencies of iron and vitamin A especially are highly prevalent in pregnant and lactating Indonesian women. There is evidence for a role of maternal nutrition during pregnancy in infant birth weight and subsequent growth. Vitamin A status during pregnancy has been shown to affect vitamin A levels in breast milk and possibly vitamin A stores of infants. Anaemia and possibly iron deficiency during pregnancy have been associated with negative pregnancy outcomes, although strong evidence is lacking. Both iron and vitamin A are important nutrients in growth and immunity and have been shown to improve growth in some studies, while vitamin A has been shown to reduce mortality and morbidity in infants. Vitamin A plays a role in iron metabolism and has been shown to reduce anaemia in vitamin A deficient pregnant women. Thus, we hypothesised that growth retardation of the foetus *in utero* and of the infant postnatally may be prevented by improving the iron and vitamin A status of their mothers during pregnancy.

7 Research objectives (outline of the thesis)

The following research questions have been addressed and are described in the thesis.

- To investigate the effect of supplementation with vitamin A and iron during pregnancy on infant vitamin A and iron status (**Chapter 2**).
- To investigate the effect of weekly supplementation with vitamin A plus iron compared to iron alone during pregnancy on growth performance, nutritional status and morbidity of infants during the first year of life (**Chapter 3**).
- To investigate patterns and determinants of growth of infants during the first year of life (**Chapter 4**).
- To investigate the effect of supplementation with vitamin A and iron during pregnancy on mental and psychomotor development of infants (**Chapter 5**).

Chapter 6 consists of a general discussion and conclusions of all results described in this thesis. The methodological aspects of the study, general conclusions, implications for health policy and programmes and future research written jointly with Siti Muslimatun are also presented in Chapter 6.

8 Study site and design

From November 1997 until November 1999, a community-based study was carried out in 9 rural villages in Leuwiliang, a rural area in West Java, Indonesia. Indonesia has a population of 207 million and infant mortality rate is 43 per 1000 live births [127]. Leuwiliang, one of the subdistricts of Bogor, has about 140000 inhabitants, measures 158 km^2 and is situated 26 km from Bogor centre. It has 19 villages and most of the villages are situated on an altitude of 500-700 m. The average number of persons per household was 5 [128]. The maximum distance between study villages was 20 km. Only the main road had asphalt, the remaining roads were made from stone or soil.

For the study, 3 groups of women were recruited at approximately 18 weeks of pregnancy. Women from 5 villages were assigned randomly to 2 groups on an individual basis to receive weekly supplementation from enrolment until delivery. Supplementation comprised 2 tablets each containing 60 mg elemental iron as ferrous sulfate and 250 μg folic acid with or without 2400 retinol equivalent (RE) vitamin A. This amount was below the safe level of intake of 7500 RE vitamin A during pregnancy [129]. As advised by the Indonesian Ministry of Health, based on the RDA of vitamin A for Indonesian pregnant women (700 RE) and the expected absorption and dietary intake of vitamin A, we intended to supplement 6000 RE weekly (3000 RE per tablet). However, analysis of the supplements ~1 year after production, revealed that the tablets contained ~2400 RE; thus we considered to have given 4800 RE weekly. Both types of supplements were similar in physical appearance. The pregnant women received the supplement once a week between

09.00 and 12.00 h from voluntary health workers who ensured that the women swallowed the tablets in their presence.

Subjects from the other 4 villages were assigned to a third group, referred to as the 'daily' group. These subjects were participating in the ongoing national supplementation programme and received iron plus folic acid tablets through medical services. This programme has been implemented since 1970 [130]. It would therefore have been unethical to include an iron placebo group in the present study. However, compliance was expected to be low [131] thus this group was included as a 'control' group for comparison with the weekly iron supplementation group. We recruited this group from different villages in order to avoid confusion about the iron tablets among midwives, voluntary health workers and other persons involved in the research. Following government guidelines, women start receiving supplementation during their first visit for antenatal care at the Posyandu (integrated health services post) and should receive 90-120 iron plus folic acid tablets, each tablet containing 60 mg elemental iron and 250 μg folic acid, to be taken daily throughout pregnancy. These tablets were similar in appearance to the tablets administered weekly. By including this 'daily' group, the study also intended to test the hypothesis that iron supplementation once a week instead of daily would be a method of choice for reducing iron deficiency anaemia in pregnant women [132,133]. The theory behind this was that intermittent iron supplementation would avoid the postulated mucosal block of iron absorption [134] and would have the advantage of higher compliance due to decreased side effects [133,135], easier distribution and lower costs. In addition, previous studies had shown that weekly iron supplementation had similar effects on iron status as daily iron supplementation [136-138].

For the pregnant women, a sample size of 63 per group was calculated based on 5% significance level (two-tailed test), power of 80%, difference in haemoglobin concentration of 5 g/L and standard deviation of 10 g/L. For the infants, a sample size of 51 per group was based on 5% significance level (two-tailed test), power of 80%, difference in length at 1 year of age of 1.5 cm and standard deviation of 2.7 cm. A sample size of 120 women per group was chosen to allow for a 50% drop out during pregnancy and during follow-up period until their infants reached 1 year of age.

In addition to this thesis, another thesis entitled 'Nutrition of Indonesian women during pregnancy and lactation: a focus on vitamin A and iron' by Siti Muslimatun is based on the described study.

References

1. Mora JO, Nestel PS. Improving prenatal nutrition in developing countries: strategies, prospects, and challenges. Am J Clin Nutr 2000;71:1353S-1363S.
2. Harding J. The nutritional basis of the fetal origins of adult disease. Int J Epidemiol 2001;30:15-23.
3. Administrative Committee on Coordination (ACC)/Sub-Committee on Nutrition (SCN). Third report of the world nutrition situation. Geneva: ACC/SCN, 1997.

4. ACC/SCN. Fourth report on the world nutrition situation. Nutrition throughout the life cycle. Geneva: ACC/SCN in collaboration with IFPRI, 2000.
5. Kosen S, Herman S, Schultink W. An overview of studies on iron deficiency in Indonesia. Nutr Res 1998;18:1935-1941.
6. Stoltzfus RJ, Hakimi M, Miller KW, et al. High dose vitamin A supplementation of breast-feeding Indonesian mothers: effects on the vitamin A status of mother and infant. J Nutr 1993;123:666-675.
7. Suharno D, West CE, Muhilal, et al. Cross-sectional study on the iron and vitamin A status of pregnant women in West Java, Indonesia. Am J Clin Nutr 1992;56:988-993.
8. Tanumihardjo SA, Muherdiyantiningsih, Permaesih D, et al. Assessment of the vitamin A status in lactating and nonlactating, nonpregnant Indonesian women by use of the modified-relative-dose-response (MRDR) test. Am J Clin Nutr 1994;60:142-147.
9. World Health Organization (WHO). Global database on child growth and malnutrition. Geneva: WHO, 2000.
10. WHO. Measuring change in nutritional status: guidelines for assessing the nutritional impact of supplementary feeding programmes for vulnerable groups. Geneva: WHO, 1983.
11. Kusin JA, Kardjati S. Maternal and child nutrition in Madura, Indonesia. Amsterdam: Royal Tropical Institute, 1994.
12. Waterlow JC, Schürch B. Causes and mechanisms of linear growth retardation. Proceedings of an I/D/E/C/G workshop held in London January 15-18, 1993. Eur J Clin Nutr 1994;48:S1-S216.
13. de-Onis M, Blossner M, Villar J. Levels and patterns of intrauterine growth retardation in developing countries. Eur J Clin Nutr 1998;52(suppl 1):S5-15.
14. Arifeen SE, Black RE, Caulfield LE, et al. Infant growth patterns in the slums of Dhaka in relation to birth weight, intrauterine growth retardation, and prematurity. Am J Clin Nutr 2000;72:1010-1017.
15. Ricci JA, Becker S. Risk factors for wasting and stunting among children in Metro Cebu, Philippines. Am J Clin Nutr 1996;63:966-975.
16. Brown JL, Pollitt E. Malnutrition, poverty and intellectual development. Sci Am 1996;274:38-43.
17. Pollitt E. Functional significance of the covariance between protein energy malnutrition and iron deficiency anemia. J Nutr 1995;125:2272S-2277S.
18. Tomkins A. The risk of morbidity in a stunted child. In: Waterlow JC, ed. Linear growth retardation in less developed countries. Nestlé Nutrition Workshop Series 14. New York: Raven Press, 1988.
19. Bairagi R, Chowdhury MK, Kim YJ, Curlin GT. Alternative anthropometric indicators of mortality. Am J Clin Nutr 1985;42:296-306.
20. Chen LC, Chowdhury A, Huffman SL. Anthropometric assessment of energy-protein malnutrition and subsequent risk of mortality among preschool aged children. Am J Clin Nutr 1980;33:1836-1845.
21. Barker DJ. Maternal nutrition, fetal nutrition, and disease in later life. Nutrition 1997;13:807-813.
22. Allen LH, Uauy R. Guidelines for the study of mechanisms involved in the prevention or reversal of linear growth retardation in developing countries. Eur J Clin Nutr 1994;48(suppl 1):S212-S216.
23. Waterlow JC. Introduction. Causes and mechanisms of linear growth retardation (stunting). Eur J Clin Nutr 1994;48(suppl 1):S1-S4.
24. Martorell R, Khan LK, Schroeder DG. Reversibility of stunting: epidemiological findings in children from developing countries. Eur J Clin Nutr 1994;48(suppl 1):S45-S57.
25. Droomers M, Gross R, Schultink W, Sastroamidjojo S. High socioeconomic class preschool children from Jakarta, Indonesia are taller and heavier than NCHS reference population. Eur J Clin Nutr 1995;49:740-744.
26. de-Onis M, Villar J, Gulmezoglu M. Nutritional interventions to prevent intrauterine growth retardation: evidence from randomized controlled trials. Eur J Clin Nutr 1998;52(suppl 1):S83-S93.
27. Kusin JA, Kardjati S, Houtkooper JM, Renqvist UH. Energy supplementation during pregnancy and postnatal growth. Lancet 1992;340:623-626.
28. Ramakrishnan U, Manjrekar R, Rivera J, Gonzales CT, Martorell R. Micronutrients and pregnancy outcome: A review of the literature. Nutr Res 1999;19:103-159.
29. Adair LS, Guilkey DK. Age-specific determinants of stunting in Filipino children. J Nutr 1997;127:314-320.

30. Keller W. The epidemiology of stunting. In: Waterlow JC, ed. Linear growth retardation in less developed countries. Nestlé Nutrition Workshop Series 14. New York: Raven Press, 1988.
31. Kramer MS. Effects of energy and protein intakes on pregnancy outcome: an overview of the research evidence from controlled clinical trials. Am J Clin Nutr 1993;58:627-635.
32. Prentice AM, Cole TJ, Foord FA, Lamb WH, Whitehead RG. Increased birthweight after prenatal dietary supplementation of rural African women. Am J Clin Nutr 1987;46:912-925.
33. Mora JO, de-Paredes B, Wagner M, et al. Nutritional supplementation and the outcome of pregnancy. I. Birth weight. Am J Clin Nutr 1979;32:455-462.
34. Zachman RD. Vitamin A. In: Tsang RC, Nichols BL, eds. Nutrition during infancy. Philadelphia: Hanley & Belfus, inc., 1988:253-263.
35. West CE. Meeting requirements for vitamin A. Nutr Rev 2000;58:341-345.
36. Azais-Braesco V, Pascal G. Vitamin A in pregnancy: requirements and safety limits. Am J Clin Nutr 2000;71:1325S-1333S.
37. Wallingford JC, Underwood BA. Vitamin A deficiency in pregnancy, lactation, and the nursing child. In: Bauerfeind JC, ed. Vitamin A deficiency and its control. New York: Academic Press, 1986:101-152.
38. Maden M. The role of retinoic acid in embryonic and post-embryonic development. Proc Nutr Soc 2000;59:65-73.
39. Antipatis C, Grant G, Ashworth CJ. Moderate maternal vitamin A deficiency affects perinatal organ growth and development in rats. Br J Nutr 2000;84:125-132.
40. Ghebremeskel K, Burns L, Burden TJ, et al. Vitamin A and related essential nutrients in cord blood: relationships with anthropometric measurements at birth. Early Hum.Dev 1994;39:177-188.
41. Rondo PH, Abbott R, Rodrigues LC, Tomkins AM. Vitamin A, folate, and iron concentrations in cord and maternal blood of intra-uterine growth retarded and appropriate birth weight babies. Eur J Clin Nutr 1995;49:391-399.
42. Panth M, Shatrugna V, Yasodhara P, Sivakumar B. Effect of vitamin A supplementation on haemoglobin and vitamin A levels during pregnancy. Br J Nutr 1990;64:351-358.
43. Howells DW, Haste F, Rosenberg D, Brown IR, Brooke OG. Investigation of vitamin A nutrition in pregnant British Asians and their infants. Hum Nutr Clin Nutr 1986;40:43-50.
44. Katz J, West KP-Jr, Khatry SK, et al. Maternal low-dose vitamin A or {beta}-carotene supplementation has no effect on fetal loss and early infant mortality: a randomized cluster trial in Nepal. Am J Clin Nutr 2000;71:1570-1576.
45. Ortega RM, Andres P, Martinez RM, Lopez-Sobaler AM. Vitamin A status during the third trimester of pregnancy in Spanish women: influence on concentrations of vitamin A in breast milk. Am J Clin Nutr 1997;66:564-568.
46. Stoltzfus RJ. Vitamin A deficiency in the mother-infant dyad. SCN News, Maternal and child nutrition (ACC/SCN) 1994;11:25-27.
47. Christian P, West KP-Jr, Khatry SK, et al. Vitamin A or beta-carotene supplementation reduces but does not eliminate maternal night blindness in Nepal. J Nutr 1998;128:1458-1463.
48. West KP-Jr, Katz J, Khatry SK, et al. Double blind, cluster randomised trial of low dose supplementation with vitamin A or beta carotene on mortality related to pregnancy in Nepal. The NNIPS-2 Study Group. BMJ 1999;318:570-575.
49. Christian P, West KP-Jr, Khatry SK, et al. Maternal night blindness increases risk of mortality in the first 6 months of life among infants in Nepal. J Nutr 2001;131:1510-1512.
50. Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. Am J Clin Nutr 1994;59:517S-522S.
51. Shah RS, Rajalakshmi R, Bhatt RV, et al. Liver stores of vitamin A in human fetuses in relation to gestational age, fetal size and maternal nutritional status. Br J Nutr 1987;58:181-189.
52. Shah RS, Rajalakshmi R. Vitamin A status of the newborn in relation to gestational age, body weight, and maternal nutritional status. Am J Clin Nutr 1984;40:794-800.
53. Shirali GS, Oelberg DG, Mehta KP. Maternal-neonatal serum vitamin A concentrations. J Pediatr Gastroenterol Nutr 1989;9:62-66.
54. Jansson L, Nilsson B. Serum retinol and retinol-binding protein in mothers and infants at delivery. Biol Neonate 1983;43:269-271.
55. Whittaker PG, Lind T, Williams JG. Iron absorption during normal human pregnancy: a study using stable isotopes. Br J Nutr 1991;65:457-463.

56. Barrett JF, Whittaker PG, Williams JG, Lind T. Absorption of non-haem iron from food during normal pregnancy. BMJ 1994;309:79-82.
57. International Nutritional Anemia Consultative Group (INACG). Iron deficiency in women: a report of the INACG. Washington, DC: Nutrition Foundation, 1981.
58. Beard JL, Dawson H, Pinero DJ. Iron metabolism: a comprehensive review. Nutr Rev 1996;54:295-317.
59. Benito P, Miller D. Iron absorption and bioavailability: an updated review. Nutr Res 1998;18:581-603.
60. Yip R. Prevention and control of iron deficiency in developing countries (UNICEF). Curr Issues Public Health 1996;2:253-263.
61. Chanarin I, Rothman D. Further observations on the relation between iron and folate status in pregnancy. BMJ 1971;2:81-84.
62. Garn SM, Keating MT, Falkner F. Hematological status and pregnancy outcomes. Am J Clin Nutr 1981;34:115-117.
63. Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1986;1:992-995.
64. Scholl TO, Hediger ML. Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. Am J Clin Nutr 1994;59:492S-500S.
65. Singla PN, Tyagi M, Kumar A, Dash D, Shankar R. Fetal growth in maternal anaemia. J Trop Pediatr 1997;43:89-92.
66. Scholl TO, Hediger ML, Fischer RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr 1992;55:985-988.
67. Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and rerinatal mortality? J Nutr 2001;131:590S-601S.
68. Morton RE, Nysenbaum A, Price K. Iron status in the first year of life. J Pediatr Gastroenterol Nutr 1988;7:707-712.
69. Singla PN, Tyagi M, Shankar R, Dash D, Kumar A. Fetal iron status in maternal anemia. Acta Paediatr 1996;85:1327-1330.
70. Hokama T, Takenaka S, Hirayama K, et al. Iron status of newborns born to iron deficient anaemic mothers. J Trop Pediatr 1996;42:75-77.
71. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 2000;71:1280S-1284S.
72. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997;66:1178-1182.
73. British Nutrition Foundation. Iron, nutritional and physiological significance. London: Chapman & Hall, 1995.
74. Brown KH. WHO/UNICEF review on complementary feeding and suggestions for future research: WHO/UNICEF guidelines on complementary feeding. Pediatrics 2000;106:1290-1291.
75. Dettwyler KA, Fishman C. Infant feeding practices and growth. Ann Rev Anthropol 1992;21:171-204.
76. WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. Lancet 2000;355:451-455.
77. Allen LH. Maternal micronutrient malnutrition: effects on breast milk and infant nutrition and priorities for intervention. SCN News, Maternal and child nutrition (ACC/SCN) 1994;21:6-10.
78. Butte NF, Villalpando S, Wong WW, et al. Human milk intake and growth faltering of rural Mesoamerindian infants. Am J Clin Nutr 1992;55:1109-1116.
79. Butte NF, Garza C, Stuff JE, Smith EO, Nichols BL. Effect of maternal diet and body composition on lactational performance. Am J Clin Nutr 1984;39:296-306.
80. Dewey KG. Complementary feeding and infant growth and body composition. Pediatrics 2000;106:1281.
81. Kusin JA, Kardjati S, Renqvist UH, Van SWM, Koetsier D. Infant nutrition and growth. In: Kusin JA, Kardjati S, eds. Maternal and child nutrition in Madura, Indonesia. Amsterdam: Royal Tropical Institute, 1994:175-240.

82. Lutter CK, Mora JO, Habicht JP, Rasmussen KM, Robson DS, Herrera MG. Age-specific responsiveness of weight and length to nutritional supplementation. Am J Clin Nutr 1990;51:359-364.
83. Allen LH. Nutritional influences on linear growth: a general review. Eur J Clin Nutr 1994;48:75S-89S.
84. Walker SP, Grantham MS, Himes JH, Powell CA, Chang SM. Early childhood supplementation does not benefit the long-term growth of stunted children in Jamaica. J Nutr 1996;126:3017-3024.
85. Muhilal, Murdiana A, Azis I, Saidin S, Jahari AB, Karyadi D. Vitamin A-fortified monosodium glutamate and vitamin A status: a controlled field trial. Am J Clin Nutr 1988;48:1265-1270.
86. WHO. Vitamin A supplements. A guide to their use in the treatment and prevention of vitamin A deficiency and xerophthalmia. Geneva: WHO, 1988.
87. Sommer A, West KP-Jr. Vitamin A deficiency. Health, survival, and vision. New York/Oxford: Oxford University Press, 1996.
88. Stephensen CB. Vitamin a, infection, and immune function. Annu Rev Nutr 2001;21:167-192.
89. Humphrey JH, Agoestina T, Wu L, et al. Impact of neonatal vitamin A supplementation on infant morbidity and mortality. J Pediatr 1996;128:489-496.
90. Sommer A, Katz J, Tarwotjo I. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 1984;40:1090-1095.
91. Beaton GH, Martorell R, Aronson KJ, et al. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. ACC/SCN State-of-the-art series, Nutrition Policy discussion paper no.13. Geneva: ACC/SCN, 1993.
92. Rice AL, Stoltzfus RJ, de-Francisco A, Chakraborty J, Kjolhede CL, Wahed MA. Maternal vitamin A or beta-carotene supplementation in lactating bangladeshi women benefits mothers and infants but does not prevent subclinical deficiency. J Nutr 1999;129:356-365.
93. Roy SK, Islam A, Molla A, Akramuzzaman SM, Jahan F, Fuchs G. Impact of a single megadose of vitamin A at delivery on breastmilk of mothers and morbidity of their infants. Eur J Clin Nutr 1997;51:302-307.
94. Humphrey JH, Rice AL. Vitamin A supplementation of young infants. Lancet 2000;356:422-424.
95. WHO/CHD Immunisation-Linked Vitamin A Supplementation Study Group. Randomised trial to assess benefits and safety of vitamin A supplementation linked to immunisation in early infancy. Lancet 1998;352:1257-1263.
96. West KP-Jr, Katz J, Shrestha SR, et al. Mortality of infants <6 mo of age supplemented with vitamin A: a randomized, double-masked trial in Nepal. Am J Clin Nutr 1995;62:143-148.
97. Donnen P, Brasseur D, Dramaix M, et al. Vitamin A supplementation but not deworming improves growth of malnourished preschool children in eastern Zaire. J Nutr 1998;128:1320-1327.
98. Muhilal, Permeisih D, Idjradinata YR, Muherdiyantiningsih, Karyadi D. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. Am J Clin Nutr 1988;48:1271-1276.
99. Mwanri L, Worsley A, Ryan P, Masika J. Supplemental vitamin A improves anemia and growth in anemic school children in Tanzania. J Nutr 2000;130:2691-2696.
100. West KP-Jr, LeClerq SC, Shrestha SR, et al. Effects of vitamin A on growth of vitamin A-deficient children: field studies in Nepal. J Nutr 1997;127:1957-1965.
101. Grajeda R, Perez ER, Dewey KG. Delayed clamping of the umbilical cord improves hematologic status of Guatemalan infants at 2 mo of age. Am J Clin Nutr 1997;65:425-431.
102. Michaelsen KF, Milman N, Samuelson G. A longitudinal study of iron status in healthy Danish infants: effects of early iron status, growth velocity and dietary factors. Acta Paediatr 1995;84:1035-1044.
103. Lonnerdal B. Effects of maternal dietary intake on human milk composition. J Nutr 1986;116:499-513.
104. Siimes MA, Salmenpera L, Perheentupa J. Exclusive breast-feeding for 9 months: risk of iron deficiency. J Pediatr 1984;104:196-199.
105. Dewey KG, Cohen RJ, Rivera LL, Brown KH. Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras. Am J Clin Nutr 1998;67:878-884.
106. Dallman PR. Iron deficiency and the immune response. Am J Clin Nutr 1987;46:329-334.

107. Angeles IT, Schultink JW, Matulessi P, Gross R, Sastroamidjojo S. Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. Am J Clin Nutr 1993;58:339-342.
108. Chwang LC, Soemantri AG, Pollitt E. Iron supplementation and physical growth of rural Indonesian children. Am J Clin Nutr 1988;47:496-501.
109. Lawless JW, Latham MC, Stephenson LS, Kinoti SN, Pertet AM. Iron supplementation improves appetite and growth in anemic Kenyan primary school children. J Nutr 1994;124:645-654.
110. Tomkins A, Watson F. Malnutrition and infection, a review. ACC/SCN state-of-the-art series, nutrition policy discussion paper no.5. Geneva: ACC/SCN, 1989.
111. Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. Lancet 1993;341:1-4.
112. Lozoff B, Jimenez E, Wolf AW. Long-term developmental outcome of infants with iron deficiency. N Engl J Med 1991;325:687-694.
113. 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. A report of the International Nutritional Anemia Consultative Group. Washington, DC: International Life Science Institute, 1998.
114. Nabarro D, Howard P, Cassels C, Pant M, Wijga A, Padfield N. The importance of infections and environmental factors as possible determinants of growth retardation in children. In: Waterlow JC, ed. Linear growth retardation in less developed countries. Nestlé Nutrition Workshop Series vol. 14. New York: Raven Press, 1988.
115. Engle PL, Bentley M, Pelto G. The role of care in nutrition programmes: current research and a research agenda. Proc Nutr Soc 2000;59:25-35.
116. Stephensen CB. Burden of infection on growth failure. J Nutr 1999;129:534S-538S.
117. Brown KH, Stallings RY, de-Kanashiro HC, Lopez-de RG, Black RE. Effects of common illnesses on infants' energy intakes from breast milk and other foods during longitudinal community-based studies in Huascar (Lima), Peru. Am J Clin Nutr 1990;52:1005-1013.
118. Black RE, Brown KH, Becker S. Effects of diarrhea associated with specific enteropathogens on the growth of children in rural Bangladesh. Pediatrics 1984;73:799-805.
119. Rowland MG, Cole TJ, Whitehead RG. A quantitative study into the role of infection in determining nutritional status in Gambian village children. Br J Nutr 1977;37:441-450.
120. Rowland MG, Rowland SG, Cole TJ. Impact of infection on the growth of children from 0 to 2 years in an urban West African community. Am J Clin Nutr 1988;47:134-138.
121. Roodenburg AJ, West CE, Yu S, Beynen AC. Comparison between time-dependent changes in iron metabolism of rats as induced by marginal deficiency of either vitamin A or iron. Br J Nutr 1994;71:687-699.
122. Roodenburg AJ, West CE, Hovenier R, Beynen AC. Supplemental vitamin A enhances the recovery from iron deficiency in rats with chronic vitamin A deficiency. Br J Nutr 1996;75:623-636.
123. Bloem MW, Wedel M, Egger RJ, et al. Iron metabolism and vitamin A deficiency in children in northeast Thailand. Am J Clin Nutr 1989;50:332-338.
124. Bloem MW, Wedel M, van-Agtmaal EJ, Speek AJ, Saowakontha S, Schreurs WH. Vitamin A intervention: short-term effects of a single, oral, massive dose on iron metabolism. Am J Clin Nutr 1990;51:76-79.
125. Mejia LA, Chew F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. Am J Clin Nutr 1988;48:595-600.
126. Suharno D, West CE, Muhilal, Karyadi D, Hautvast JG. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet 1993;342:1325-1328.
127. World Bank. Indonesia at a glance and country brief, 2000. In: www.worldbank.org, accessed on 11/09/2001.
128. Kabupaten dati II Bogor. Kabupaten Bogor dalam angka tahun 1996. Kerjasama BPS kabupaten Bogor dengan BADAN perencanaan pembangunan Daerah, 1997.
129. WHO. Safe vitamin a dosage during pregnancy and lactation. Recommendations and report of a consultation. Geneva: WHO/Ottawa: Micronutrient Initiative, 1998.
130. Muhilal, Sumarno I, Komari. Review of surveys and supplementation studies of anaemia in Indonesia. Food Nutr Bull 1996;17:3-6.

131. Schultink W, van-der-Ree M, Matulessi P, Gross R. Low compliance with an iron-supplementation program: a study among pregnant women in Jakarta, Indonesia. Am J Clin Nutr 1993;57:135-139.
132. Gross R, Angeles-Agdeppa A, Schultink JW, Dillon D, Sastroamdijojo S. Daily versus weekly iron supplementation: Programmatic and economic implications for Indonesia. Food Nutr Bull 1997;18:64-70.
133. Schultink JW, Gross R. Iron deficiency alleviation in developing countries. Nutr Res Rev 1996;9:281-293.
134. Viteri FE, Liu X, Tolomei K, Martin A. True absorption and retention of supplemental iron is more efficient when iron is administered every three days rather than daily to iron-normal and iron-deficient rats. J Nutr 1995;125:82-91.
135. Viteri FE. Weekly compared with daily iron supplementation [comment]. Am J Clin Nutr 1996;63:610-612.
136. Liu XN, Liu PY. The effectiveness of weekly iron supplementation regimen in improving the iron status of Chinese children and pregnant women. Biomed Environ Sci 1996;9:341-347.
137. Ridwan E, Schultink W, Dillon D, Gross R. Effects of weekly iron supplementation on pregnant Indonesian women are similar to those of daily supplementation. Am J Clin Nutr 1996;63:884-890.
138. Viteri FE, Ali F, Tujague J. Long-term weekly iron supplementation improves and sustains nonpregnant women's iron status as well or better than currently recommended short-term daily supplementation. J Nutr 1999;129:2013-2020.

2

Vitamin A and iron supplementation of Indonesian pregnant women benefits vitamin A status of their infants

Marjanka K. Schmidt, Siti Muslimatun, Clive E. West, Werner Schultink and Joseph G.A.J. Hautvast

ABSTRACT Many Indonesian infants have an inadequate nutritional status, which may be due in part to inadequate maternal nutrition during pregnancy. This study was designed to investigate whether infant nutritional status can be improved by vitamin A and iron supplementation during gestation. Mothers of these infants from 5 villages had been randomly assigned on an individual basis to supervised, double blind supplementation once weekly from ~18 weeks of pregnancy until delivery. Supplementation comprised 120 mg iron and 500 μg folic acid with or without 4800 retinol equivalent vitamin A. Mothers of infants from 4 other villages who participated in the national iron/folic acid supplementation programme were also recruited; intake of tablets was not supervised. Anthropometric and biochemical parameters of infants and their mothers were assessed ~4 months after delivery. Infants of mothers supplemented with vitamin A plus iron had higher serum retinol concentrations than infants of mothers supplemented with iron alone. However the proportion of infants with serum retinol concentrations <0.70 μmol/L was >70% in all groups. Maternal and infant serum retinol concentrations were correlated. Iron status, weight and length of infants were similar in all groups. Iron status of girls was better than that of boys, but boys were heavier and longer. We conclude that supplementation with vitamin A in conjunction with iron supplementation of women during pregnancy benefits vitamin A status of their infants. However considering the large proportion of infants with marginal serum retinol concentrations it may still be necessary to increase their vitamin A intake.

In Indonesia many infants have an inadequate nutritional status as shown by the high prevalence of micronutrient deficiencies, underweight and stunting [1]. Low nutrient stores and growth retardation of infants may partly be caused by inadequate nutrition of their mothers during pregnancy [2,3]. A large proportion of Indonesian pregnant women suffer from iron deficiency anaemia and marginal vitamin A deficiency [1,4,5].

Adequate maternal vitamin A status during pregnancy is important for the development of the foetus and sufficient levels of vitamin A in breast milk [6]. The relationship between vitamin A status of pregnant women and that of their newborn infants is indicated by associations of serum retinol levels during pregnancy with foetal liver retinol concentrations and by a high correlation of vitamin A concentrations in cord and maternal serum [7,8]. In addition vitamin A supplementation of pregnant women has been shown to increase cord levels of vitamin A [9]. Vitamin A intake and status in the third trimester of pregnancy have been found to affect concentrations of vitamin A in breast milk [10].

Iron deficiency anaemia during pregnancy is associated with higher risk of low birth weight and preterm delivery [11]. In addition evidence is accumulating that maternal iron deficiency in pregnancy reduces foetal iron stores and this may influence development during the postnatal period [12]. In a placebo-controlled study in Niger, iron supplementation during pregnancy improved infant length and APGAR scores at birth, and serum ferritin concentration 3 months after birth [13]. Under conditions of poor diet quality, iron alone may not be effective in correcting iron deficiency anaemia during pregnancy [14]. Vitamin A supplementation has been found to improve haemoglobin concentrations of pregnant women [9,15] and vitamin A plus iron supplementation to benefit iron status of children [16]. Roodenburg *et al.* [17] confirmed the benefit of vitamin A for iron status in a study in rats and suggested that mobilisation of iron was improved by vitamin A supplementation.

In Indonesia iron supplementation during pregnancy is standard practice and therefore it is not ethical to conduct a study in which pregnant women are not provided with iron [14]. However compliance in the governmental programme is low and we found that supervised weekly iron supplementation performed similarly to the ongoing iron supplementation programme with respect to improving maternal iron status during pregnancy [18]. Considering the adverse effects of iron deficiency on mental and motor development of infants [19] it is important to assess the impact of any new iron supplementation regime on infant iron status.

We hypothesised that improving the vitamin A and iron status of pregnant women may also lead to an improvement in iron status, vitamin A status, length and weight of their infants after birth. The present study was designed to investigate whether infant nutritional status can be improved by weekly supplementation of their mothers during pregnancy with vitamin A and iron.

Materials and methods

Study design

The present study was carried out in infants from 9 rural villages in Leuwiliang subdistrict, Bogor district, West Java, Indonesia. Mothers of these infants had been supplemented with iron and folic acid, either with or without vitamin A, during pregnancy or had access to iron/folic acid tablets through the ongoing government programme. Explanation of the study had been given to the women at enrolment and only women who gave written informed consent participated. The research proposal was approved by the Ministry of Health Indonesia and the Ethical Committees of the Medical Faculty at the University of Indonesia and Wageningen University.

Initially 366 women were enrolled to participate in the study (**Figure 2.1**). A sample size of 120 women per group had been chosen to allow for 50% drop-out during the study period [18]. The present article reports the effect of supplementation on the nutritional status of infants at ~4 months of age. The results of the effect of supplementation with iron and vitamin A during pregnancy on biochemical status near term, including details of the study design, have been published elsewhere [18]. Briefly, women, who were 16-20 weeks pregnant, aged 17-35 years and parity <6, from 5 villages were randomly assigned on an individual basis to double-blind, weekly supplementation until delivery. Supplementation consisted of 2 tablets each containing 60 mg iron as ferrous sulphate and 250 μg folic acid with or without 2400 retinol equivalent (RE) vitamin A. WHO guidelines [20] allow a maximum weekly dose of 7500 RE. The intention was to supplement pregnant women with 6000 RE per week, based on advice of the Indonesian Ministry of Health. However owing to losses during storage vitamin A supplementation was found to be 4800 RE weekly. Tablet intake was supervised by volunteer health workers and monitored by field assistants. Health personnel and participants received clear instructions in order to avoid additional iron tablet intake. The average period of supplementation in these 'weekly' groups was 20 weeks; thus mothers had taken about 40 tablets. Women from the other 4 villages, referred to as the 'daily' group, were recruited at the same time. These women had free access to iron tablets from the health care services following government policy that pregnant women should receive 90-120 iron/folic acid tablets, containing 60 mg iron as ferrous sulphate and 250 μg folic acid. Tablet intake in this group was not supervised because this was an effectiveness trial comparing the weekly, supervised iron supplementation to the current programme. Adherence of tablet intake was assessed though interview during a postnatal home visit which revealed that the median tablet intake was 50 while only 17% of the subjects took >90 tablets [18].

nearest 0.1 cm by using a plastic measuring tape. At enrolment, height of mothers had been measured to the nearest 0.1 cm using a standing height measurement microtoise.

Infant health

Physicians from the community health centre (Puskesmas) examined the general health of the infants before blood sampling. Signs and symptoms examined were: running nose, fever, cough, upper respiratory tract infection, diarrhoea, skin disease such as diaper rash and dermatitis, and ear discharge. Besides the individual categories, the term 'sick' was defined as having at least 1 of the signs or symptoms mentioned above. Medical treatment was provided when necessary. At the time of the anthropometric assessment, the field assistants interviewed the mothers to assess whether they were still breast-feeding.

Data analysis

Normality of data distribution was checked by visual evaluation using a histogram with a normal curve. Serum ferritin and sTfR concentrations were not normally distributed; therefore these data were logarithmically transformed and reported as mean with 95% CI. In the analyses the weekly vitamin A plus iron group was compared with the weekly iron group to evaluate the effect of vitamin A supplementation. In addition, the weekly iron group was compared to the 'daily' group to evaluate the different iron supplementation regimes. Differences in age, anthropometric and biochemical variables between groups were evaluated by independent *t*-test. ANOVA was used to evaluate differences between groups or genders correcting for gender or group and age. Differences in proportions were evaluated by χ^2-test. Trends in biochemical variables of infants by age were evaluated using linear regression. Association of parameters of biochemical status within infants and between infants and their mothers was estimated by the Pearson correlation coefficient. To establish the determinants of infant serum ferritin and retinol concentrations stepwise multiple linear regression was applied. Because the weekly iron and 'daily' groups did not differ with respect to anthropometric and biochemical variables, groups were defined as vitamin A supplementation (1) or not (0), i.e. weekly vitamin A plus iron group, 1; weekly iron group, 0; 'daily' group, 0. Results did not change if we included the 3 groups as 2 dummy variables. Initially, variables of mothers during pregnancy, mothers at ~4 months postpartum and infants were included in the model. However, analysis revealed that association between maternal variables during pregnancy and infant variables were poor. Therefore, it was decided to include only variables of mothers at ~4 months postpartum and infants in the model. Variables of infants included in both models were age at the time of blood collection, gender, neonatal weight, weight at ~4 months of age, and haemoglobin, serum ferritin, serum sTfR and serum retinol concentrations. Variables of mothers included in both models were haemoglobin, serum ferritin, serum sTfR and serum retinol concentrations, and weight ~4 months postpartum and height. Prevalence of upper respiratory infection was included in the model for serum retinol concentrations. Neonatal length and length at ~4 months of age were not included in the model because they were highly correlated with neonatal weight and weight at ~4 months of age respectively ($r > 0.8$). Also, maternal mid-upper arm circumference was not included because it was highly correlated with maternal weight ~4 months postpartum ($r > 0.8$). Variables that were found to be significantly associated with the dependent variable in the stepwise multiple regression analysis were entered again in a model for each dependent variable and are presented. The SPSS software package (Windows version 7.5.2. SPSS Inc., Chicago, IL, USA) was used for all statistical analyses and a P value of <0.05 was considered as significant.

Results

Women were recruited in 6 rounds from November 1997 until May 1998, and blood samples for the postpartum assessment were taken between August 1998 and February 1999. From enrolment during pregnancy until ~4 months after delivery [3.7 (1.9-6.3) months; mean (range)] 22 (18%), 20 (17%) and 20 (16%) mothers and infants dropped out in the weekly vitamin A and iron group, weekly iron group and 'daily' group respectively. Drop-out did not differ among groups and was not related to gender of the infants. Of the infants eligible for the assessment at ~4 months of age, 28 (28%), 25 (25%) and 29 (28%) in the weekly vitamin A and iron, weekly iron and 'daily' groups respectively did not provide a blood sample or did not attend the assessment. We present data of infants (n=222) from whom sufficient blood was drawn for the assessment of haemoglobin concentration, and their mothers (**Figure 2.1**). Age, parity, anthropometric and biochemical variables of mothers and gender and anthropometric variables of infants either at the postnatal visit (measured n=326) or at ~4 months of age (measured n=276) did not significantly differ (corrected for age) between subjects included or excluded (data not shown). However, infants for whom data are reported had higher neonatal length than infants for whom data are not reported (49.6 ± 0.2 cm, n=222 versus 48.9 ± 0.2 cm, n=80; $P < 0.05$; mean ± SE).

Age and gender of infants was similar in all groups (**Table 2.1**). Infants in the weekly vitamin A plus iron group had higher serum retinol concentrations than the infants in the weekly iron group (**Table 2.1**). Serum retinol concentrations did not differ between the weekly iron and 'daily' group. The proportion of infants with a serum retinol concentration ≤0.35 μmol/L was 3%, 11%, 20% and with a serum retinol concentration ≤0.70 μmol/L was 71%, 84% and 78% in the weekly vitamin A and iron, weekly iron and 'daily' groups respectively.

Iron status, length and weight of infants did not differ between the weekly groups or between the weekly iron group and the 'daily' group (**Table 2.1**). The proportion of infants with a haemoglobin concentration <100 g/L and <110 g/L was similar in all groups, 29% and 68% respectively while the lowest value was 83 g/L. Only 3 infants had a serum ferritin concentration <12 μg/L. Serum ferritin and sTfR concentrations of infants were inversely correlated ($r = -0.420$, $P < 0.01$) in all groups. Iron status and serum retinol concentrations of mothers and infants did not differ between mothers in the 'daily' group who had taken less than 50 tablets and those who had taken more than 50 tablets during pregnancy.

Table 2.1 Biochemical and anthropometric characteristics of infants according to group or gender at ~4 months of age. From ~18 weeks of gestation their mothers had been supplemented with 2 tablets weekly containing 60 mg ferrous sulfate and 250 µg folic acid with or without 2400 retinol equivalent vitamin A. The 'daily' group had free access to iron tablets from the Indonesian governmental health services[1]

	Vitamin A + iron		Iron		'Daily'		Boys		Girls	
n (boys/girls)	72 (31/41)		76 (33/43)		74 (39/35)		103		119	
	Mean	SE / 95% CI	Mean	SE / 95% CI	Mean	SE / 95% CI	Mean	SE / 95% CI	Mean	SE / 95% CI
Age, months	3.6	0.1	3.8	0.1	3.7	0.1	3.8	0.1	3.7	0.1
Haemoglobin, *g/L*	105.0	1.0	105.0	0.9	105.7	1.2	105.6	0.9	104.9	0.8
Serum ferritin,[2] *µg/L*	74.0	60.3-90.7	72.3	60.6-86.4	73.3	60.0-89.7	60.3**	52.5-69.2	87.1	75.9-102.3
Serum sTfR,[3] *mg/L*	2.03	1.92-2.15	2.08	1.94-2.23	2.19	2.06-2.33	2.29**	2.19-2.40	1.95	1.86-2.04
Serum retinol,[4] *µmol/L*	0.62	0.02	0.54*	0.02	0.55*	0.02	0.61**	0.02	0.54	0.02
Length, *cm*	60.7	0.3	61.2	0.3	60.8	0.4	62.1**	0.2	59.8	0.2
Weight, *kg*	6.1	0.1	6.2	0.1	6.2	0.1	6.55**	0.06	5.83	0.06

[1]Data are presented as mean with SE or geometric mean with 95% confidence interval; sTfR, soluble transferrin receptor; [2]n=64, 69, 69, 93, 109; [3]n=61, 58, 56, 86, 89; [4]n=58, 63, 60, 88, 93; *Significantly different from weekly vitamin A and iron group corrected for gender, P <0.01 (ANOVA); **Significantly different between boys and girls corrected for group and age, P <0.01 (ANOVA).

Serum ferritin concentrations decreased (**Figure 2.2**) while haemoglobin (r =0.173) and serum sTfR concentrations (r =0.293) increased significantly (*P* <0.01) with age. Serum retinol concentrations did not change with age. Girls had a better iron status, defined by higher serum ferritin and lower serum sTfR concentrations, than boys. However boys had a higher serum retinol concentration and were longer and heavier than girls (**Table 2.1**).

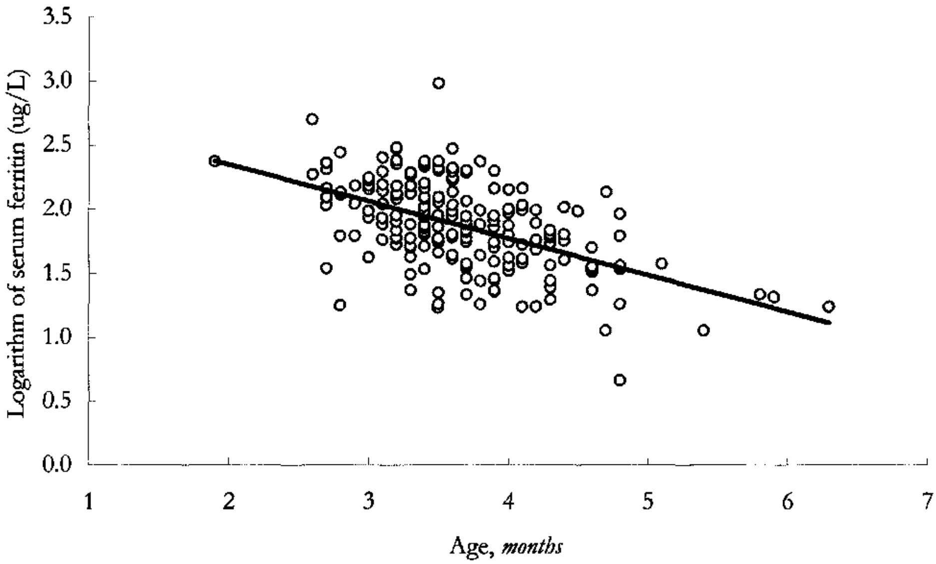

Figure 2.2 Relationship between serum ferritin concentration and age of infants (r =-0.520, *P* <0.01).

At ~4 months postpartum, iron status, serum retinol concentration and anthropometric variables of mothers did not differ between the weekly groups or between the weekly iron group and the 'daily' group (**Table 2.2**). At ~4 months postpartum serum retinol concentrations of mothers and infants were correlated (r =0.374, *P* <0.01) in all groups. Haemoglobin concentrations of mothers and infants were positively correlated (r =0.248, *P* <0.01), but if evaluated per group only reached significance in the weekly iron and vitamin A group and the 'daily' group.

Maternal serum retinol concentrations at enrolment and infant serum retinol concentrations were positively correlated in the vitamin A and iron group, weekly iron group and the 'daily' group (r =0.127, not significant; r =0.288, *P* <0.05; and r =0.438, *P* <0.01 respectively). None of the variables of iron status of mothers during pregnancy was correlated with infant nutritional status. Also when comparing anaemic (haemoglobin concentration <110 g/L) with non-anaemic mothers or comparing mothers with serum ferritin concentration <12 μg/L with those ≥12 μg/L, no differences in nutritional status of infants were found.

Table 2.2 Biochemical and anthropometric characteristics of mothers at ~4 months postpartum who had been supplemented with 2 tablets weekly each containing 60 mg ferrous sulfate and 250 μg folic acid with or without 2400 retinol equivalent vitamin A. The 'daily' group had free access to iron tablets from the Indonesian governmental health services[1]

	Vitamin A + iron		Iron		'Daily'	
n	72		76		74	
	Mean	SE / 95% CI	Mean	SE / 95% CI	Mean	SE / 95% CI
Haemoglobin,[2] *g/L*	118.8	1.4	120.4	1.6	118.7	1.5
Serum ferritin,[3] *μg/L*	12.3	9.5-15.8	10.8	8.5-13.7	14.5	11.5-18.2
Serum sTfR,[4] *mg/L*	2.07	1.90-2.26	2.04	1.86-2.23	1.99	1.82-2.17
Serum retinol,[5] *μmol/L*	1.14	0.04	1.11	0.05	1.03	0.05
Weight, *kg*	46.8	0.7	46.8	0.7	47.4	0.8
MUAC, *cm*	24.3	0.3	24.6	0.2	25.1	0.3
Height, *cm*	149.1	0.6	149.0	0.5	149.0	0.6

[1]Data are presented as mean with SE or geometric mean with 95% confidence interval; sTfR, soluble transferrin receptor; MUAC, mid-upper arm circumference; [2]n=71, 74, 74; [3]n=71, 75, 73; [4]n=69, 74, 73; [5]n=71, 73, 71.

Neonatal weight and length of infants were similar in all groups, 3.18 ± 0.50 kg (mean ± SD) and 49.6 ± 2.3 cm respectively. The time between birth and measurement of neonatal weight and length (7.3 ± 6.3 days) varied largely due to inadequate communication and difficulty in reaching the subjects. Only 20% of the infants were reached within 72 hours, measuring 3.01 kg and 48.6 cm, and 63% were reached within 7 days, measuring 3.07 kg and 49.0 cm.

A general health check was obtained from 217 of the 222 infants. Data could not be obtained from 5 infants because of the temporary absence of the physician. The proportion of infants with various signs and symptoms of disease were: running nose, 36%; fever, 7%; cough, 8%; upper respiratory tract infection, 5%; diarrhoea, 4%; skin diseases such as diaper rash and dermatitis, 24%; ear discharge, 2%; and sick (by definition having 1 or more sign or symptom of disease), 56%. Prevalence of these signs and symptoms and the proportion sick were similar in all groups. The serum retinol concentration of infants with upper respiratory tract infection (0.42 ± 0.04 μmol/L;

mean ± SE; n=11) was lower (*P* <0.01) than that of infants without (0.58 ± 0.01 µmol/L; n=167). In fact all infants with upper respiratory tract infection had a serum retinol concentration <0.70 µmol/L. Haemoglobin concentrations were consistently lower in infants with running nose, fever, cough, upper respiratory tract infection, diarrhoea, skin diseases and ear discharge. Infants defined as sick had a haemoglobin concentration 3.3 g/L less than their healthy counterparts (*P* <0.01; corrected for age). Serum sTfR and ferritin concentrations did not significantly differ between infants with or without any sign or symptom, after correction for sex and age, except that infants with skin diseases had higher (*P* <0.05) serum ferritin concentrations [(89.4 (71.3-112.1), geometric mean (95% CI)] than those without [68.7 (60.5-78.0)].

Table 2.3 Determinants of serum ferritin and retinol concentrations in infants from all groups at ~4 months of age[1]

	Coefficient (b)	SE	*P* value	R^2
Serum ferritin concentration[2], *µg/L* (n=172)				0.466
Age of infant at blood drawing, *months*	-0.200	0.032	0.000	
Neonatal weight, *kg*	0.194	0.041	0.000	
Gender (boy=0, girl=1)	0.124	0.040	0.002	
Serum sTfR concentration of infant[2], *mg/L*	-0.560	0.197	0.005	
Haemoglobin concentration of infant, *g/L*	-0.006	0.002	0.008	
Height of mother, *cm*	-0.012	0.004	0.002	
Serum retinol concentration, *µmol/L* (n=168)				0.351
Serum retinol concentration of mother, *µmol/L*	0.161	0.031	0.000	
Upper respiratory tract infection (not present=0, present=1)	-0.152	0.051	0.003	
Vitamin A supplementation (no vitamin A=0, vitamin A=1)	0.071	0.024	0.004	
Haemoglobin concentration of infant, *g/L*	0.004	0.001	0.005	
Infant weight ~4 months of age, *kg*	0.042	0.015	0.006	
Serum sTfR concentration of infant[2], *mg/L*	0.266	0.144	0.021	

[1]Stepwise multiple regression analysis; biochemical variables of mothers and infants were assessed ~4 months after delivery; sTfR, soluble transferrin receptor; [2]logarithmic values.

Determinants of serum retinol and ferritin concentrations in infants were evaluated by multiple regression analysis and are presented in **Table 2.3**. Age, neonatal weight and gender were found to be the strongest determinants of serum ferritin concentrations. Serum retinol concentrations of mothers ~4 months postpartum, upper respiratory tract infection and vitamin A supplementation showed the strongest association with serum retinol concentrations in infants. Serum retinol concentrations and weight of infants were also associated.

Discussion

The present study shows that vitamin A supplementation during pregnancy benefited serum retinol concentrations of infants at ~4 months of age. This is consistent with our finding that mothers in the weekly vitamin A and iron group maintained their serum retinol concentrations near term in contrast to mothers in the weekly iron group where levels decreased [18]. Our finding is also in line with the study of Katz *et al.* [22], showing that weekly supplementation with 7000 RE vitamin A of Nepalese women before and during pregnancy increased serum retinol concentrations in their infants at 3 months of age.

The difference in serum retinol concentrations of the infants in our study could have resulted from differences in the vitamin A stores built up during gestation, from differences in retinol concentrations in breast milk, or both [6,23]. It is not yet known to what extent vitamin A is transferred via the placenta [23]; however, the vitamin A status of pregnant women and that of their newborn infants are associated [7,8]. In addition, in an intervention study, Panth *et al.* [9] showed that vitamin A supplementation of pregnant women increased cord levels of vitamin A. A single dose of 60000 or 90000 RE to lactating women has been shown to increase their breast milk levels and improved infant vitamin A status even up to 6 to 8 months after supplementation [24,25]. Although women in our study were supplemented weekly during pregnancy the amount of vitamin A given (20 weeks x 4800 RE = 96000 RE) as well as the difference in serum retinol concentrations of infants were comparable [24,25]. In addition all infants but 1 were breast-fed and not given vitamin A-rich foods (data not shown). Therefore, we can assume that they depended on their stores and breast milk intake for vitamin A supply.

Vitamin A supplementation did not affect infant iron status, which is not surprising considering the small increase in haemoglobin and decrease in serum ferritin concentrations in their mothers during pregnancy [18]. Nutritional status of infants in the weekly iron group was similar to that of infants in the 'daily' group. This is in line with our finding that, in terms of improving iron status of mothers during pregnancy, supervised weekly iron supplementation performed no better or no worse than the national programme [18]. Infant and maternal iron status were not associated except for an association of haemoglobin concentrations of mothers ~4 months postpartum and infants. The relatively small range of maternal indicators of iron status and amount of iron taken during pregnancy, as compared to a placebo-controlled study in Niger in which pregnant women were supplemented with 100 mg Fe/d [13], may explain the lack of a relationship with infant iron status.

Infants are born with low reserves of vitamin A, even if their mothers have an adequate vitamin A status [6,26]. Therefore, a cut-off level of 0.35 μmol/L for infants after 1 month of age has been proposed [27]. Serum retinol concentrations of our infants were low but consistent with values in Bangladeshi, Indonesian and Zambian infants [28-30], although in the last study only 1% had serum retinol concentrations <0.35 μmol/L. In our study 3% of infants from mothers supplemented with vitamin A had levels <0.35 μmol/L as compared to >10% in the other 2 groups. It would be useful to compare serum retinol concentrations of our infants with those from developed countries, but unfortunately we are not aware of any publications reporting such data. The high proportion of infants with serum retinol concentrations ≤0.70 μmol/L would indicate this population as having a severe public health problem [31], although it should be taken into account that these criteria are based on children aged 6-71 months of age.

Results reported from various studies on the effect of vitamin A on growth of children are not consistent [32]. However, vitamin A supplementation has been found to improve growth of children in an area with vitamin A deficiency [33] or of children with xerophthalmia [34]. The association between weight and serum retinol concentrations suggests that vitamin A plays a role in growth, but probably the increase in serum retinol concentration was too small to have a significant effect. Supplementation of vitamin A has been shown to reduce duration of respiratory tract infection [28]. However, the negative association between serum retinol concentrations and respiratory tract infection might also be a consequence of infection-induced changes in nutrient transport [35].

Values of parameters of iron status we have reported are normal for infants of these ages [36]. Serum ferritin concentration as an indicator of iron status has been found to decrease with age and to be determined by gender and neonatal weight [37]. Although half of the mothers were anaemic or iron deficient during pregnancy [18], it seems that their infants had sufficient iron status at least until 4 months of age. We do not know to what extent serum ferritin concentrations of our infants were influenced by infection. Signs and symptoms of disease, except for skin disease, did not affect serum ferritin concentration of infants, but we did not monitor infection, for example, by measuring serum C-reactive protein concentrations. However, concentrations of sTfR provide a quantitative measure of iron status that is not affected by infection [21]. In general, sTfR concentrations in our infants were low, which is indicative of an adequate iron status, and only 4 infants had concentrations higher than reported reference values for children of 6 months to 2 years (1.48-3.51 mg/L) [21]. The strong inverse correlation of concentrations of serum ferritin and sTfR also suggests that the high serum ferritin values were mostly a reflection of high stores [38].

In conclusion, in a population with marginal vitamin A deficiency, weekly supplementation of women during pregnancy with 4800 RE vitamin A in the presence of concurrent iron supplementation benefits vitamin A status but not iron status, length or weight of their infants. Iron status of infants at ~4 months of age was considered sufficient irrespective of supplementation regime. The difference in serum retinol levels between infants from vitamin A supplemented pregnant mothers and their counterparts were similar as found in studies which provided supplementation to mothers during lactation [24,25]. Therefore, supplementation during pregnancy could be an alternative or an addition to supplementation during lactation and has the advantage that it can be implemented in the existing iron supplementation programme. However, considering the large proportion of infants with serum retinol concentration ≤0.70 μmol/L, we need to investigate the relevance of marginal serum retinol concentrations further in infants younger than 6 months, and whether it is necessary to increase vitamin A intake during infancy.

Acknowledgements
We are grateful to all participants, field workers, midwives and medical doctors of Leuwiliang for their contribution to this project. We are especially thankful to Bapak Nurdin Hadiat for his expertise and in particular for his careful drawing of blood. We thank the heads of Health Centres for providing the facilities during data collection.

References

1. ACC/SCN. Fourth report on the world nutrition situation. Nutrition throughout the life cycle. Geneva: ACC/SCN in collaboration with IFPRI, 2000.
2. Frongillo EA-Jr. Symposium: Causes and Etiology of Stunting. Introduction. J Nutr 1999;129:529S-530S.
3. Waterlow JC, Schürch B. Causes and mechanisms of linear growth retardation. Proceedings of an I/D/E/C/G workshop held in London January 15-18, 1993. Eur J Clin Nutr 1994;48:S1-S216.
4. Kosen S, Herman S, Schultink W. An overview of studies on iron deficiency in Indonesia. Nutr Res 1998;18:1935-1941.
5. Suharno D, West CE, Muhilal, et al. Cross-sectional study on the iron and vitamin A status of pregnant women in West Java, Indonesia. Am J Clin Nutr 1992;56:988-993.
6. Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. Am J Clin Nutr 1994;59:517S-522S.
7. Shah RS, Rajalakshmi R, Bhatt RV, et al. Liver stores of vitamin A in human fetuses in relation to gestational age, fetal size and maternal nutritional status. Br J Nutr 1987;58:181-189.
8. Shah RS, Rajalakshmi R. Vitamin A status of the newborn in relation to gestational age, body weight, and maternal nutritional status. Am J Clin Nutr 1984;40:794-800.
9. Panth M, Shatrugna V, Yasodhara P, Sivakumar B. Effect of vitamin A supplementation on haemoglobin and vitamin A levels during pregnancy. Br J Nutr 1990;64:351-358.
10. Ortega RM, Andres P, Martinez RM, Lopez-Sobaler AM. Vitamin A status during the third trimester of pregnancy in Spanish women: influence on concentrations of vitamin A in breast milk. Am J Clin Nutr 1997;66:564-568.
11. Scholl TO, Hediger ML. Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. Am J Clin Nutr 1994;59:492S-500S.
12. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 2000;71:1280S-1284S.
13. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997;66:1178-1182.
14. Yip R. Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. Am J Clin Nutr 2000;72:272S-278S.
15. Suharno D, West CE, Muhilal, Karyadi D, Hautvast JG. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet 1993;342:1325-1328.
16. Mejia LA, Chew F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. Am J Clin Nutr 1988;48:595-600.

17. Roodenburg AJ, West CE, Hovenier R, Beynen AC. Supplemental vitamin A enhances the recovery from iron deficiency in rats with chronic vitamin A deficiency. Br J Nutr 1996;75:623-636.
18. Muslimatun S, Schmidt MK, Schultink W, et al. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J Nutr 2001;131:85-90.
19. 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. A report of the International Nutritional Anemia Consultative Group. Washington, DC: International Life Science Institute, 1998.
20. WHO. Safe vitamin a dosage during pregnancy and lactation. Recommendations and report of a consultation. Geneva: WHO/Ottawa: Micronutrient Initiative, 1998.
21. Suominen P, Punnonen K, Rajamaki A, Majuri R, Hanninen V, Irjala K. Automated immunoturbidimetric method for measuring serum transferrin receptor. Clin Chem 1999;45:1302-1305.
22. Katz J, West KP-Jr, Khatry SK, et al. Maternal low-dose vitamin A or {beta}-carotene supplementation has no effect on fetal loss and early infant mortality: a randomized cluster trial in Nepal. Am J Clin Nutr 2000;71:1570-1576.
23. Azais-Braesco V, Pascal G. Vitamin A in pregnancy: requirements and safety limits. Am J Clin Nutr 2000;71:1325S-1333S.
24. Rice AL, Stoltzfus RJ, de-Francisco A, Chakraborty J, Kjolhede CL, Wahed MA. Maternal vitamin A or beta-carotene supplementation in lactating bangladeshi women benefits mothers and infants but does not prevent subclinical deficiency. J Nutr 1999;129:356-365.
25. Stoltzfus RJ, Hakimi M, Miller KW, et al. High dose vitamin A supplementation of breast-feeding Indonesian mothers: effects on the vitamin A status of mother and infant. J Nutr 1993;123:666-675.
26. Olson JA, Gunning DB, Tilton RA. Liver concentrations of vitamin A and carotenoids, as a function of age and other parameters, of American children who died of various causes. Am J Clin Nutr 1984;39:903-910.
27. Lindblad BS, Patel M, Hamadeh M, et al. Age and sex are important factors in determining normal retinol levels. J Trop Pediatr 1998;44:96-99.
28. Rahman MM, Mahalanabis D, Alvarez JO, et al. Acute respiratory infections prevent improvement of vitamin A status in young infants supplemented with vitamin A. J Nutr 1996;126:628-633.
29. Hadi H, Stoltzfus RJ, Dibley MJ, et al. Vitamin A supplementation selectively improves the linear growth of indonesian preschool children: results from a randomized controlled trial. Am J Clin Nutr 2000;71:507-513.
30. Hautvast JL, Tolboom JJ, West CE, Kafwembe EM, Sauerwein RW, van Staveren WA. Malaria is associated with reduced serum retinol levels in rural Zambian children. Int J Vitam Nutr Res 1998;68:384-388.
31. WHO. Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. WHO/NUT/96.10. Geneva: WHO, 1996.
32. Allen LH. Nutritional influences on linear growth: a general review. Eur J Clin Nutr 1994;48:75S-89S.
33. Muhilal, Permeisih D, Idjradinata YR, Muherdiyantiningsih, Karyadi D. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. Am J Clin Nutr 1988;48:1271-1276.
34. West KP-Jr, LeClerq SC, Shrestha SR, et al. Effects of vitamin A on growth of vitamin A-deficient children: field studies in Nepal. J Nutr 1997;127:1957-1965.
35. Stephensen CB, Gildengorin G. Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. Am J Clin Nutr 2000;72:1170-1178.
36. Siimes MA, Saarinen UM, Dallman PR. Relationship between hemoglobin concentration and transferrin saturation in iron-sufficient infants. Am J Clin Nutr 1979;32:2295-2300.
37. Wharf SG, Fox TE, Fairweather TS, Cook JD. Factors affecting iron stores in infants 4-18 months of age. Eur J Clin Nutr 1997;51:504-509.
38. Virtanen MA, Viinikka LU, Virtanen MK, et al. Higher concentrations of serum transferrin receptor in children than in adults. Am J Clin Nutr 1999;69:256-260.

3

Randomised double-blind trial of the effect of vitamin A supplementation of Indonesian pregnant women on morbidity and growth of their infants during the first year of life

Marjanka K. Schmidt, Siti Muslimatun, Werner Schultink, Clive E. West and Joseph G.A.J. Hautvast

ABSTRACT

Objective: To investigate whether supplementation with vitamin A together with iron of Indonesian pregnant women decreases morbidity and improves growth of their infants during the first year of life.

Design: Women from a rural area in West Java, Indonesia, had been randomly assigned on an individual basis to double blind supplementation once weekly from ~18 weeks of pregnancy until delivery. Supplementation comprised 120 mg iron and 500 μg folic acid with or without 4800 RE vitamin A. Their newborn infants were followed up during the first year of life: weight, length, morbidity and food intake were assessed monthly.

Results: Infants whose mothers had taken vitamin A supplements during pregnancy had similar weight, length, weight gain and growth as their counterparts during the follow-up period. The proportions of infants with reported symptoms of morbidity were similar in the vitamin A plus iron group and the iron group. In addition immunisation coverage and feeding mode did not differ between the groups. All infants were breast-fed but exclusive breast-feeding rapidly declined at 4 months of age. Infants with serum retinol concentrations >0.70 μmol/L increased their weight and length more during the first 6 months of life and had higher weight-for-age z-scores during the first year of life than infants with serum retinol concentrations ≤0.70 μmol/L. Serum retinol concentrations were not associated with morbidity.

Conclusion: In this study, vitamin A supplementation in conjunction with iron supplementation of pregnant women did not improve growth or reduce morbidity of their infants during the first year of life.

Vitamin A is one of the essential nutrients for health and development of infants [1], which is shown by the reduction in mortality and morbidity by vitamin A supplementation of infants aged 6 months and older in populations with vitamin A deficiency [2,3]. However the impact of vitamin A supplementation on mortality and morbidity of infants younger than 6 months is less conclusive [4-6]. Vitamin A supplementation during lactation has been shown to improve vitamin A status and reduce morbidity of infants through its effect on breast milk [7-9].

As reviewed before [10] the effect of vitamin A supplementation on growth is inconsistent. However vitamin A supplementation has been shown to improve growth of children in populations with vitamin A deficiency or xerophthalmia [11-14], suggesting that vitamin A supplementation may be beneficial for growth in populations in which vitamin A is a limiting factor.

In Indonesia, a large proportion of infants have micronutrient deficiencies and are underweight or stunted [15,16]. Inadequate maternal nutrition during pregnancy is thought to be one of the contributors to this [17-19]. Vitamin A supplementation of women before and during pregnancy has been shown to reduce maternal night blindness and mortality [20,21] and improve serum retinol levels in infants [22,23] (Chapter 2). However it did not reduce foetal loss or early infant mortality [22] and other outcomes of infants have not been reported so far.

Indonesia is one of the many countries that use iron supplementation for controlling maternal anaemia during pregnancy [24]. Supervised weekly iron supplementation has been shown to perform similar to the ongoing governmental iron supplementation programme in preventing iron deficiency anaemia [25]. In this study, we investigated whether vitamin A supplementation in concurrence of iron supplementation to Indonesian pregnant women decreases morbidity and improves growth of their infants during the first year of life.

Subjects and methods

Study design

The study was carried out in infants from 5 rural villages in Leuwiliang subdistrict, Bogor district, West Java, Indonesia. At ~17 weeks of pregnancy, their mothers had been randomly assigned on an individual basis to a double blind, supervised supplementation once weekly from enrolment until delivery. Supplementation comprised 120 mg iron as ferrous sulphate and 500 μg folic acid or the same amounts of iron and folic acid plus 4800 RE vitamin A. Details of the study design and the effect of supplementation on maternal biochemical status near term and infant biochemical status at ~4 months of age have been published elsewhere [23,25] (Chapter 2). After birth, infants were followed up during the first year of life. Growth, morbidity and nutrition were assessed monthly, while biochemical status was assessed at ~4 months of age. The study had been explained to the women at enrolment and only women who gave written informed consent were enrolled. The research was approved by the Ministry of Health Indonesia and the Ethical Committees of the Medical Faculty at the University of Indonesia and Wageningen University.

Monthly assessment

Weight and length of infants were measured and mothers interviewed for morbidity and food intake of their infants during their monthly visit to their neighbourhood health post (Posyandu). In principal visits for each health post were planned on the same date of each month. However, in practice visits were made within 1 week before or after the planned date. It was attempted to reach individual mothers who were not able to attend during a visit at their home.

Anthropometry Two pairs of trained field assistants measured weight and length of infants. Weight was measured to the nearest 50 g by using a baby weighing scale (Misaki, Japan) which was tared each time before use. Calibration with a standard weight (5 kg) at regular intervals showed that all scales were stable and precise. Recumbent length was measured to the nearest 0.1 cm by using a wooden length board (SEAMEO TROPMED). Infants were measured with light clothing. Neonatal weight and length of infants had been measured by 2 of the authors (MKS and SM) during a postnatal home visit [23] (Chapter 2). All field assistants had received training and were supervised every month by the 2 authors mentioned above. At each supervision the supervisors also independently measured length and weight in a random sub-sample of ~5% of the infants (total n=163). The mean technical error, expressed as a standard deviation ($SD=\sqrt{\sum d^2/2n}$, d=difference between paired measurements, n=number of infants) was 44 g and 0.70 cm. In addition SD of pooled duplicate measurements in a random sample by the field assistants (recorded n=25) was 35 g and 0.46 cm. The reliability coefficients of the anthropometric measurements were high, 0.967 and 0.997 for length and weight respectively [26].

Morbidity At the time of the anthropometric assessment the same field assistants interviewed the caretaker, in all but 2 cases the mother, of the infant about symptoms of morbidity in the 14 days preceding the visit. More specifically, mothers were asked to recall: diarrhoea, defined as ≥3 stools that were more liquid than usual in one day; fever, mother's evaluation of infant's body temperature above normal (hot to the touch; 'panas'); running nose ('pilek'), nasal discharge; cough, persistent coughing; difficulty breathing, breathing with severe noise or wheezing or difficulty inhaling; ear discharge, fluid or pus draining from at least one ear; or vomiting. Questions were asked in Bahasa Indonesia or in the local language (Sundanese) if the mother did not understand the term. First the interviewer asked the mother for any symptoms and then asked her specifically for each category and for how many days the infant suffered from that specific symptom. Episodes were defined as a period of sickness separated by at least 3 days. Morbidity incidence rates were determined as total number of episodes divided by the number of days at risk. Days at risk were defined as the number of days on which morbidity was assessed (number of interviews x 14 days) minus the number of days not at risk, i.e. the total of days on which a symptom was reported plus 3 days for each separate episode.

In addition mothers were asked whether the infant had received immunisation since the last interview. Data on immunisation were crosschecked against the Posyandu record of growth and immunisation. If inconsistencies were found data from the record were used. Mortality of infants was recorded at a home visit after death had been reported.

Breast-feeding and food intake Together with the morbidity interview the mother was asked whether the infant was still breast-fed and whether the infant received any other liquid or food in the 14 days preceding the visit. Data on breast-feeding and food intake had also been collected at the postnatal home visit. The type of liquid or food was recorded using a pre-coded questionnaire. Infants were categorised as exclusively breast-fed, predominantly breast-fed (i.e. receiving breast milk plus water, tea, coffee or fruit juices, but no artificial milk), complementary fed, or non breast-fed [27].

Biochemical measures
At ~4 months of age, non-fasting venous blood samples (~2 mL) were taken from infants and collected in a tube without anticoagulant between 09.00 and 12.00 h. It was not possible to obtain blood from all subjects due to maternal refusal and in some cases the amount of blood obtained was insufficient for all analyses. Haemoglobin was determined using the cyanmethemoglobin method (Test 3317; Merck, Darmstadt, Germany) at the Nutrition Research and Development Center laboratory, Bogor. For serum ferritin, soluble transferrin receptor and retinol analyses, blood was allowed to clot before it was placed in a cool box with cooling elements for transport to the laboratory in Bogor. Blood samples were centrifuged at 3000 *g* for 10 minutes at room temperature and serum was distributed among three vials. Serum was kept at -20°C for 1 month and subsequently at -79°C. All analyses were carried out within 1 year of blood collection and have been described earlier [23] (Chapter 2).

Data analysis
Infants whose mothers had been supplemented during pregnancy with vitamin A plus iron (n=107) or iron alone (n=105) and who attended at least once the assessment at the health post were included for analysis (**Figure 3.1**). Infant's weights and lengths were converted into z-scores using the NCHS/WHO reference data incorporated in the Epi-Info software (Epi Info2000 version 1.0.5. CDC, Atlanta). NCHS/WHO reference data were also used to construct a reference curve weighed for the number of boys and girls in the study population. For analysis, follow-up data of infants were grouped into monthly age groups of 0 (0-0.99 months) to 12 (12-12.99 months). For additional analysis and presentation, morbidity and breast-feeding data were pooled into 4 age categories reflecting the different periods of mode of feeding and vulnerability to morbidity: 0-3, 4-6, 7-9 and 10-12 months. Differences in age, anthropometric and biochemical variables between groups were evaluated by independent *t*-test. Differences in proportion were evaluated by χ^2-test. Data of morbidity were not normally distributed, therefore differences between groups were evaluated using non-parametric tests. For the analysis of growth and morbidity in relationship with serum retinol concentrations data of all infants were pooled and evaluated by *t*-test, ANOVA and multiple regression analysis as appropriate. The SPSS software package (Windows version 7.5.2. SPSS Inc., Chicago, IL) was used for all statistical analyses and a P value of <0.05 was considered as significant.

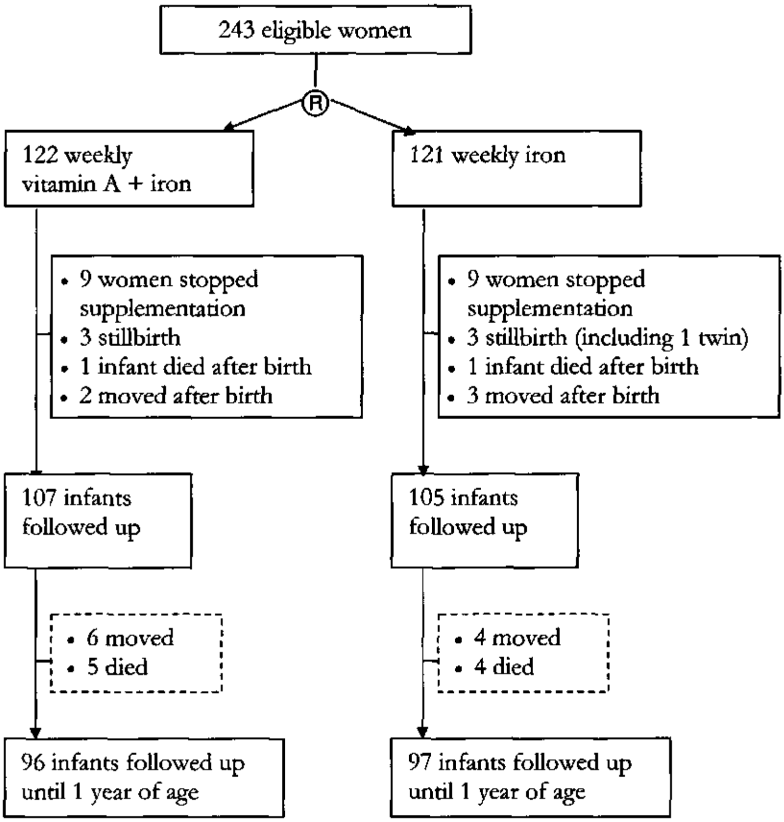

Figure 3.1 Study profile.

Results

Infants were followed up at least until 1 year of age from May 1998 until November 1999. During this period 11 infants in the vitamin A plus iron group and 8 infants in the iron group were lost to follow-up as a result of emigration from the research area or death (**Figure 3.1**). Infants were measured monthly but, because visits were not always on the same date, and due to temporary absence, refusal of the mother or sickness of the infant, some of the infants were not measured. In total 2330 anthropometric assessments, including 219 measurements at the postnatal visit, and 2188 morbidity interviews were made during the first year of follow-up.

The coverage of anthropometric measurements of infants at each monthly visit was >80%. Coverage or gender of infants did not differ between the vitamin A plus iron group and iron group at any monthly visit. We obtained 11-14 anthropometry measurements from 81.6% of the infants, 8-10 from 11.8% and 2-7 from 6.6%. The mean (SE) number of measurements of 11.5 (0.3) and period of follow-up of 11.3 (0.3) months in the vitamin A plus iron group were similar to those in the iron group, 11.9 (0.2) and 11.6 (0.2) months respectively.

Length and weight of infants who were supplemented with vitamin A during gestation did not differ from their counterparts at any age during the first year of life (**Figure 3.2**). During the 1 year follow-up the mean increase in length and weight gain of infants in the vitamin A plus iron group, 20.8 (0.5) cm (SD) and 4.78 (0.12) kg respectively, were similar to those in the iron group, 21.3 (0.4) cm and 4.83 (0.11) kg respectively. Selection of infants who had been followed up for ≥10 months or excluding infants who dropped out because of death or migration did not alter these results. Weight and length of all infants started to deviate below the NSCH/WHO reference population at 5 months of age (**Figure 3.2**). At 12 months of age 23 and 33% of the infants had z-scores <-2 for height-for-age and weight-for-age respectively.

The proportion of infants with reported health complaints were similar in both groups (**Table 3.1**). Except that there were small inconsistent differences in reporting of cough between groups at 0-6 months of age (**Table 3.1**). The results were consistent if expressed and analysed as number of days, number of episodes or number of days per episode or incidence ratio (**Table 3.2**). Symptoms of morbidity were not related to gender of the infant.

Immunisation coverage was similar in both groups, 90.5% of the infants had at least received 1 vaccination of BCG (bacille Calmette-Guérin), oral polio and DTP (diphtheria, pertussis and tetanus) during the first year of life. Only 6.7% of the infants had already fully complied with the immunisation schedule, i.e. 1 BCG, 3 DTP, 4 polio, 1 measles and 3 hepatitis, at 1 year of age. During the study, 9 infants in the vitamin A plus iron group and 9 infants in the iron group received a vitamin A supplement from the midwives. This occurred at a mean age of 10.4 (3.9) months.

At the time of the postnatal visit, 208 of the 219 mothers were already breast-feeding their infants but 26 of these discarded some of the colostrum. A large proportion (84%) of mothers also gave some form of prelacteal feeding to their infants: formula milk, 7%; water, 14%; honey, 71%; banana, 14%; or other food or drinks, 34%. All but 1 infant were breast-fed at least until 6 months of age. The proportion of infants that were exclusively breast-fed, predominantly breast-fed, complementary fed or non breast-fed did not differ between the groups (**Table 3.3**). If we consider an infant no longer to be breast-fed exclusively because of having received prelacteal feeding, the proportion of infants that were exclusively breast-fed was small in both groups (**Table 3.3**). In both groups exclusive breast-feeding, whether or not taking into account prelacteal feeding, declined rapidly from 4 months of age while complementary feeding increased (**Table 3.3**).

Boys increased their length more and gained more weight than girls during the first year of life (**Table 3.4**). During the first 6 months of life, infants with serum retinol concentrations >0.70 μmol/L increased their weight and length more than those with serum retinol concentrations ≤0.70 μmol/L (**Table 3.4**). These differences remained significant if correcting for neonatal weight or length, serum ferritin and haemoglobin concentrations, supplementation group and gender. In addition infants with serum retinol concentrations >0.70 µmol/L had higher z-scores for weight-for-age during the first year of life (**Figure 3.3**); similar trends were seen for height-for-age and weight-for-height z-scores. Infants with serum retinol concentrations >0.70 µmol/L had similar prevalences of morbidity and did not differ with respect to type of feeding compared to infants with serum retinol concentrations ≤0.70 µmol/L (data not shown).

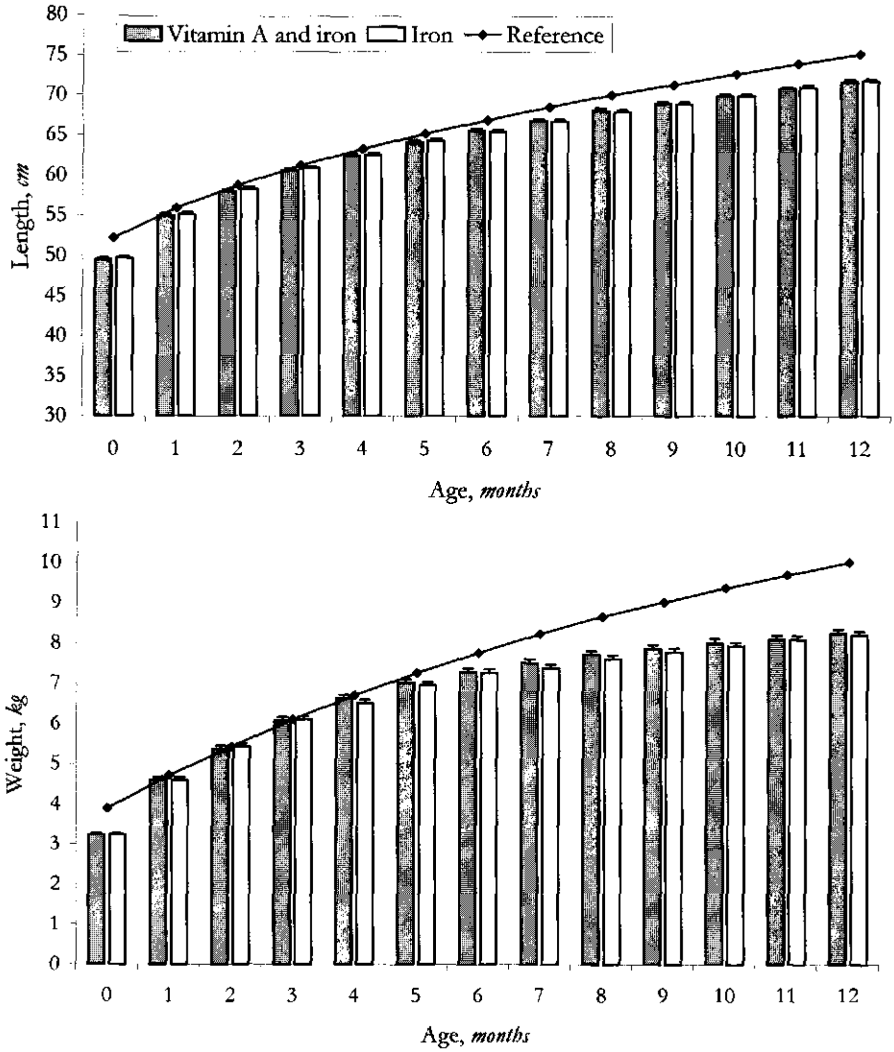

Figure 3.2 Mean (SE) length and weight of infants from 0 to 12 months in the vitamin A plus iron group, iron group and reference values based on the NCHS standards.

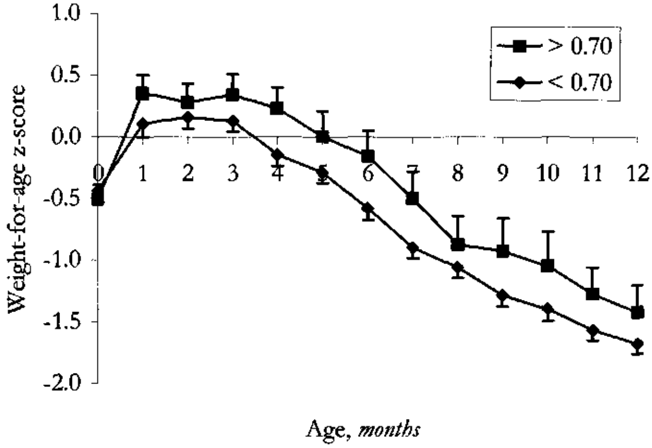

Figure 3.3 Mean (SE) weight-for-age Z-scores at 0 to 12 months of age of infants with serum retinol concentrations >0.70 μmol/L and ≤0.70 μmol/L; differences evaluated by t-test, at 4 months: P =0.066, at 6 months: P =0.045, at 7 months: P =0.052.

Table 3.1 Proportion of infants in the vitamin A plus iron group and iron group suffering from selected symptoms of morbidity by age category

	Vitamin A + iron				Iron			
Age category (months)	0-3	4-6	7-9	10-12	0-3	4-6	7-9	10-12
n infant	105	99	98	96	103	100	100	96
n interview	273	281	275	254	274	283	284	264
%								
Running nose	49.1	63.0	56.4	66.5	42.0	64.3	62.7	59.1
Fever	30.0	48.8	42.5	42.5	28.5	49.1	50.4	40.9
Cough	20.1*	26.0†	27.3	29.9	13.5	35.0	29.6	29.9
Diarrhoea	8.8	13.9	11.6	10.6	5.8	14.8	14.1	14.0
Vomiting	5.5	6.0	7.3‡	13.0	4.4	6.7	13.0	14.0
Difficulty breathing	3.3	3.6	5.6	4.7	3.3	2.5	6.7	4.2
Ear discharge	0	0.4	0.7	0.4	0	0	0	0

*Difference between the vitamin A plus iron group and iron group by χ^2-test, P =0.040, †P =0.022, ‡P =0.026.

Table 3.2 Morbidity incidence (episodes per 100 infant days) of infants in the vitamin A plus iron group and iron group during the follow-up period

	Number of episodes per 100 infant days		Incidence risk ratio (95% confidence interval)
	Vitamin A plus iron	Iron	
Running nose	6.53	6.17	1.06 (0.95-1.18)
Fever	3.80	3.91	0.97 (0.86-1.11)
Cough	2.21	2.31	0.96 (0.81-1.13)
Diarrhoea	0.86	0.93	0.92 (0.72-1.18)
Vomiting	0.58	0.72	0.81 (0.61-1.07)
Difficulty breathing	0.27	0.30	0.90 (0.59-1.38)

Table 3.3 Proportion of different feeding modes of infants in the vitamin A plus iron group and iron group by age category

	Vitamin A + iron				Iron			
Age category (months)	0-3	4-6	7-9	10-12	0-3	4-6	7-9	10-12
%								
Taking into account prelacteal feeding								
Exclusively breastfeeding†	9.1	0.4	0	0	13.2	2.5*	0	0
Predominantly breastfeeding†	49.1	13.8	4.4	1.6	44.5	13.0	6.0	3.4
Not taking into account prelacteal feeding								
Exclusively breastfeeding†	54.2	8.2	1.5	0	53.3	10.2	2.1	0.7
Predominantly breastfeeding‡	4.0	6.0	2.9	1.6	4.4	5.3	3.9	2.7
Complementary feeding†	41.8	85.4	94.2	94.9	41.6	83.4	92.6	93.6
Non breastfeeding‡	0	0.4	1.4	3.5	0.7	1.1	1.4	3.0

*Different between groups by χ^2-test, P =0.034; †Trends in vitamin A plus iron group and iron group by χ^2-test, P =0.000; ‡Trends in vitamin A plus iron group by χ^2-test, P =0.030.

Table 3.4 Weight gain and length increase by gender and serum retinol concentrations of infants during the first year of life*

	Weight gain (kg)		Length increase (cm)	
	0-12 months	0-6 months	0-12 months	0-6 months
Gender†				
Boys	5.27 ± 0.09 (71)[1]	4.28 ± 0.09 (78)[2]	22.7 ± 0.3 (71)[3]	16.5 ± 0.2 (78)[4]
Girls	4.84 ± 0.08 (92)	3.94 ± 0.07 (101)	21.9 ± 0.2 (91)	15.8 ± 0.2 (100)
Serum retinol concentrations††				
≤0.70 μmol/L	4.94 ± 0.08 (80)	3.99 ± 0.07 (87)[5]	22.1 ± 0.3 (79)	15.7 ± 0.2 (86)[6]
>0.70 μmol/L	5.20 ± 0.23 (21)	4.34 ± 0.19 (23)	22.8 ± 0.5 (21)	16.6 ± 0.4 (23)

*Mean ± SE (n); †Significant difference between gender by *t*-test or ††serum retinol concentrations by ANOVA corrected for gender, [1]P =0.001, [2]P =0.002, [3]P =0.023, [4]P =0.017, [5]P =0.036, [6]P =0.048.

Discussion

In this study we demonstrated that weekly supplementation of pregnant women in Indonesia with vitamin A did not improve growth or reduce morbidity in their infants during the first year of life. This is in line with the findings of Katz *et al.* [22], who found that foetal loss and early infant mortality were not reduced when Nepalese women were supplemented weekly with vitamin A during pregnancy. However vitamin A supplementation was beneficial in terms of reducing mortality in pregnant women [20]. In addition vitamin A supplementation has been shown to reduce morbidity and mortality in children under 5 years old [2,3]. However it should be noted that this reduction in morbidity and mortality is only seen in children above the age of 6 months. Earlier we reported a significant but small difference in serum retinol concentrations of infants of the vitamin A plus iron group and iron group [23] (Chapter 2). However the amount of vitamin A given may have been too small to provoke an additional impact on infant growth or morbidity. On the other hand even infants given a large dose of vitamin A only had a small increase in serum retinol concentration and similar morbidity compared to infants given a placebo [28]. In addition a large trial in which infants were supplemented 4 times with 25000 IU vitamin A and their mothers once with 200000 IU did not show any effect on anthropometric status or morbidity of infants, although vitamin A status at 6 months of age improved [5].

In our study, we supplemented pregnant women with 4800 RE vitamin A each week. This amount is in line with WHO guidelines [29], which allow a maximum weekly dose of 7500 RE, and does not exceed the Indonesian RDA of 700 RE per day [30]. Although the amount of vitamin A given may have been too small to have an impact on infant growth or morbidity, intake during pregnancy cannot be increased because of the teratogenic effects of vitamin A [29].

Iron supplementation of both groups could partly explain the lack of effect of vitamin A supplementation on growth and morbidity [31]. In a study in Tanzanian school children, vitamin A plus iron supplementation also did not have an impact an growth compared to iron supplementation [13]. Increase in iron stores of the infant through maternal iron supplementation during pregnancy may also have improved growth of our infants [32,33].

The relationship of xerophthalmia, which is indicative of severe vitamin A deficiency, with stunting, wasting and smaller increases in length and weight than non-xeropthalmic counterparts has been established [34,35]. We have shown that infants with serum retinol concentrations >0.70 μmol/L increased their weight and length more and had higher weight-for-age z-scores until 1 year of age as compared to their counterparts. More of these infants were in the vitamin A plus iron group (26%) than in the iron group (16%); however, this difference was not significant (P =0.241). Infants started growth faltering and becoming underweight at 4-6 months of age as is typically seen in Southeast Asian and populations of other developing countries, and has been associated with factors such as nutrition, morbidity, prenatal nutrition, birth weight and parental height [36-39]. The use of the NCHS/WHO reference has been criticised because it is not based on breast-fed infants, but if we compared our infants to the WHO 12 months-breast-fed pooled data the same trends were visible, although the deterioration was smaller (data not shown) [40].

The use of parental report as a method to establish morbidity has been suggested to be one of the reasons for the inconsistencies found in the effect of vitamin A supplementation on morbidity in community-based studies [41]. However mothers have been found to report sickness of their children quite well [42] and as education levels and other characteristics of mothers in both groups were comparable (data not shown), we do not expect that over- or under-reporting would be different between the groups. Morbidity prevalence could have been underestimated because we interviewed mothers only once a month about the past 14 days [43]. However incidence of cough was comparable to that reported for infants from developing countries [5]. Also other studies in which infants were supplemented with vitamin A did not show an effect on diarrhoea or respiratory diseases [44,45], or only an effect on duration of acute respiratory infections [46].

The type of feeding during the first year of life and immunisation coverage were comparable between the groups, thus these could not have accounted for the lack of difference in growth and morbidity of infants. We conducted 2-weeks recalls using a pre-coded questionnaire, because a trial of 24-hour recalls in mothers showed that this would be a more reliable measure of intake of foods other than breast milk. Although our method had the disadvantage of relying more on the mothers memory, obtaining dietary information for the 24 hours before each visit may overestimate exclusive breast-feeding because some infants may receive fluids or foods on less than daily basis [47].

Until 5 months of age infants in this population grew well, but thereafter growth faltering occurred, therefore further research is necessary regarding improvement of growth of infants after 4-6 months of age. The association between serum retinol concentrations and growth indicates that vitamin A plays a role in growth [34]. However our study showed that in populations that are marginal vitamin A deficient [25] vitamin A supplementation in conjunction with iron supplementation during pregnancy on community level has no impact on growth or morbidity of infants.

Acknowledgements

We are grateful to all mothers and infants who participated in this study for their willingness to attend our monthly measurements. We thank the field workers, voluntary health workers and midwives of Leuwiliang for their contribution to this project.

References

1. Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. Am J Clin Nutr 1994;59:517S-522S.
2. Beaton GH, Martorell R, Aronson KJ, et al. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. ACC/SCN State-of-the-art series, Nutrition Policy discussion paper no 13. Geneva: ACC/SCN, 1993.
3. Glasziou PP, Mackerras DE. Vitamin A supplementation in infectious diseases: a meta-analysis. BMJ 1993;306:366-370.
4. Humphrey JH, Rice AL. Vitamin A supplementation of young infants. Lancet 2000;356:422-424.
5. WHO/CHD Immunisation-Linked Vitamin A Supplementation Study Group. Randomised trial to assess benefits and safety of vitamin A supplementation linked to immunisation in early infancy. Lancet 1998;352:1257-1263.
6. West KP-Jr, Katz J, Shrestha SR, et al. Mortality of infants <6 mo of age supplemented with vitamin A: a randomized, double-masked trial in Nepal. Am J Clin Nutr 1995;62:143-148.
7. Rice AL, Stoltzfus RJ, de-Francisco A, Chakraborty J, Kjolhede CL, Wahed MA. Maternal vitamin A or beta-carotene supplementation in lactating bangladeshi women benefits mothers and infants but does not prevent subclinical deficiency. J Nutr 1999;129:356-365.
8. Roy SK, Islam A, Molla A, Akramuzzaman SM, Jahan F, Fuchs G. Impact of a single megadose of vitamin A at delivery on breastmilk of mothers and morbidity of their infants. Eur J Clin Nutr 1997;51:302-307.
9. Stoltzfus RJ, Hakimi M, Miller KW, et al. High dose vitamin A supplementation of breast-feeding Indonesian mothers: effects on the vitamin A status of mother and infant. J Nutr 1993;123:666-675.
10. Allen LH. Nutritional influences on linear growth: a general review. Eur J Clin Nutr 1994;48:75S-89S.
11. Donnen P, Brasseur D, Dramaix M, et al. Vitamin A supplementation but not deworming improves growth of malnourished preschool children in eastern Zaire. J Nut 1998;128:1320-1327.
12. Muhilal, Permeisih D, Idjradinata YR, Muherdiyantiningsih, Karyadi D. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. Am J Clin Nutr 1988;48:1271-1276.
13. Mwanri L, Worsley A, Ryan P, Masika J. Supplemental vitamin A improves anemia and growth in anemic school children in Tanzania. J Nutr 2000;130:2691-2696.
14. West KP-Jr, LeClerq SC, Shrestha SR, et al. Effects of vitamin A on growth of vitamin A-deficient children: field studies in Nepal. J Nutr 1997;127:1957-1965.
15. WHO. Global database on child growth and malnutrition. Geneva: WHO, 2000.
16. ACC/SCN. Fourth report on the world nutrition situation. Nutrition throughout the life cycle. Geneva: ACC/SCN in collaboration with IFPRI, 2000.

17. Frongillo EA-Jr. Symposium: Causes and Etiology of Stunting. Introduction. J Nutr 1999;129:529S-530S.
18. Waterlow JC, Schürch B. Causes and mechanisms of linear growth retardation. Proceedings of an I/D/E/C/G workshop held in London January 15-18, 1993. Eur J Clin Nutr 1994;48:S1-S216.
19. Kusin JA, Kardjati S, Houtkooper JM, Renqvist UH. Energy supplementation during pregnancy and postnatal growth. Lancet 1992;340:623-626.
20. West KP-Jr, Katz J, Khatry SK, et al. Double blind, cluster randomised trial of low dose supplementation with vitamin A or beta carotene on mortality related to pregnancy in Nepal. The NNIPS-2 Study Group. BMJ 1999;318:570-575.
21. Christian P, West KP- Jr, Khatry SK, et al. Vitamin A or beta-carotene supplementation reduces but does not eliminate maternal night blindness in Nepal. J Nutr 1998;128:1458-1463.
22. Katz J, West KP-Jr, Khatry SK, et al. Maternal low-dose vitamin A or {beta}-carotene supplementation has no effect on fetal loss and early infant mortality: a randomized cluster trial in Nepal. Am J Clin Nutr 2000;71:1570-1576.
23. Schmidt MK, Muslimatun S, West CE, Schultink W, Hautvast JGAJ. Vitamin A and iron supplementation of Indonesian pregnant women benefits vitamin A status of their infants. Br J Nutr 2001;86:in press.
24. Yip R. Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. Am J Clin Nutr 2000;72:272S-278S.
25. Muslimatun S, Schmidt MK, Schultink W, et al. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J Nutr 2001;131:85-90.
26. Marks GC, Habicht JP, Mueller WH. Reliability, dependability, and precision of anthropometric measurements. The Second National Health and Nutrition Examination Survey 1976-1980. Am J Epidemiol 1989;130:578-587.
27. Labbok M, Krasovec K. Toward consistency in breastfeeding definitions. Stud Fam Plann 1990;21:226-230.
28. Rahman MM, Mahalanabis D, Wahed MA, Islam MA, Habte D. Administration of 25,000 IU vitamin A doses at routine immunisation in young infants. Eur J Clin Nutr 1995;49:439-445.
29. WHO. Safe vitamin a dosage during pregnancy and lactation. Recommendations and report of a consultation. Geneva: WHO/Ottawa: Micronutrient Initiative, 1998.
30. LIPI. Widya Karya Pangan dan Gizi. Indonesia: LIPI, 1994.
31. Angeles IT, Schultink JW, Matulessi P, Gross R, Sastroamidjojo S. Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. Am J Clin Nutr 1993;58:339-342.
32. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 2000;71:1280S-1284S.
33. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997;66:1178-1182.
34. Sommer A, West KP-Jr. Vitamin A deficiency. Health, survival, and vision. New York/Oxford: Oxford University Press, 1996.
35. Tarwotjo I, Katz J, West KP-Jr, Tielsch JM, Sommer A. Xerophthalmia and growth in preschool Indonesian children. Am J Clin Nutr 1992;55:1142-1146.
36. Adair LS, Guilkey DK. Age-specific determinants of stunting in Filipino children. J Nutr 1997;127:314-320.
37. Dewey KG. Cross-cultural patterns of growth and nutritional status of breast-fed infants. Am J Clin Nutr 1998;67:10-17.
38. Hop LT, Gross R, Giay T, Sastroamidjojo S, Schultink W, Lang NT. Premature Complementary Feeding Is Associated with Poorer Growth of Vietnamese Children. J Nutr 2000;130:2683-2690.
39. Kusin JA, Kardjati S, Renqvist UH, Van SWM, Koetsier D. Infant nutrition and growth. In: Kusin JA, Kardjati S, eds. Maternal and child nutrition in Madura, Indonesia. Amsterdam: Royal Tropical Institute, 1994:175-240.
40. WHO Working Group on Infant Growth. An evaluation of infant growth; A summary of analyses performed in preparation for the WHO Expert Committee on Physical status: the use and interpretation of anthropometry in infants. Geneva: Nutrition Unit of the WHO, 1994.

41. Semba RD. Vitamin A, immunity, and infection. Clin Infect Dis 1994;19:489-499.
42. Kalter HD, Gray RH, Black RE, Gultiano SA. Validation of the diagnosis of childhood morbidity using maternal health interviews. Int J Epidemiol 1991;20:193-198.
43. Ruel MT, Rivera JA, Santizo MC, Lonnerdal B, Brown KH. Impact of zinc supplementation on morbidity from diarrhea and respiratory infections among rural Guatemalan children. Pediatrics 1997;99:808-813.
44. Coutsoudis A, Adhikari M, Pillay K, Kuhn L, Coovadia HM. Effect of vitamin A supplementation on morbidity of low-birth-weight neonates. S Afr Med J 2000;90:730-736.
45. Rahmathullah L, Underwood BA, Thulasiraj RD, Milton RC. Diarrhea, respiratory infections, and growth are not affected by a weekly low-dose vitamin A supplement: a masked, controlled field trial in children in southern India. Am J Clin Nutr 1991;54:568-577.
46. Rahman MM, Mahalanabis D, Alvarez JO, et al. Acute respiratory infections prevent improvement of vitamin A status in young infants supplemented with vitamin A. J Nutr 1996;126:628-633.
47. Victoria CG, Morris SS, Barros FC, Horta BL, Weiderpass E, Tomasi E. Breast-feeding and growth in Brazilian infants. Am J Clin Nutr 1998;67:452-458.

4

Patterns and determinants of growth faltering and nutritional status in Indonesian infants

Marjanka K. Schmidt, Siti Muslimatun, Clive E. West, Werner Schultink, Rainer Gross and Joseph G.A.J. Hautvast

ABSTRACT One of the health problems in Indonesia is the high prevalence of stunting in infants. In order to design appropriate interventions, growth patterns and the contribution of prenatal and postnatal factors to growth faltering and nutritional status of Indonesian infants were investigated. Newborn infants, from women recruited at ~18 weeks of pregnancy from 9 rural villages in West Java, Indonesia, were followed up to 12 to 15 months of age. Growth, morbidity, breast-feeding and food intake were assessed monthly. At enrolment, mothers were interviewed and anthropometry assessed. At birth, weight and length of infants and feeding practices were assessed. Determinants of growth and nutritional status were evaluated using multiple linear regression. Infants were born with reasonable weight and length while growth started to falter at 6-7 months of age. Stunting was highly prevalent at 12 months of age. Almost all infants were breast-fed and received complementary feeding. Morbidity was negatively and food intake positively associated with growth and nutritional status of infants. The most important determinants of increases in weight and length and nutritional status were neonatal weight and length, reflecting prenatal factors. Maternal factors and postnatal factors were of equal strength in predicting growth and nutritional status of infants. In order to have the highest chance of achieving a measurable effect on infant growth, interventions should be aimed at both improving infant nutritional status and ensuring that women enter pregnancy with good nutritional status.

About one third of Indonesian infants and children are underweight or stunted [1,2]. Stunting indicates a public health problem because of its association with an increased risk of morbidity and mortality, delayed motor and mental development, and reduced physical capacity [2,3]. Most growth faltering, resulting in underweight and stunting, occurs from birth until about 2 years of age and may already start during the gestation period [3]. During infancy and childhood, inadequate intakes of nutrients and frequent or prolonged episodes of infections may exacerbate the effects of foetal growth retardation [2,4]. Infection can affect nutrition through anorexia, impaired nutrient absorption, micronutrient losses in urine, increased metabolism or impaired transport to target tissues [5,6]. However, the severity of the effect of morbidity on growth depends on the pathogens and population involved [5,7].

Breast-feeding has been found to decrease the risk of morbidity and mortality in infants in developing countries [8,9] and morbidity in developed countries [10]. In addition growth of breast-fed infants differs from those who are formula-fed [11]. However the effects of duration of breast-feeding, early introduction of food or liquids, and complementary feeding on growth differ among populations [12-18]. In addition, to nutrition and morbidity, factors such as maternal nutrition during pregnancy and parental anthropometric variables could account for differences in growth patterns during infancy and childhood [3,4,19].

Stunting is an irreversible process and therefore it is important to take preventive measures during gestation or early infancy [20]. However, determinants of stunting depend on the ecological setting [21] and, in order to improve intervention strategies, it is important to know the relative contribution of prenatal versus postnatal nutrition to growth faltering during infancy [22]. Up to now, little information is available from Indonesia on growth of infants. We have shown that infant iron and vitamin A status are associated with their nutritional status and growth [23,24] (Chapter 2 & 3), however, other determinants have not yet been analysed and published. Therefore, the aim of this article is to present growth patterns and the influence of prenatal and postnatal factors on growth faltering and nutritional status of Indonesian infants.

Methodology

Study design

Pregnant women from 9 rural villages in Leuwiliang subdistrict, Bogor district, West Java, Indonesia were enrolled in 6 rounds from November 1997 until May 1998 and their infants were born from February until November 1998. After birth all eligible infants were followed up monthly up to at least 12 months of age. Thereafter infants, whose mothers were still willing to participate, continued to be measured into childhood until the end of the study period (November 1999). Growth, morbidity, breast-feeding and food intake were assessed monthly. In addition, mothers were interviewed at enrolment for socio-economic background and pregnancy history, at delivery for infant food intake and other variables and at 13 months after delivery about the use of contraceptives and pregnancy status. Selected anthropometric measurements of women [25] were assessed at enrolment, near term and ~4 months postpartum. The data presented in this paper were collected in an intervention trial investigating the effect of vitamin A and iron supplementation during gestation on infant growth. The results of the intervention and details of the study design have been published earlier [23-26] (Chapter 2 & 3). Explanation of the study had been given to the women at enrolment and all women who participated gave written informed consent. The Ministry of Health Indonesia and the Ethical Committees of the Medical Faculty at the University of Indonesia and Wageningen University approved the research proposal.

Study population

Of 366 women recruited at ~18 weeks of pregnancy, 318 infants were eligible for follow-up after birth. Drop-out of subjects had occurred because of withdrawal from supplementation (n=18), moving outside the research area (n=15), stillbirth (n=13) or neonatal death (n=2) [23,25] (Chapter 2). During the follow-up period from birth to 12 months, 16 infants moved outside the research area and 12 infants died. Neonatal weight or length and gender ratio did not differ between infants that dropped out and infants that were followed up to 12 months (n=290). All 318 infants experienced at least 1 anthropometric assessment. Infants who were measured 1-9 (n=46), 10-13 (n=100) or 14-19 (n=172) times did not differ with respect to neonatal weight or length, weight gain or length increase during the first 6 months of life and gender ratio. The average number of measurements was 13.2 ± 3.6.

Assessment of infants

Weight and length of infants were measured and mothers interviewed for morbidity and food intake of their infants during their monthly visit to their neighbourhood health post (Posyandu). In principle, visits for each health post were planned on the same date of each month. However, in practice visits were made within 1 week before or after the planned date. It was attempted to reach individual mothers who were not able to attend during a visit at their home.

Anthropometry Two pairs of trained field assistants measured weight and length of infants using standardised methods [27]. Weight was measured to the nearest 50 g by using a baby weighing scale (Misaki, Japan), which was tared each time before use. Calibration with a standard weight (5 kg) at regular intervals showed that all scales were stable and precise. Recumbent length was measured to the nearest 0.1 cm by using a wooden length board [28]. Infants were measured with light clothing. Neonatal weight and length had been measured by 2 of the authors (MKS and SM) during a postnatal home visit [23] (Chapter 2). All field assistants had received training and were supervised every month by the 2 authors mentioned above. Independent measurements performed in a random sub-sample of ~5% of the infants revealed a high reliability of the measurements [24] (Chapter 3).

Morbidity At the time of the anthropometric assessment the same field assistants interviewed the caretaker, in all but 2 cases the mother, of the infant about symptoms of morbidity in the 14 days preceding the visit. More specifically, mothers were asked to recall: diarrhoea, defined as ≥3 stools in 1 day that were more liquid than usual; fever, mother's evaluation of infant's body temperature above normal (hot to the touch; 'panas'); running nose ('pilek'), nasal discharge; cough, persistent coughing; difficulty breathing, breathing with severe noise or wheezing or difficulty inhaling; ear discharge, fluid or pus draining from at least 1 ear; vomiting. Questions were asked in Bahasa Indonesia or in the local language (Sundanese) if the mother did not understand the term. First the interviewer asked the mother for any symptoms and then asked her specifically for each category and for how many days the infant suffered from that specific symptom. Episodes were defined as a period of sickness separated by at least 3 days. Mortality of infants was recorded at a home visit after death had been reported.

Breast-feeding and food intake Together with the morbidity interview the mother was asked whether the infant was still breast-fed and whether the infant received any other liquid or food in the 14 days preceding the visit. Data on breast-feeding and food intake, including food given to the infant before or at the time of starting the breast-feeding i.e. prelacteal feeding, had also been collected at the postnatal home visit. The type of liquid or food was recorded using a pre-coded questionnaire. For breast-feeding status, infants were categorised as exclusively breast-fed, predominantly breast-fed (i.e. receiving breast milk plus water, tea, coffee or fruit juices, but no artificial milk), complementary fed, or non breast-fed [29]. Here, we refer to infants breast-fed exclusively as those infants who were exclusively breast-fed regardless of whether they received prelacteal feeding. For data analysis, infant food intake was categorised as follows, *animal products*: meat (beef or chicken), liver, egg, fish; *biscuits*: biscuit, bread, noodles, porridge (bean or wheat-flour); *fruit*: banana and other; *rice*: rice, rice porridge, rice steamed in banana leaves ('lontong'); *tempeh*: tempeh or tofu ('tahu'); *vegetables*: carrot, dark green leafy vegetables, cabbages, (french) beans, potato and other; *snacks*: fried (sweet) potato or cassava (in small quantities not with the meal), bakso (flour and meat balls), cookies (other than biscuit), chips, krupuk, agar-agar, candies; *milk*: milk, infant formula, fortified infant food; *liquids*: water, tea and/or coffee with or without sugar, honey, fruit or vegetable juice.

Data analysis

Data of 318 infants eligible for follow-up were pooled for analysis irrespective of the treatment that their mothers had received during pregnancy. Growth, morbidity, iron status and feeding mode of infants did not differ from 0 to 12 months of age among the 3 treatment groups, although serum retinol concentrations were improved by vitamin A plus iron supplementation [23,24] (Chapter 2 & 3). In addition no differences in growth were found among groups up to 18 months of age (unpublished data). Therefore, we consider that pooling of data of infants for analysis of determinants of growth faltering is justified. Weight and length were converted into z-scores using the NCHS/WHO reference data incorporated in the Epi-Info software (Epi Info2000 version 1.0.5. CDC, Atlanta). Stunting was defined as a height-for-age z-score of <-2, underweight as weight-for-age z-score <-2 and wasting as weight-for-height z-score <-2. For analysis, follow-up data of infants were grouped into monthly age groups of 0 (0 until 0.99 months, neonatal) to 18 (18 until 18.99 months). However, we considered that only up to 15 months of age the number of follow-up (anthropometric) data of infants that was available for analysis was still reasonable (n=128), therefore, descriptive data are presented until that age. For further analysis, we present the follow-up data up to 12 months to reduce the risk of selection bias and to increase power by having more infants in the analysis.

For the analysis of association of symptoms of morbidity or food groups with growth, nutritional status or feeding practices and for multiple linear regression, morbidity symptoms and food intake were converted into a ratio correcting for compliance to the assessments as follows: the total number of episodes of a symptom or the total number of times a certain food group was reported to be consumed by the infant was divided by the total number of assessments this infant had during the follow-up period.

Comparisons of continuous, normally distributed data between groups were done using *t*-test or ANOVA if correction for other variables was necessary. Comparisons of continuous, non-normally distributed data and categorical data between groups were done using Mann Whitney U-test and χ^2-test respectively. Associations of normally and non-normally distributed data were evaluated using Pearson's correlation and Spearman rank correlation, respectively. For correction of gender in the association between growth and morbidity or food intake partial correlation was applied. Determinants of growth and nutritional status of infants were evaluated using backward multiple linear regression (threshold 0.1). Variables included in all models are those presented in **Table 4.1** and were entered as dichotomous or continuous variables. However, mid-upper arm circumference was not included in any model because it was highly correlated with maternal weight ($r > 0.8$). Occupation of father was entered as dichotomous (0=daily worker, 1=monthly worker or trader) or as 2 dummy variables, but with neither method became significant in the model. Also the variables: prelacteal feeding (0,1), colostrum intake (0,1) and number of months the infant was exclusively breast-fed (not taking into account prelacteal feeding) were included in all models. When neonatal weight and length were both included in all models, neonatal weight was found to be a significant predictor in the models for weight gain and weight-for-age z-scores, neonatal length in the models for length increase and height-for-age z-scores, and both variables in the model for weight-for-height z-scores. Because neonatal weight and length were highly correlated ($r > 0.8$), models were rerun only including the significant variable (neonatal weight or length, or both in the case of weight-for-length). In order to limit the number of variables and to avoid collinearity, we selected 1 variable for morbidity symptoms and 1 for food groups to be included in the models. The selection was based on a comparison of correlation coefficients of each morbidity or food variable with the dependent variable. Fever was most associated with growth and nutritional status of infants and was therefore included in all models. Of the food groups, fruit was most associated with weight gain, weight-for-age and height-for-age z-scores, biscuits with length increase and snacks with weight-for-height z-scores, thus these were included in the respective models. Statistical analysis was carried out with the SPSS software package (Windows version 7.5.2. SPSS Inc., Chicago, IL).

Results

General characteristics of the study population are shown in **Table 4.1**. The number of boys and girls was not significantly different. Twenty-seven percent of the infants were first-borns. Among multiparous mothers, the average period between the present and the previous delivery was 44 months, 66 mothers had delivered ≤24 months and 166 >24 months ago. At 13 months after delivery 4 mothers of the 281 interviewed reported to be pregnant again.

Table 4.1 General characteristics of subjects followed up[1]

Boy / girl (n)	151 / 167
Birth order	1.5 ± 1.3
Gestational age, *weeks*	37.8 ± 2.6
Maternal age at enrolment, *years*	24.3 ± 4.6
Maternal height at enrolment, *cm*	149.1 ± 4.9
Maternal weight at enrolment, *kg*	48.9 ± 6.6
Maternal MUAC at enrolment, *cm*	24.6 ± 2.4
Maternal education (%)	
Elementary school	74.8
Higher education	25.2
Occupation of father (%)	
Daily worker (labourer, farmer, driver)	46.5
Monthly worker (government or private employee, retired)	34.0
Trader	19.5
Housing (%)	
Good housing (brick wall, tile roof and cemented floor)	67
Poorer housing (wall, roof and/or floor made of materials with less quality)	33

[1]Data are presented as mean ± SD unless stated otherwise.

Growth and nutritional status of infants Most of the increase in length and weight of infants occurred during the first 6 months (**Table 4.2**). At the neonatal assessment only 7% of the infants were stunted, 5% were underweight and none were wasted. This situation was similar at 6 months of age. However at 12 months of age, 24% was stunted, 32%

underweight and 4% wasted while at 15 months of age these proportions had increased to 32%, 40% and 6%, respectively. Weight-for-age and height-for-age z-scores started to decrease significantly ($P < 0.05$) from 3-4 months of age and weight-for-height z-scores from 4-5 months of age (**Figure 4.1**). However if compared with neonatal nutritional status, growth started to falter around 6-7 months of age as shown by the lower z-scores. Wasting was the least prevalent: mean weight-for-height z-scores were higher ($P < 0.0001$) than weight-for-age and height-for-age z-scores from 0 to 15 months of age (**Figure 4.1**). Stunting was more prevalent than underweight up to 7 months of age as shown by the lower ($P < 0.01$) height-for-age z-scores than weight-for-age z-scores, while the situation was the reverse ($P < 0.01$) from 9 to 15 months of age. Boys had higher weight and length than girls from 0 to 15 months (**Figure 4.2**). This difference was mainly due to the higher birth weight and length of boys, and greater increases in weight [0.39 (0.20-0.57) kg, mean (95%CI), $P < 0.0001$)] and length [0.7 (0.2-1.2) cm, $P < 0.01$)] during the first 6 months of life. Height-for-age, weight-for-age and weight-for-height z-scores from 0 to 15 months did not differ significantly between boys and girls. Compared with the 12-months breast-fed pooled data set of WHO [11], length and weight of boys and girls started to falter from the reference curve around 6-7 months of age. At 12 months of age, mean weight-for-age and height-for-age z-scores were –1.25 and –1.17 for boys and –1.12 and –1.13 for girls, respectively.

Table 4.2 Weight, length and growth of Indonesian young infants from birth to 15 months of age[1]

Age	n	Weight, *kg*	Length, *cm*
≤72 hours (birth)	68	3.0 ± 0.3	48.5 ± 1.7
0 months (neonatal)	312*	3.2 ± 0.5	49.7 ± 2.2
6 months	267	7.3 ± 0.9	65.5 ± 2.4
12 months	248	8.2 ± 0.9	71.7 ± 2.4
15 months	128**	8.7 ± 0.9	74.4 ± 2.5
Period, months		Weight increase, *kg*	Length increase, *cm*
0 – 6	263*	4.1 ± 0.8	16.0 ± 2.1
6 – 12	223	1.0 ± 0.5	6.4 ± 1.2
12 – 15	117**	0.4 ± 0.3	2.4 ± 1.1

[1]Mean ± SD; *length of 1 infant missing, ** weight of 2 infants missing.

Nutrition of infants After birth, a large proportion (84%) of the infants received prelacteal feeding which in most cases was honey. Also some mothers reported to have given water with or without sugar, coffee, biscuit, banana or formula milk. A small proportion (15%)

of mothers reported that they discharged part of the first colostrum. All infants but 1 were breast-fed after birth and another mother stopped breast-feeding soon after delivery. During the first months of life, 80% of the infants were exclusively breast-fed, however this proportion decreased rapidly with age (**Figure 4.3**). The average period of exclusive breast-feeding was 1.96 (1.95) months. Almost all women continued breast-feeding their infants during the study follow-up (**Figure 4.3**). The small proportion of mothers that breast-fed exclusively or predominantly after 6 months postpartum were mostly mothers that had returned to exclusive or predominant breast-feeding after having tried unsuccessfully to introduce food to their infants. Most infants were first introduced to liquids, fruit (often banana) and biscuits while foods such as rice, vegetables, tempeh and snacks were introduced at a later age (**Figure 4.4**). From 4 months of age intake of foods increased rapidly. Intake of milk products increased during the first 5 months but declined thereafter (**Figure 4.4**).

Fifteen percent of the infants at 4 months of age did not have a good appetite as reported by their mothers and this increased gradually to 50% at 12 months of age. There were no significant differences between boys and girls in feeding practices. However, compared with mothers of girls, mothers of boys tended to discharge part of the first colostrum less often (11% vs. 19%, $P = 0.073$) and give prelacteal feeding less often (79% vs. 87%, $P = 0.067$). Boys also tended ($P < 0.1$) to have higher ratios for intake of animal products, biscuits and fruit groups (data not shown).

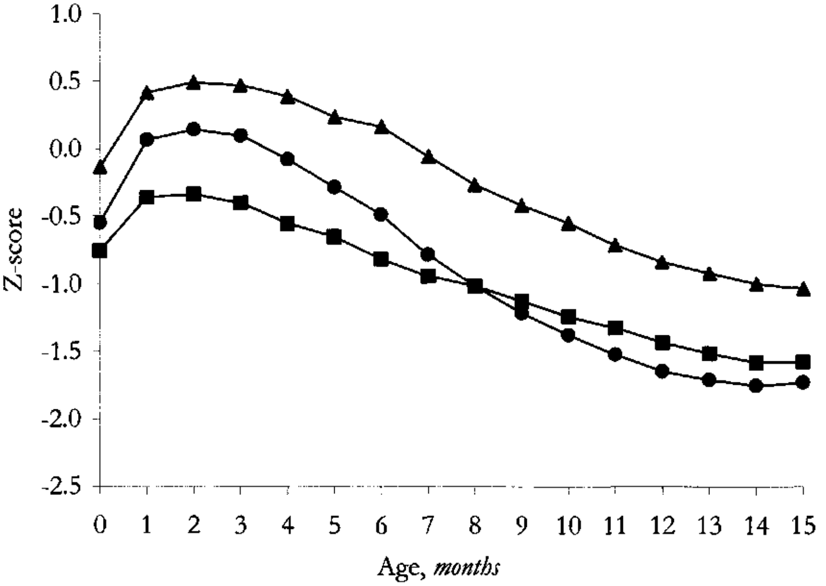

Figure 4.1 Height-for-age (■), weight-for-age (●) and weight-for-height (▲) z-scores of infants from 0 to 15 months of age.

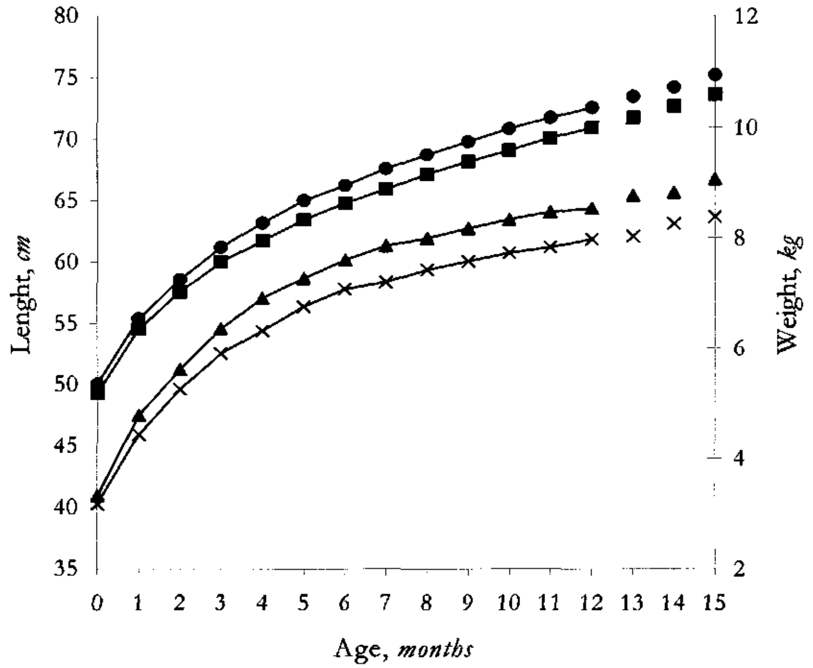

Figure 4.2 Length of boys (●) and girls (■), and weight of boys (▲) and girls (X) from 0 to 15 months of age with growth curve that has been constructed only until 12 months of age because thereafter the number of children measured was <100.

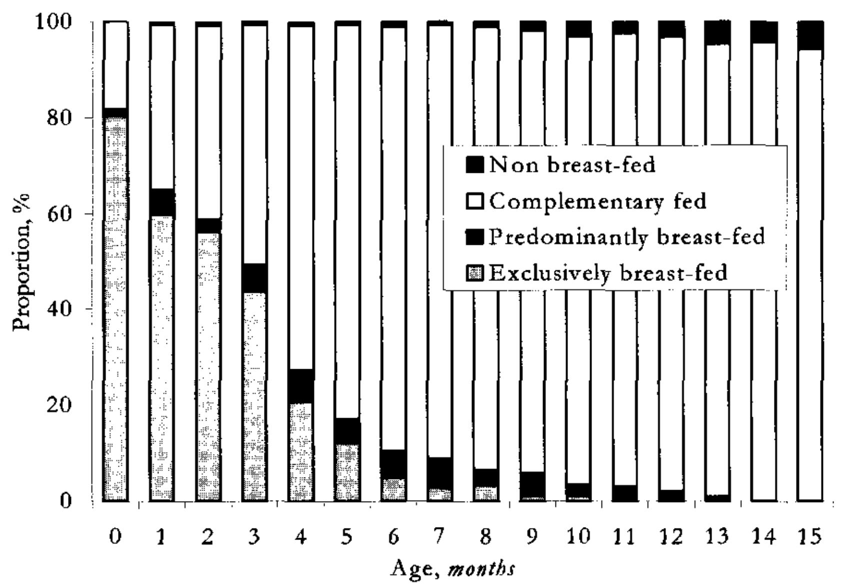

Figure 4.3 Proportion of infants exclusively breast-fed, predominantly breast-fed, complementary fed or non breast-fed at monthly intervals from 0 to 15 months of age.

Figure 4.4 Proportion of infants receiving foods from various food groups (as described in the methodology section) at monthly intervals from 0 (excluding the prelacteal feeding) to 15 months of age. Food groups: animal products (+), biscuits (▲), fruit (●), liquid (O), milk (✳), rice (◆), snacks (■), tempeh ( ), vegetables (-).

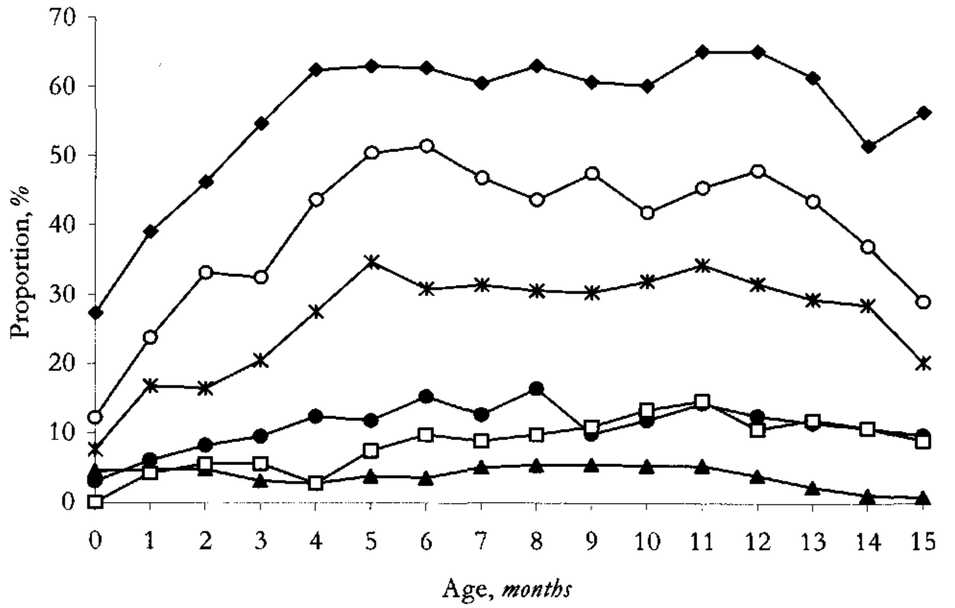

Figure 4.5 Proportion of infants suffering from selected symptoms of morbidity at monthly intervals from 0 to 15 months of age. Morbidity symptoms: running nose (◆), fever (O), coughing (✳), diarrhea (●), vomiting (□), difficulty breathing (▲).

Table 4.3 Association of growth and nutritional status with variables of morbidity symptoms and food intake of infants[1]

	Increases in weight or length, 0-12 months		Z-scores at 12 months of age		
	Weight	Length	Weight-for-age	Height-for-age	Weight-for-height
n	243	242	248	248	248
Symptoms of morbidity					
Fever	-0.166*	0.056	-0.149*	0.044	-0.261**
Running nose	-0.134*	-0.062	-0.063	0.048	-0.134*
Coughing	-0.149*	0.004	-0.116†	-0.000	-0.163*
Food intake					
Animal products	0.123†	0.118†	0.117†	0.083	0.085
Fruit	0.184**	0.170**	0.182**	0.165*	0.109†
Biscuits	0.173**	0.237**	0.119†	0.147*	0.040
Snacks	0.091	0.009	0.100	-0.005	0.145*

[1]Partial correlation coefficients controlling for gender; **significant association $P < 0.01$, *$P < 0.05$, †$P < 0.10$.

Morbidity of infants Prevalence of all reported symptoms of morbidity in infants increased up 4 to 5 months of age and then remained at a similar level until 12 months of age (**Figure 4.5**). Running nose, fever and coughing were the most reported symptoms of morbidity and about 10% of the infants suffered from diarrhoea and/or vomiting. Prevalence of ear discharge was rare. Morbidity prevalence was not significantly related to season or gender.

Associations among anthropometry, feeding and morbidity of infants The most prevalent symptoms of morbidity, i.e. fever, running nose and coughing, were significantly associated with weight increase, and weight-for-age and weight-for-height z-scores (**Table 4.3**). Consumption ratio of the animal products, fruit and biscuits food groups were significantly associated with increases in weight and length, and weight-for-age and height-for-age z-scores while snacks consumption was specifically associated with weight-for-height z-scores (**Table 4.3**). Feeding practices such as prelacteal feeding, partly discharging first colostrum, or exclusive breast-feeding for ≥4 months were not associated with increases in length or weight, or nutritional status. Infants who had received prelacteal feeding suffered more from cough and difficulty breathing and partly discharging first colostrum was associated with difficulty breathing, fever and running nose. However, if we corrected these associations for gender, only the association between prelacteal feeding and coughing remained significant (data not shown).

Multiple regression models explained 25% and 36% of the variation in increases of weight and of length from 0 to 12 months, respectively, and 25% of weight-for-age, 35% of height-for-age and 19% of weight-for-height z-scores at 12 months of age, respectively. Maternal weight and height at ~18 weeks of pregnancy, housing, and postnatal variables such as fever and fruit, biscuits or snacks were of similar strength in predicting increases in length and weight and nutritional status of infants (**Table 4.4**). However, gender and especially neonatal weight or length were stronger predictors of increases in length and weight and nutritional status of infants. Male gender was associated with lower weight-for-age and height-for-age z-scores. While neonatal weight and length were negative predictors of increases in weight and length respectively, they were positive predictors of nutritional status of infants. As also shown in the correlation analysis (**Table 4.3**), fever was negatively associated with weight increase and weight-for-age and weight-for-height z-scores, but not with length increase and height-for-age z-scores. Neonatal length was a stronger predictor of length increase and height-for-age z-scores than neonatal weight for weight gain and weight-for-age z-scores (**Table 4.4**). Height-for-age, weight-for-age, and weight-for-height z-scores at 12 months of age were strongly correlated (0.897, 0.947 and 0.841, respectively; P <0.01), with those at 15 months of age. In addition, models for increases in weight and length from 0 to 15 months and nutritional status at 15 months were similar to those presented, except that higher maternal education, instead of better housing, was a positive predictor in all models.

Discussion

Infants in our study began life with reasonable neonatal weight and length while growth began to falter around 6-7 months of life. Only a small proportion of the infants was wasted but stunting was highly prevalent. The most important determinants of increases in weight and length and nutritional status were neonatal weight and length, reflecting prenatal factors [19,30]. Maternal factors and postnatal factors were of equal strength in predicting growth and nutritional status of infants.

Growth faltering during the second half of infancy is often seen in Asian populations [31,32]. However, infants in our study started growth faltering a few months later and, although stunting was highly prevalent at 12 months of age, the proportion of stunting was lower than reported earlier in Madurese infants [4]. Once an infant has become stunted it will not recover [20] and often height-for-age z-scores remain at the same level until 5 years of age [7,32]. Nutritional status of infants at 15 months of age was highly correlated with that at 12 months and determinants were similar, indicating that infants continued on the same tract into childhood.

Neonatal weight and length were negatively associated with increases in weight and length but positively with z-scores of nutritional status at 12 months of age. Thus, infants with lower birth weight experience catch up growth, but they will not achieve the same weight or length at 12 months of age, as infants born heavier and longer [33]. This phenomenon of slower growth of initially larger infants is normally seen in developed as well as in developing countries [8,34]. In general, boys are taller and heavier than girls while lower prevalence of stunting in girls than in boys (23 vs. 27% respectively in our population) during the first 1.5 years of age have been reported in other Asian populations as well [34].

Neonatal length was more predictive of length increase and height-for-age z-scores than neonatal weight for weight increase and weight-for-age z-scores. It seems therefore that the prenatal environment influenced length more than weight. This is supported by the fact that morbidity was associated with weight gain and weight-for-age z-scores but not with length increase and height-for-age z-scores. In addition, it is likely that breast milk intake protected infants from any long-term growth faltering due to sickness and/or decreased nutrient intake [6,35,36].

Breast-feeding not only protects infants against infections and diseases but also provides psychological advantages [17,34]. Almost all infants were breast-fed until 12 months of age and received colostrum. However, exclusive breast-feeding was rare in the population studied and it declined rapidly from birth to 6 months of age. Exclusive breast-feeding for ≥4 months [37] did not show an advantage above complementary or predominantly breast-feeding in terms of growth or morbidity. Comparison of the infants studied with the 12-months pooled breast-fed data set [11] and the NCHS reference data showed that the lower height-for-age and weight-for-age z-scores before 6-7 months of age were due to the different growth pattern of breast-fed infants. However, after 6-7 months the infants studied also started to falter from the reference breast-fed curve. Subsequent research has shown that the pattern of growth is not sensitive to the duration of breast-feeding [13]. However, we found a small negative association between the duration of exclusive breast-feeding and weight-for-height z-scores of the infants studied. This effect has also been observed in European infants but was no longer detectable after 24 months of age [14]. No harmful effects of prelacteal feeding have been reported previously in Indonesian infants [33]. However, Vietnamese children that received complementary feeding before 3 months of age grew less well and had higher morbidity [15] and in the present study a relationship was found between prelacteal feeding and coughing. Therefore, we would advice to discourage this habit in line with current recommendations for infant feeding [10].

Moderate maternal malnutrition does not affect breast milk volume [38] while infant growth is not related to energy derived from breast milk [39]. However, micronutrient levels in breast milk may play a role in infant growth [4]. Levels of vitamin A and iron in breast milk of our mothers were relatively low [26]. Only vitamin A levels of breast milk were significantly correlated with increases in weight and length and nutritional status of infants. We did not include this variable in our analysis because it greatly reduced the number of subjects available for the model. However, if we did include this variable, the proportion explained by the models for weight and length increase increased to 32% and 46%, respectively, with ß values of 0.131 and 0.201, respectively.

Table 4.4 Regression analysis to explain the influence of prenatal, maternal and postnatal determinants on growth from 0 to 12 months of age and nutritional status at 12 months of age of infants[1]

	Increase in weight, 0 - 12 months				Increase in length, 0 - 12 months			
n	227				226			
	b	SE	ß	*P*	b	SE	ß	*P*
Housing (0=poorer housing, 1=good housing)	0.197	0.105	0.112	0.060				
Maternal height, *cm*	0.021	0.011	0.126	0.065	0.096	0.024	0.219	0.000
Maternal weight, *kg*	0.019	0.009	0.157	0.025				
Gender (0=boy, 1=girl)	-0.444	0.100	-0.266	0.000	-1.109	0.244	-0.248	0.000
Neonatal weight, *kg*	-0.502	0.106	-0.291	0.000	-	-	-	-
Neonatal length, *cm*	-	-	-	-	-0.502	0.055	-0.504	0.000
Fever, number of episodes/assessments	-0.648	0.256	-0.148	0.012				
Fruit*	0.797	0.241	0.194	0.001	-	-	-	-
Biscuits*	-	-	-	-	2.313	0.635	0.197	0.000

	Weight-for-age z-score				Height-for-age z-score				Weight-for-height z-score			
n	227				226				226			
	b	SE	ß	*P*	b	SE	ß	*P*	b	SE	ß	*P*
Housing (0=lesser housing, 1=good housing)	0.191	0.100	0.114	0.056	0.158	0.092	0.095	0.087				
Maternal height, *cm*	0.019	0.011	0.124	0.069	0.035	0.009	0.229	0.000				
Maternal weight, *kg*	0.019	0.008	0.162	0.021					0.021	0.007	0.195	0.002
Gender (0=male, 1=female)	0.216	0.095	0.136	0.024	0.312	0.088	0.198	0.000				
Neonatal weight, *kg*	0.469	0.101	0.285	0.000	-	-	-	-	0.730	0.207	0.481	0.001
Neonatal length, *cm*	-	-	-	-	0.172	0.019	0.492	0.000	-0.147	0.043	-0.459	0.001
Exclusively breast-fed, *months*									-0.042	0.022	-0.117	0.059
Fever, number of episodes/assessments	-0.591	0.244	-0.142	0.016					-0.957	0.229	-0.255	0.000
Fruit*	0.759	0.230	0.194	0.001	0.596	0.211	0.154	0.005				
Snacks*	-	-	-	-	-	-	-	-	0.634	0.288	0.136	0.029

[1]Variabels with dash (-) are not included in the model; *Fruit, biscuits and snacks: number of times intake was reported (yes or no) per number of assessments (as explained in the methodology section).

Kusin *et al.* [40] reported that attained weight of Indonesian infants was correlated with breast milk intake, but not with food intake. In this study, an association was found between intake of certain food groups and increases in weight and length of infants. After 6 months growth of breast-fed infants may be limited by the micronutrient content of complementary foods [12,22]. It may well be that dietary patterns rather than nutrient intakes are important in relation to linear growth [7]. In our study, especially fruit, meat and biscuits intake were significantly associated with better growth. We only assessed food intake of infants qualitatively, but we speculate that food intake may have been insufficient to support growth of infants after 7 months. Kusin *et al.* [33] reported that food given to infants might have insufficient energy density. Decreased appetite of infants as reported by mothers in our study, may have been due to anorexia as a result of morbidity or insufficient micronutrient intake [4,6].

Although infants in our study had reasonable neonatal weight and length, meaning that prenatal factors were favourable, as compared with for example Indian infants [30], neonatal weight and length were still more important determinants of infant growth than postnatal factors. Linear growth was less sensitive to postnatal factors such as morbidity than increase in weight. In addition, part of infant growth faltering could be attributed to maternal nutritional status as has been shown earlier [4,19]. Therefore, it is important not only to improve infant nutritional status, but also to ensure that women enter pregnancy with good nutritional status. As we reported earlier, infants with adequate vitamin A status grew ~1 cm more and gained ~300 g more in the first half year of life than infants with marginal vitamin A deficiency [24] (Chapter 3). In all infants vitamin A levels in breast milk were associated with growth. Because micronutrient levels in breast milk of the mothers of these Indonesian infants were relatively low and also because intake of nutrients through food may be inadequate, increasing micronutrient intake of mothers and infants through supplements or fortified foods should be considered. Because our models explained only one third of growth faltering of infants, other factors such as intake of food supplying protein and energy, housing, hygiene and care giving [41] should be considered.

Acknowledgements

We are grateful to all participants, field assistants and health personal of subdistrict Leuwiliang, Bogor for their contribution to this project.

References

1. World Health Organization. Global database on child growth and malnutrition. Geneva: WHO, 2000.
2. ACC/SCN. Fourth report on the world nutrition situation. Nutrition throughout the life cycle. Geneva: ACC/SCN in collaboration with IFPRI, 2000.
3. Waterlow JC, Schürch B. Causes and mechanisms of linear growth retardation. Proceedings of an I/D/E/C/G workshop held in London January 15-18, 1993. Eur J Clin Nutr 1994;48:S1-S216.
4. Kusin JA, Kardjati S. Maternal and child nutrition in Madura, Indonesia. Amsterdam: Royal Tropical Institute, 1994.
5. Stephensen CB. Burden of infection on growth failure. J Nutr 1999;129:534S-538S.

6. Brown KH, Stallings RY, de-Kanashiro HC, Lopez-de RG, Black RE. Effects of common illnesses on infants' energy intakes from breast milk and other foods during longitudinal community-based studies in Huascar (Lima), Peru. Am J Clin Nutr 1990;52:1005-1013.
7. Allen LH. Nutritional influences on linear growth: a general review. Eur J Clin Nutr 1994;48:75S-89S.
8. Dettwyler KA, Fishman C. Infant feeding practices and growth. Ann Rev Anthropol 1992;21:171-204.
9. WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. Lancet 2000;355:451-455.
10. Work Group on Breastfeeding. Breastfeeding and the Use of Human Milk. Pediatrics 1997;100:1035-1039.
11. WHO Working Group on Infant Growth. An evaluation of infant growth; A summary of analyses performed in preparation for the WHO Expert Committee on Physical status: the use and interpretation of anthropometry in infants. Geneva: Nutrition Unit of the WHO, 1994.
12. Brown KH. WHO/UNICEF review on complementary feeding and suggestions for future research: WHO/UNICEF guidelines on complementary feeding. Pediatrics 2000;106:1290-1291.
13. Cohen RJ, Brown KH, Canahuati J, Rivera LL, Dewey KG. Effects of age of introduction of complementary foods on infant breast milk intake, total energy intake, and growth: a randomised intervention study in Honduras. Lancet 1994;344:288-293.
14. Haschke F, van't Hof MA, the Euro-Growth Study Group. Euro-Growth references for breast-fed boys and girls: Influence of breast-feeding and solids on growth until 36 months of age. J Pediatr Gastroenterol Nutr 2000;31(suppl 1):S60-S71.
15. Hop LT, Gross R, Giay T, Sastroamidjojo S, Schultink W, Lang NT. Premature complementary feeding is associated with poorer growth of Vietnamese children. J Nutr 2000;130:2683-2690.
16. Onyango AW, Esrey SA, Kramer MS. Continued breastfeeding and child growth in the second year of life: a prospective cohort study in western Kenya. Lancet 1999;354:2041-2045.
17. Victoria CG, Morris SS, Barros FC, Horta BL, Weiderpass E, Tomasi E. Breast-feeding and growth in Brazilian infants. Am J Clin Nutr 1998;67:452-458.
18. Villalpando S, Lopez AM. Growth faltering is prevented by breast-feeding in underprivileged infants from Mexico City. J Nutr 2000;130:546-552.
19. Dewey KG. Cross-cultural patterns of growth and nutritional status of breast-fed infants. Am J Clin Nutr 1998;67:10-17.
20. Martorell R, Khan LK, Schroeder DG. Reversibility of stunting: epidemiological findings in children from developing countries. Eur J Clin Nutr 1994;48(suppl 1):S45-S57.
21. Allen LH, Uauy R. Guidelines for the study of mechanisms involved in the prevention or reversal of linear growth retardation in developing countries. Eur J Clin Nutr 1994;48(suppl 1):S212-S216.
22. Dewey KG. Complementary feeding and infant growth and body composition. Pediatrics 2000;106:1281
23. Schmidt MK, Muslimatun S, West CE, Schultink W, Hautvast JGAJ. Vitamin A and iron supplementation of Indonesian pregnant women benefits vitamin A status of their infants. Br J Nutr 2001;86:in press.
24. Schmidt MK, Muslimatun S, Schultink W, West CE, Hautvast JGAJ. Randomised double-blind trial of the effect of vitamin A supplementation of Indonesian pregnant women on morbidity and growth of their infants during the first year of life. Eur J Clin Nutr 2002:in press.
25. Muslimatun S, Schmidt MK, Schultink W, et al. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J Nutr 2001;131:85-90.
26. Muslimatun S, Schmidt MK, West CE, Schultink W, Hautvast JGAJ, Karyadi D. Weekly vitamin A and iron supplementation during pregnancy increases vitamin A concentration of breast milk but not iron status in Indonesian lactating women. J Nutr 2001;131:2664-2669.
27. Jeliffe DB. The assessment of the nutritional status of the community. Monograph series WHO no 53. Geneva: WHO, 1996.
28. Gross R, Kielmann A, Korte R, Schoeneberger H, Schultink W. Nutrition baseline surveys in communities. Bangkok: SEAMEO-TROPMED and Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), 1997.

29. Labbok M, Krasovec K. Toward consistency in breastfeeding definitions. Stud Fam Plann 1990;21:226-230.
30. Arifeen SE, Black RE, Caulfield LE, et al. Infant growth patterns in the slums of Dhaka in relation to birth weight, intrauterine growth retardation, and prematurity. Am J Clin Nutr 2000;72:1010-1017.
31. Kolsteren PW, Kusin JA, Kardjati S. Pattern of linear growth velocities of infants from birth to 12 months in Madura, Indonesia. Trop Med Int Health 1997;2:291-301.
32. Shrimpton R, Victora CG, de Onis M, Lima RC, Blossner M, Clugston G. Worldwide timing of growth faltering: implications for nutritional interventions. Pediatrics 2001;107:e75.
33. Kusin JA, Kardjati S, Renqvist UH, Van SWM, Koetsier D. Infant nutrition and growth. In: Kusin JA, Kardjati S, eds. Maternal and child nutrition in Madura, Indonesia. Amsterdam: Royal Tropical Institute, 1994:175-240.
34. Adair LS, Guilkey DK. Age-specific determinants of stunting in Filipino children. J Nutr 1997;127:314-320.
35. Lutter CK, Habicht JP, Rivera JA, Martorell R. The relationship between energy intake and diarrhoeal disease in their effects on child growth: biological model, evidence, and their implications for public health policy. Food Nutr Bull 1992;14:36-42.
36. Dickin KL, Brown KH, Fagbule D, et al. Effect of diarrhoea on dietary intake by infants and young children in rural villages of Kwara State, Nigeria. Eur J Clin Nutr 1990;44:307-317.
37. Garza C, de-Onis M. A new international growth reference for young children. Am J Clin Nutr 1999;70:169S-172S.
38. Butte NF, Garza C, Stuff JE, Smith EO, Nichols BL. Effect of maternal diet and body composition on lactational performance. Am J Clin Nutr 1984;39:296-306.
39. Butte NF, Villalpando S, Wong WW, et al. Human milk intake and growth faltering of rural Mesoamerindian infants. Am J Clin Nutr 1992;55:1109-1116.
40. Kusin JA, Kardjati S, van-Steenbergen WM, Renqvist UH. Nutritional transition during infancy in East Java, Indonesia: 2. A longitudinal study of growth in relation to the intake of breast milk and additional foods. Eur J Clin Nutr 1991;45:77-84.
41. Engle PL, Bentley M, Pelto G. The role of care in nutrition programmes: current research and a research agenda. Proc Nutr Soc 2000;59:25-35.

5

Mental and psychomotor development of infants of mothers supplemented with iron and vitamin A during pregnancy in Indonesia

Marjanka K. Schmidt, Siti Muslimatun, Werner Schultink, Clive E. West and Joseph G.A.J. Hautvast

ABSTRACT Maternal nutrition is important for foetal development but impact on functional outcome of infants is still unclear. This study investigated the effects of vitamin A and iron supplementation during gestation on infant mental and psychomotor development. In addition, associations between maternal vitamin A and iron status during pregnancy and infant development were explored. Mothers of infants from 5 villages had been randomly assigned on an individual basis to supervised, double blind supplementation once weekly from ~18 weeks of pregnancy until delivery. Supplementation comprised 120 mg iron and 500 μg folic acid with (n=94) or without (n=94) 4800 RE vitamin A. Mothers of infants from 4 other villages who participated in the national iron/folic acid supplementation program, but whose intake of supplements was not supervised, were also recruited (n=88). Mental and psychomotor development of infants was assessed using the Bayley test. Infants were tested either at 6 or 12 months of age. Biochemical parameters of mothers were assessed at enrolment, near term and ~4 months postpartum and of infants at ~4 months of age. We found no impact of supplementation with vitamin A during gestation on mental or psychomotor development of infants. In addition, infants whose mothers had received weekly iron supplementation during pregnancy had similar mental and psychomotor indices as those whose mothers had participated in the governmental iron supplementation programme. Infants of mothers with adequate vitamin A status near term (n=145) had higher mental development indices than infants of mothers with marginal vitamin A status near term (n=59). Thus, vitamin A status of mothers during pregnancy would appear to play a role in mental development of infants but further research is necessary to test this hypothesis.

The importance of nutrition, especially iron, on infant development and the consequences for later health have been recognised [1-3]. Studies in humans and animals have shown that poor nutrition during gestation may not only impair foetal growth, but also have an impact on development during the prenatal period [4-6]. There is evidence for a role of iron and possibly of vitamin A in brain development [7-9]. Many studies have shown that iron-deficient anaemic infants scored lower on the mental and psychomotor development indices of the Bayley test than did iron-sufficient infants [10,11]. An intervention study in Indonesia has shown that iron supplementation can improve mental and psychomotor development of iron-deficient anaemic infants [12].

A large proportion of Indonesian pregnant women suffer from iron deficiency anaemia and marginal vitamin A deficiency [13-15]. Iron deficiency anaemia during pregnancy is associated with higher risk of low birth weight and pre-term delivery [16]. In addition, maternal iron deficiency in pregnancy may reduce foetal and infant iron stores [17,18]. Iron status of pregnant women and children have been found to be improved by vitamin A supplementation [19,20]. In addition, adequate maternal vitamin A status during pregnancy is important for the development of the foetus and to attain sufficient levels of vitamin A in breast milk [21].

Nutritional status of women postpartum may affect caring capacity and thus mental and psychomotor development of their infants [22]. Malnutrition has been related to infant development in many trials [23,24], but none has specifically studied the impact of vitamin A plus iron supplementation during pregnancy on infant development.

In Indonesia, iron supplementation during pregnancy is standard practice and thus it is not ethical to conduct a study in which pregnant women are not provided with iron [25]. However, compliance with supplement intake is found to be low [26] and supervised weekly iron supplementation may be an alternative method of choice [27,28]. Hence, iron deficiency during gestation may affect iron stores of infants and iron deficiency anaemia has adverse effects on infant development, thus it is important to evaluate any new regimen of iron supplementation during pregnancy on infant outcome.

This study investigated whether adding vitamin A supplementation to weekly iron supplementation during pregnancy improved mental and psychomotor development of infants and whether weekly iron supplementation and the ongoing governmental program performed similarly or not with respect to infant development. In addition, the relationship between maternal vitamin A and iron status during pregnancy and infant development was investigated.

Methodology

Study design

Pregnant women from 9 rural villages in Leuwiliang subdistrict, Bogor district, West Java, Indonesia were enrolled in 6 rounds from November 1997 until May 1998 and their infants were born from February until November 1998. After birth, all eligible infants were followed up monthly up to at least 12 months of age. Mothers of these infants had been supplemented with iron and folic acid, either with or without vitamin A, during pregnancy or had access to iron plus folic acid tablets through the ongoing government program. Details of the study design and the impact of treatment on maternal biochemical status near term and postpartum, breast milk composition, and infant's biochemical status, growth and morbidity have been published earlier [27,29-31] (Chapter 2 & 3). Briefly, women who were 16-20 weeks pregnant, aged 17-35 years and parity <6, from 5 villages were randomly assigned on an individual basis to double-blind, weekly supplementation until delivery. Supplementation comprised 120 mg iron as ferrous sulphate and 500 μg folic acid or the same amounts of iron and folic acid plus 4800 retinol equivalent (RE) vitamin A. Tablet intake was supervised by volunteer health workers and monitored by field assistants. Health personal and participants received clear instructions in order to avoid additional iron tablet intake. The average period of supplementation in these weekly groups was 20 weeks. Women from the other 4 villages, referred to as the 'daily' group, were recruited at the same time. These women had free access to iron tablets from the health care services following government policy that pregnant women should receive 90-120 iron/folic acid tablets, each containing 60 mg iron as ferrous sulphate and 250 μg folic acid. Tablet intake in this group was not supervised because this was an effectiveness trial comparing the weekly, supervised iron supplementation to the current program. Adherence of tablet intake was assessed though interview during a postnatal home visit which revealed that the median tablet intake was 50 while only 17% of the subjects took >90 tablets [27]. The purpose and procedure of the study were explained to the women at enrolment and only women who gave written informed consent were enrolled. The Ministry of Health Indonesia and the Ethical Committees of the Medical Faculty at the University of Indonesia and Wageningen University approved the research proposal.

Characteristics of the study population

Of 366 women recruited at ~18 weeks of pregnancy, 318 infants were eligible for follow up after birth. Drop out of subjects had occurred because of withdrawal from supplementation (n=18), moving outside the research area (n=15), stillbirth (n=11) or twins (n=3, only 1 infant survived), or neonatal death (n=2) [27,30] (Chapter 2). Mothers were interviewed at enrolment for socio-economic background and pregnancy history and at a postnatal visit at home for infant food intake and other variables using pre-coded questionnaires.

Development of infants

Infants were tested for mental and psychomotor development between January and May 1999 using the Bayley I test [32]. The Bayley test was administered by 2 experienced psychologists who had shown previously that the reproducibility between them was high (L. Karyadi, personal communication). The psychologists were masked with respect to which of the 3 treatment groups the infants belonged. The test had been translated into Bahasa Indonesia. Both psychologists were trilingual (Bahahsa Indonesia, Sundanese, and English). Half of the infants were tested at 6 (5.5-6.5) months of age and half at 12 (11.5-12.5) months of age. The tests at 6 months of age were delayed to 7 months in some infants because of logistic problems. If an infant was ill, the mother was asked to return to enable her infant to be tested at a later session. Tests were administered on the floor in 2 separate quiet rooms in a house in 1 of the participating villages.

The Bayley Mental Scale assesses sensory-perceptual acuity, discrimination and the ability to respond to such discrimination; early acquisition of object constancy and memory, learning, and problem solving ability; vocalisation and verbal communication; and evidence of the ability to generalise and classify [32]. Examples of tests are the infant's reaction to sound of bell, dangling a ring, box of blocks, pushing a toy car, or saying 'dada' or 'mama'. The Bayley Motor Scale provides a measure of the degree of control of the body and the infant's level of maturation in a wide range of gross-motor and fine-motor movements [32]. Examples of tests are if the infant is able to role from back to stomach, sit independently and pick up blocks or small tablets. From the infant's performance on the tests, aggregate scores were calculated, the 'raw' scores, from which age adjusted indices of mental development and psychomotor development, were derived. Three infants, 1 in each treatment group, had raw mental scores that were too low to be converted to the mental development index. These indices were set to 50, the lowest score that can be obtained in the mental development index.

Biochemical measures

Non-fasting venous blood samples were taken from women (~5 mL) at enrolment, near term and ~4 months postpartum and from infants at ~4 months of age (~2 mL) and collected in tubes without anticoagulant between 09.00 and 12.00 h. It was not possible to obtain blood samples in all cases due to maternal refusal and, in some cases, the amount of blood obtained was insufficient for all analyses. Haemoglobin was determined using the cyanmethemoglobin method (Test 3317; Merck, Darmstadt, Germany) at the Nutrition Research and Development Center laboratory, Bogor. For serum ferritin, soluble transferrin receptor and retinol analyses, blood was allowed to clot before it was placed in a cool box with cooling elements for transport to the laboratory in Bogor. Blood samples were centrifuged at 3000 *g* for 10 minutes at room temperature and serum was distributed among 3 vials. Serum was kept at -20°C for 1 month and subsequently at -79°C. The methods used have been described earlier with all analyses been carried out within 1 year of blood collection [30] (Chapter 2).

Anthropometry

Weight and length of infants were measured using standardised methods [33]. Weight and length of the newborn infant were measured by 2 of the authors (MKS and SM) at the time of the postnatal home visit [30] (Chapter 2). Thereafter 2 pairs of trained field assistants measured weight and length of infants when their mothers paid the monthly visit to the health service post (Posyandu) until 1 year of age. Weight was measured to the nearest 50 g by using a baby weighing scale (Misaki, Japan) which was tared each time before use. Calibration with a standard weight (5 kg) at regular intervals showed that all scales were stable and precise. Recumbent length was measured to the nearest 0.1 cm by using a wooden length board [34]. Infants were measured with light clothing. All field assistants had received training and were supervised every month by MKS and SM [31] (Chapter 3). At enrolment, near term and ~4 months postpartum body weight of the mothers was measured to the nearest 0.1 kg by using a UNICEF electronic weighing scale (model 890, SECA, Hamburg, Germany), height to the nearest 0.1 cm using a standing height measurement microtoise and mid-upper arm circumference (MUAC) to the nearest 0.1 cm by using a plastic measuring tape.

Data analysis

In the analyses the weekly vitamin A plus iron group was compared with the weekly iron group to evaluate the effect of vitamin A supplementation. The weekly iron group was compared to the 'daily' group to evaluate the different iron supplementation regimes. In addition, the relationship of maternal vitamin A and iron status with development and growth of infants was evaluated. Weight and length were converted into z-scores using the NCHS/WHO reference data incorporated in the Epi-Info software (Epi Info2000 version 1.0.5. CDC, Atlanta). Stunting was defined as a height-for-age z-score of <-2, underweight as weight-for-age z-score <-2 and wasting as weight-for-height z-score <-2. Anthropometric data of infants was grouped into monthly age groups of 0 (0 until 0.99 months) to 12 (12 until 12.99 months). Comparisons of continuous, normally distributed data between groups were done using *t*-test or ANOVA if correction for other variables was necessary. Comparisons of continuous, non-normally distributed data and categorical data between groups were done using Mann Whitney U-test and χ^2-test respectively. Associations of normally and non-normally distributed data were evaluated using Pearson's correlation and Spearman rank correlation respectively. Maternal vitamin A and iron status in relation to infant mental and psychomotor development indices was evaluated by ANCOVA (GLM procedure). The weekly iron and 'daily' groups did not differ with respect to anthropometric and biochemical variables of mothers and infants or infant development. Thus, treatment group was defined as having received vitamin A supplementation or not (weekly vitamin A plus iron group=1, weekly iron group=0, 'daily' group=0). Other variables included were those associated with mental or psychomotor indices, i.e. age of infant (6 or 12 months), consultation during pregnancy (as a proxy of health conscious behaviour) and maternal age. Birth order was highly correlated with age of mothers (r =0.75) and was therefore not included in the model. First, a full factorial model was applied and then the not significant interaction terms were excluded from the model step by step. Statistical analysis was carried out with the SPSS software package (Windows version 10.0.5 SPSS Inc., Chicago, IL).

Results

Of the 318 newborn infants initially eligible, 16 infants moved outside the research area and 12 infants died during the follow up period from birth to 1 year of age. In addition, 14 infants did not attend the test because they were sick, their mothers refused or were not present. Data are presented of the 276 infants that participated in assessment of the Bayley test. More infants were tested at 6 months (n=166) than at 12 months (n=110) of age, however, infants from the 2 age groups were evenly distributed over the 3 treatment groups (weekly vitamin A plus iron, weekly iron, 'daily'). Infants that were tested did not differ with respect to gender, birth order, neonatal weight and length, and maternal age, weight, height, biochemical variables, education and housing from those (n=42) that were not tested (data not shown). Except that mothers of infants that were tested had higher serum retinol concentrations at enrolment than mothers of infants that were not tested (1.00 ± 0.02 versus 0.90 ± 0.04 µmol/L, mean ± SE; P <0.05). Mental and psychomotor development indices of the Bayley test did not differ between psychologists (**Table 5.1**). One infant could not be tested for the mental test.

Table 5.1 Comparison of mental and psychomotor development indices of Indonesian infants measured by 2 psychologists who conducted the Bayley test[1]

Infant	Psychologist A	Psychologist B
n	126	150
Age, *months*	8.5 ± 0.2	8.7 ± 0.2
Mental 'raw' scores	82.8 ± 1.5[2]	81.9 ± 1.8
Mental development index	95.2 ± 1.2[2]	93.0 ± 1.6
Psychomotor 'raw ' scores	35.9 ± 0.7	35.8 ± 0.9
Psychomotor development index	103.1 ± 1.8	102.2 ± 1.4

[1]Mean ± SEM, [2]n=125.

Table 5.2 General characteristics of infants whose mental and psychomotor development was assessed using the Bayley test in the weekly vitamin A plus iron, weekly iron and 'daily' group[1]

	Weekly vitamin A plus iron	Weekly iron	'Daily'
Infant characteristics			
Boy / girl (n)	41/53	38/56	49/39
Birth order	1.4 ± 0.1	1.5 ± 0.1	1.7 ± 0.2
Neonatal weight, *kg*	3.2 ± 0.1	3.2 ± 0.1	3.3 ± 0.1
Neonatal length, *cm*	49.5 ± 0.2	49.7 ± 0.2	49.8 ± 0.3
Anaemic: haemoglobin concentrations <100 g/L, % (n)[2]	31 (68)	29 (73)	28 (68)
Marginal vitamin A deficient: serum retinol concentrations <0.70 µmol/L, % (n)[2]	69 (54)	80 (60)	74 (57)
Gestational period, *weeks*	37.9 ± 0.3	38.5 ± 0.3	37.3 ± 0.3*
Maternal characteristics			
Age at enrolment, *years*	24.2 ± 0.5	24.1 ± 0.5	24.9 ± 0.5

Weight at enrolment, *kg*	49.2 ± 0.8	48.4 ± 0.6	49.1 ± 0.8
Height at enrolment, *cm*	149.1 ± 0.6	149.1 ± 0.5	149.0 ± 0.5
Body mass index, *kg/m²*	22.1 ± 0.3	21.8 ± 0.2	22.1 ± 0.3
Anaemic: haemoglobin concentration <110 g/L, % (n)			
Enrolment	42 (94)	44 (91)	46 (87)
Near term	40 (77)	47 (79)	47 (60)
Iron deficiency: serum ferritin concentration <12 μg/L, % (n)			
Enrolment	45 (92)	46 (91)	46 (77)
Near term	55 (76)	50 (76)	54 (57)
Iron deficiency anaemia: anaemic and iron deficient, % (n)			
Enrolment	16 (92)	22 (88)	25 (77)
Near term	28 (76)	30 (76)	33 (57)
Marginal vitamin A deficiency: serum retinol concentration <0.70 μmol/L, % (n)			
Enrolment	12 (92)	15 (91)	13 (79)
Near term	12 (75)	32 (71)**	48 (59)**
More than 4 consultations during pregnancy, %	61	63	65
Delivery assisted by traditional birth attendant, %	82	85	74
Education beyond elementary school, %	26	30	22
Other characteristics			
Occupation of father, %			
Daily worker (labourer, farmer, driver)	47	50	46
Monthly worker (government or private employee, retired)	31	36	33
Trader	22	14	22
Household members	4.7 ± 0.2	4.7 ± 0.2	5.1 ± 0.2
House with brick wall, tile roof and cemented floor, %	67	65	70

[1]Data are presented as mean ± SE and n=276, unless stated otherwise; [2]Blood samples were taken at ~4 months of age; *Significantly different from the weekly iron group, P <0.01 (t-test); **Significantly different from the weekly vitamin A plus iron group, P <0.01 (χ^2-test).

General characteristics of the vitamin A plus iron group and the iron group were similar as well as the characteristics of the iron group compared with the 'daily' group (**Table 5.2**). Except for a small difference in the gestation period between infants from the weekly iron and 'daily' groups. In addition vitamin A deficiency near term was less prevalent in mothers in the vitamin A plus iron group than in the other 2 groups, as reported earlier [27]. Infants in the weekly vitamin A plus iron group had similar development indices as infants in the weekly iron group (**Table 5.3**). Also the infants in the weekly iron group did not differ from infants in the 'daily' group. Mental and psychomotor development indices were associated within supplementation and age groups ($0.427 \leq r \leq 0.706$, $P < 0.05$). In addition proportions of infants in the 4 categories of the Bayley reference [35] did not differ significantly among groups (**Table 5.4**). Compared with psychomotor development indices, mental development indices were lower at 6 months of age but higher at 12 months of age in all treatment groups. Mental development indices were higher at 12 months of age than at 6 months of age, whereas psychomotor development indices did not differ between both age groups (**Table 5.3**). Mental and psychomotor development indices did not differ between gender. Both indices were inversely correlated with age at the 6 months assessment, $r = -0.346$ and $r = -0.260$ ($P < 0.01$) respectively and positively correlated with age at the 12 months assessment, $r = 0.273$ and $r = 0.200$ ($P < 0.05$) respectively.

Mental development indices were significantly ($P < 0.05$) but weakly associated with birth order ($r = -0.125$), maternal age ($r = -0.139$) and consultation during pregnancy ($r = 0.125$) and psychomotor development indices with neonatal length ($r = 0.120$) in all infants. None of the other variables presented in **Table 5.2** such as education and nutritional status of women and occupation of both men and women (94% of the women were housewives) were associated with infant development. Mental and psychomotor development indices were also not associated with growth during the first year of life (data not shown).

Infants of mothers with serum retinol concentrations ≥0.70 μmol/L at near term had significantly higher mental development indices than those of mothers with serum retinol concentrations <0.70 μmol/L corrected for vitamin A supplementation (yes or no), age of infant (6 or 12 months), consultation during pregnancy and maternal age (**Table 5.5**). The association was consistent across all groups. Infants of mothers with serum ferritin concentrations <12 μg/L at near term had significantly higher mental and psychomotor development indices than those of mothers with serum ferritin concentrations ≥12 μg/L, corrected as stated above (**Table 5.5**). The association was consistent across all groups. Neither iron status nor vitamin A status of mothers at enrolment was associated with mental or psychomotor development of infants. Infant vitamin A and iron status were not associated with mental or psychomotor development indices, except for serum transferrin receptors (**Table 5.5**).

Table 5.3 Mental and psychomotor development indices of Indonesian infants at 6 or 12 months of age whose mothers had been supplemented with vitamin A plus iron or with iron alone or had participated in the governmental iron supplementation program ('daily' group)[1]

	Weekly vitamin A plus iron	Weekly iron	'Daily'	All infants
Mean age, *months* (n)	8.9 ± 0.3 (94)	8.7 ± 0.3 (94)	8.2 ± 0.3 (88[2])	8.6 ± 0.2 (276[2])
Mean age at 6 months (n)	6.4 ± 0.1 (52)	6.4 ± 0.1 (55)	6.4 ± 0.1 (59[2])	6.4 ± 0.0 (166[2])
Mean age at 12 months (n)	12.0 ± 0.0 (42)	12.0 ± 0.0 (39)	12.0 ± 0.0 (29)	12.0 ± 0.0 (110)
Mental development index	94.7 ± 1.69*	94.5 ± 1.7*	92.7 ± 2.0*	94.0 ± 1.0*
Mental development index at 6 months	85.9 ± 1.7*†	87.8 ± 1.5*†	84.1 ± 1.9*†	85.9 ± 1.0*†
Mental development index at 12 months	105.4 ± 2.3**	104.0 ± 2.8	109.8 ± 2.5	106.1 ± 1.5**
Psychomotor development index	101.3 ± 1.9	102.8 ± 1.9	103.7 ± 2.1	102.6 ± 1.1
Psychomotor development index at 6 months	103.7 ± 2.2	103.2 ± 1.8	101.4 ± 2.2	102.7 ± 1.2
Psychomotor development index at 12 months	98.3 ± 3.3	102.3 ± 3.8	108.5 ± 4.4	102.4 ± 2.2

[1]Mean ± SE; [2]1 missing value for the mental development index; *Significantly different from the psychomotor development indices in the same age and supplementation group, $P < 0.01$, **$P < 0.05$; †Significantly different from indices at 12 months of age, $P < 0.01$.

Table 5.4 Proportion of infant mental and psychomotor development indices from the three treatment groups following the Bayley categories and compared with infants from the BSID II reference [35]

	BSID II reference	Weekly vitamin A plus iron	Weekly iron	'Daily'
		%		
Mental development index				
Significant delay	1.5	6.4	1.1	9.2
Mild delay	11.1	16	25.5	25.3
Within normal limits	72.6	63.8	58.5	49.4
Accelerated	14.8	13.8	14.9	16.1
Psychomotor development index				
Significant delay	2.3	3.2	4.3	5.7
Mild delay	12.5	17	8.5	5.7
Within normal limits	68.7	46.8	63.8	59.1
Accelerated	16.5	33	23.4	29.5

Table 5.5 Association of iron and vitamin A status of pregnant women and infants with mental and psychomotor development indices of infants[1]

Mental development index		Psychomotor development index	
Maternal at near term			
Serum ferritin <12 μ/L	Serum ferritin ≥12 μ/L	Serum ferritin <12 μ/L	Serum ferritin ≥12 μ/L
96.9 ± 1.6 (110)	92.3 ± 1.7 (98)†	105.3 ± 1.8 (111)	100.8 ± 1.8 (98)†
Serum sTfR[2] ≥1.86 mg/L	Serum sTfR <1.87 mg/L	Serum sTfR ≥1.86 mg/L	Serum sTfR <1.87 mg/L
96.4 ± 1.8 (97)	92.9 ± 1.6 (97)	103.3 ± 2.0 (98)	101.8 ± 1.8 (97)
Serum retinol <0.70 μmol/L	Serum retinol ≥0.70 μmol/L	Serum retinol <0.70 μmol/L	Serum retinol ≥0.70 μmol/L
91.4 ± 2.1 (59)	96.2 ± 1.4 (145)*	98.9 ± 2.4 (60)	104.7 ± 1.6 (145)
Infant at ~4 months of age			
Haemoglobin <100 g/L	Haemoglobin ≥100 g/L	Haemoglobin <100 g/L	Haemoglobin ≥100 g/L
93.5 ± 2.0 (61)	92.4 ± 1.4 (148)	103.2 ± 2.2 (61)	102.6 ± 1.5 (148)
Serum sTfR[2] ≥2.06 mg/L	Serum sTfR <2.06 mg/L	Serum sTfR ≥2.06 mg/L	Serum sTfR <2.06 mg/L
95.0 ± 1.7 (84)	89.6 ± 1.6 (82)*	104.4 ± 2.0 (84)	100.9 ± 1.8 (82)
Serum retinol <0.70 μmol/L	Serum retinol ≥0.70 μmol/L	Serum retinol <0.70 μmol/L	Serum retinol ≥0.70 μmol/L
93.7 ± 1.5 (127)	93.1 ± 1.8 (44)	103.8 ± 1.6 (127)	103.0 ± 2.8 (44)

[1]Mean ± SE (n); [2]sTfr, soluble transferrin receptor, groups based on the median values in mothers at near term and infants as cut-off; †Significantly different indices between groups corrected for vitamin A supplementation (yes or no), age group (6 or 12 months), consultation during pregnancy (≤4 or >4 times) and maternal age, $P < 0.10$, $^{*}P < 0.05$.

Discussion

As far as we know this is the first study to examine the relationship between maternal vitamin A and iron status during pregnancy and the impact of supplementation on infant development. It has been shown that iodine deficiency *in utero* negatively affects pregnancy outcome and infant development, and there is some evidence for an effect of protein-energy malnutrition as well [36]. Iron deficiency during infancy has been shown to have long term negative consequences for development [1,3]. Although we did not find an impact of supplementation, infants of mothers with adequate vitamin A status near term had better mental development than did infants of mothers with marginal vitamin A status near term. However, it is not clear whether this was due to better caring capacity of mothers with better nutritional status [22] or to vitamin A status during pregnancy by itself. Although our results suggest that vitamin A plays a role in brain development during gestation, we did not prove that maternal vitamin A status is a determinant of infant development. Animal studies have shown that retinoic acid is important in embryonic development and that highest concentrations are found in the neural tube, which is to be developed into the spinal cord and the brain [9]. A study in vitamin A deficient pregnant rats showed that vitamin A injection during pregnancy increased brain weight relatively to their body weight compared with those that did not receive vitamin A [37].

Changes in foetal or embryonic development may be subtler than can be picked up by a mental and psychomotor development test. But it may also be that the moderate degree of iron and vitamin A deficiency in the population studied may account for the lack of effect of the supplementation [27,31]. Little is known about the functional outcomes of supplementation with micronutrient during pregnancy [38]. One study showed that zinc supplementation during pregnancy was found to be negatively associated with mental development during infancy [39]. Concerning vitamin A, only 1 study has shown that supplementation of infants with vitamin A (52 µmol) at birth improved their Bayley indices at 3 years of age [40].

The different iron treatment regimes, supervised weekly iron supplementation or participation in the governmental programme did not lead to differences in mental or psychomotor development. Iron deficiency anaemia at early age has been associated with development during infancy and childhood [41,42]. At ~4 months of age one third of the infants studied were anaemic but only 3 were iron deficient [30] (Chapter 2). We only found, unexpectedly, that infants with higher serum transferrin receptor concentrations (median values as the cut off) had higher mental development indices. Therefore we assume that higher serum transferrin receptor concentrations reflected the process of erythropoiesis rather than that of mild iron deficiency in these infants [43]. Apparently, at least until 4 months of age infants had sufficient iron stores or were protected by iron supply from breast milk [44,45]. Almost all infants were breast-fed up to at least 12 months of age, thus breast milk intake, including essential fatty acids, maternal child interaction and reduced morbidity due to breast feeding may have protected the infants in our study from retarded development [36,46].

Unexpectedly we found that infants of mothers with adequate iron stores tended to have lower mental and psychomotor development indices than did infants of mothers with low iron stores near term. However, serum transferrin receptor concentrations, a useful indicator for mild iron deficiency during pregnancy and not influenced by infection [47], were not associated with infant development. It may be that our finding was due to chance, as it is difficult to control for all confounding factors [41]. Three infants had too low scores to be converted into the mental development index (indices were set to 50), however excluding or including these infants in our data did not change the results.

Factors such as low socio-economic status, poor maternal education and lack of stimulation in the home are both associated with anaemia and poor cognitive development [41]. We did not observe an association between maternal education and infant development. However, infants of mothers that were not anaemic and/or not iron deficient at ~4 months postpartum had consistently but non-significantly higher mental and psychomotor development indices than their counterparts (data not shown).

The Bayley test is widely used to test development of infants [42]. It has however been criticised because the predictive value of the scale for later development is small and it seems that the concept of 'mental' changes with age [48], as is shown by the difference in mental development indices at 6 and 12 months of age in the present study. However, studies have shown that the Bayley test can detect differences in development due to supplementary feeding in infants [12,49] and children [40]. We assured appropriate use of the Bayley test by employing 2 Indonesian psychologists who had much experience with using it and who spoke the local language. The indices of the infants studied were in line with those in earlier studies in Indonesia and Bangladesh [12,39,40,49] and with the Bayley II reference infants [35].

In conclusion, mental and psychomotor development of infants did not differ among those whose mothers were supplemented with vitamin A plus iron or iron alone, or whose mothers participated in the governmental iron supplementation program. From the present study there is an indication that vitamin A status of mothers in the third trimester of pregnancy, the period in which in growth spurt of the human brain growth [6], plays a role in mental development of infants, but further research is necessary to test this hypothesis.

Acknowledgements

We thank all mothers and infants who participated in the Bayley tests and the voluntary health workers that brought the mothers to the location for testing. We thank Ms. Lies Karyadi and Ms. Hanny Sunggi for performing the Bayley tests.

References

1. Lozoff B, Jimenez E, Wolf AW. Long-term developmental outcome of infants with iron deficiency. N Engl J Med 1991;325:687-694.
2. 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;66:1357-1363.

3. Pollitt E. Early iron deficiency anemia and later mental retardation [comment]. Am J Clin Nutr 1999;69:4-5.
4. Barker DJ. Maternal nutrition, fetal nutrition, and disease in later life. Nutrition 1997;13:807-813.
5. Levitsky DA, Strupp BJ. Malnutrition and the brain: changing concepts, changing concerns. J Nutr 1995;125:2212S-2220S.
6. Wauben IP, Wainwright PE. The influence of neonatal nutrition on behavioral development: a critical appraisal. Nutr Rev 1999;57:35-44.
7. Chen Q, Connor JR, Beard JL. Brain iron, transferrin and ferritin concentrations are altered in developing iron-deficient rats. J Nutr 1995;125:1529-1535.
8. Felt BT, Lozoff B. Brain iron and behavior of rats are not normalized by treatment of iron deficiency anemia during early development. J Nutr 1996;126:693-701.
9. Maden M. The role of retinoic acid in embryonic and post-embryonic development. Proc Nutr Soc 2000;59:65-73.
10. Oski FA, Honig AS. The effects of therapy on the developmental scores of iron-deficient infants. J Pediatr 1978;92:21-25.
11. Lozoff B, Brittenham GM, Viteri FE, Wolf AW, Urrutia JJ. The effects of short-term oral iron therapy on developmental deficits in iron-deficient anemic infants. J Pediatr 1982;100:351-357.
12. Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. Lancet 1993;341:1-4.
13. ACC/SCN. Fourth report on the world nutrition situation. Nutrition throughout the life cycle. Geneva: ACC/SCN in collaboration with IFPRI, 2000.
14. Kosen S, Herman S, Schultink W. An overview of studies on iron deficiency in Indonesia. Nutr Res 1998;18:1935-1941.
15. Suharno D, West CE, Muhilal, et al. Cross-sectional study on the iron and vitamin A status of pregnant women in West Java, Indonesia. Am J Clin Nutr 1992;56:988-993.
16. Scholl TO, Hediger ML. Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. Am J Clin Nutr 1994;59:492S-500S.
17. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 2000;71:1280S-1284S.
18. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997;66:1178-1182.
19. Suharno D, West CE, Muhilal, Karyadi D, Hautvast JG. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet 1993;342:1325-1328.
20. Mejia LA, Chew F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. Am J Clin Nutr 1988;48:595-600.
21. Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. Am J Clin Nutr 1994;59:517S-522S.
22. Adair LS, Pollitt E. Outcome of maternal nutritional supplementation: a comprehensive review of the Bacon Chow study. Am J Clin Nutr 1985;41:948-978.
23. Grantham-McGregor S. A review of studies on the effect of severe malnutrition on mental development. J Nutr 1995;125:2233-2238.
24. Gorman KS. Malnutrition and cognitive development: evidence from experimental/quasi-experimental studies among the mild-to-moderately malnourished. J Nutr 1995;125:2239-2244.
25. Yip R. Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. Am J Clin Nutr 2000;72:272S-278S.
26. Schultink W, van-der-Ree M, Matulessi P, Gross R. Low compliance with an iron-supplementation program: a study among pregnant women in Jakarta, Indonesia. Am J Clin Nutr 1993;57:135-139.
27. Muslimatun S, Schmidt MK, Schultink W, et al. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J Nutr 2001;131:85-90.
28. Schultink JW, Gross R. Iron deficiency alleviation in developing countries. Nutr Res Rev 1996; 9:281-293.
29. Muslimatun S, Schmidt MK, West CE, Schultink W, Hautvast JGAJ, Karyadi D. Weekly vitamin A and iron supplementation during pregnancy increases vitamin A concentration of breast milk but not iron status in Indonesian lactating women. J Nutr 2001;131:2664-2669.

30. Schmidt MK, Muslimatun S, West CE, Schultink W, Hautvast JGAJ. Vitamin A and iron supplementation of Indonesian pregnant women benefits vitamin A status of their infants. Br J Nutr 2001;86:in press.
31. Schmidt MK, Muslimatun S, Schultink W, West CE, Hautvast JGAJ. Randomised double-blind trial of the effect of vitamin A supplementation of Indonesian pregnant women on morbidity and growth of their infants during the first year of life. Eur J Clin Nutr 2002:in press.
32. Bayley N. Manual for the Bayley scales of development. New York: The Psychological corporation, 1969.
33. Jeliffe DB. The assessment of the nutritional status of the community. Monograph series WHO no 53. Geneva: WHO, 1996.
34. Gross R, Kielmann A, Korte R, Schoeneberger H, Schultink W. Nutrition baseline surveys in communities. Bangkok: SEAMEO-TROPMED and Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), 1997.
35. Bayley N. Manual for the Bayley scales of development, 2 ed. San Antonio, TX: The Psychological corporation, 1993.
36. Grantham-McGregor SM, Walker SP, Chang SM. Nutritional deficiencies and later behavioral development. Proc Nutr Soc 2000;59:47-54.
37. Antipatis C, Grant G, Ashworth CJ. Moderate maternal vitamin A deficiency affects perinatal organ growth and development in rats. Br J Nutr 2000;84:125-132.
38. Ramakrishnan U, Manjrekar R, Rivera J, Gonzales CT, Martorell R. Micronutrients and pregnancy outcome: A review of the literature. Nutr Res 1999;19:103-159.
39. Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM. Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. Am J Clin Nutr 2001;74:381-386.
40. Humphrey JH, Agoestina T, Juliana A, et al. Neonatal vitamin A supplementation: effect on development and growth at 3 y of age. Am J Clin Nutr 1998;68:109-117.
41. 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-666S.
42. 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. A report of the International Nutritional Anemia Consultative Group. Washington, DC: International Life Science Institute, 1998.
43. Olivares M, Walter T, Cook JD, Hertrampf E, Pizarro F. Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy. Am J Clin Nutr 2000;72:1191-1195.
44. Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. Am J Clin Nutr 1999;70:525-535.
45. Siimes MA, Salmenpera L, Perheentupa J. Exclusive breast-feeding for 9 months: risk of iron deficiency. J Pediatr 1984;104:196-199.
46. Uauy R, de-Andraca I. Human milk and breast feeding for optimal mental development. J Nutr 1995;125:2278-2280.
47. Carriaga MT, Skikne BS, Finley B, Cutler B, Cook JD. Serum transferrin receptor for the detection of iron deficiency in pregnancy. Am J Clin Nutr 1991;54:1077-1081.
48. Pollitt E. Statistical and psychobiological significance in developmental research. Am J Clin Nutr 2001;74:281-282.
49. Husaini MA, Karyadi L, Husaini YK, Sandjaja, Karyadi D, Pollitt E. Developmental effects of short-term supplementary feeding in nutritionally-at-risk Indonesian infants. Am J Clin Nutr 1991;54:799-804.

6

General discussion

6 General discussion

6.1 Introduction

We investigated the effect of weekly supplementation during gestation with vitamin A plus iron compared with iron alone on growth, nutritional status, morbidity, and mental and psychomotor development of Indonesian infants. Our hypothesis was that increment of nutritional status of pregnant women, would result in improved nutrient stores in infants and higher breast milk concentrations of mothers. In addition, we hypothesised that this would lead to better growth and health, i.e. a reduction in morbidity and an improvement in mental and psychomotor development, of infants. We tested these hypotheses in a community-based intervention trial in Indonesian pregnant women, who were marginally iron and vitamin A deficient. We also aimed to compare the effectiveness of supervised weekly iron supplementation with the ongoing national 'daily' iron supplementation programme. We hypothesised that infants of mothers who had been supplemented weekly with iron during pregnancy would have higher iron stores and nutritional status than infants of mothers who had participated in the national iron supplementation programme. To test this hypothesis we recruited a third group of pregnant women from neighbouring villages ('daily' group). Our main conclusion is that vitamin A plus iron supplementation during gestation did not affect growth, iron status, morbidity, or mental or psychomotor development of infants (Chapters 2, 3 & 5). We found however that infants of mothers who had been supplemented with vitamin A plus iron during pregnancy had higher serum retinol levels at ~4 months of age than those of mothers who had been supplemented with iron alone (Chapter 2). Infants of mothers who had received weekly iron supplementation during pregnancy did not differ regarding any of the variables mentioned above compared with infants of mothers that had been participating in the national iron supplementation programme (Chapters 2 & 5). Growth faltering of infants started around 6 months of age and led to high prevalence of underweight (32%) and stunting (25%) at 12 months of age. Prenatal factors as reflected in neonatal weight and length of infants were important determinants of infant growth (Chapter 4).

6.2 Effects of vitamin A supplementation during pregnancy on vitamin A status, growth and health of infants

6.2.1 Vitamin A status and intake of infants

The present study and a study in Nepal [1] have shown that vitamin A supplementation during pregnancy improved vitamin A status of infants at about 3-4 months of age (Chapter 2). The difference in serum retinol concentrations of the infants of vitamin A supplemented mothers during pregnancy could have resulted from differences in the vitamin A stores built up during gestation, from differences in retinol concentrations in breast milk, or both [2,3]. It is not yet known to what extent vitamin A is transferred via the placenta [2]. We found that concentrations of vitamin A in transitional milk (4-7 days postpartum) and mature breast milk (3 months postpartum) were 47% and 17% higher, respectively, in the weekly vitamin A and iron group than in the weekly iron group [4]. In agreement with this we found that infant serum retinol concentrations in the weekly vitamin A and iron group were 15% higher than in the weekly iron group (Chapter 2). We consider therefore that weekly supplementation with 4800 IU vitamin A during the second and third trimester of pregnancy made a significant contribution to improving vitamin A status of mothers and infants.

Two earlier studies indicated that placental transfer of vitamin A can be increased if women are supplemented with vitamin A. These studies reported that vitamin A concentrations in cord blood of infants of mothers supplemented with vitamin A during pregnancy were higher than that of their counterparts [5,6]. However, the possible contribution of an increased placental transfer of vitamin A is low as compared to that supplied via breast milk. Liver samples taken at autopsy from American infants showed that median liver concentrations at birth were 11 μg retinol/g wet weight of liver [7]. Assuming that a newborn infant of 3.1 kg (Chapter 2) has a liver weight of ~150 g (~5% of the body weight), the total amount of vitamin A store would be ~1650 μg. Normally levels of vitamin A in mature milk (≥21 d postpartum) are 500-750 μg retinol/L [8]. Thus, 1 week of exclusive breast feeding, with an intake of about 700 mL breast milk per day [9], would transfer more vitamin A to the infant than that transferred via the placenta during 9 months gestation.

The vitamin A requirement of infants is 350 μg (1.2 μmol) retinol per day [10]. We did not measure intake of breast milk in our infants, because in a pilot study we found that it was not feasible. Vitamin A concentrations in transitional milk were reasonably high (3.0 ± 2.4 μmol/L, mean ± SD) but by 4 months postpartum had declined to 1.2 ± 0.8 μmol/L. At 4 months of age, about 80% of infants were already receiving complementary feeding (Chapters 2, 3 & 4), but the effective supply of vitamin A from foods was considered low [8,11,12] (Chapters 3 & 4). Thus it is likely that vitamin A intake in the infants studied was not sufficient to build up their vitamin A stores.

The high proportion of infants with serum retinol concentrations <0.70 μmol/L indicates that vitamin A deficiency constitutes a severe public health problem in this population [13]. However these criteria are based on children aged 6-71 mo of age. The functional significance of low serum retinol concentrations in young infants is still unclear [14]. As far as we know, none of the infants in the present study had signs of vitamin A deficiency, and serum retinol levels were within range of values reported in other studies [15-17]. A study in which Indian infants were followed up from birth to 6 months of age showed significant increases in serum retinol concentrations. In this study 30% of the infants had serum retinol concentrations <0.70 μmol/L at 6 months of age, although none of the infants showed signs of xerophthalmia [18].

6.2.2 Growth and health of infants

Vitamin A supplementation of marginally vitamin A deficient and iron deficient mothers during pregnancy did not improve growth or health of infants. The present study found however that vitamin A status of infants was positively associated with anthropometric parameters of infants during the first year of life (Chapters 2 & 3) and negatively associated with respiratory infections at ~4 months of age (Chapter 2). This was supported by the finding that vitamin A levels in breast milk were positively associated with growth of infants (discussion, Chapter 4). In addition we found a positive association between vitamin A status of women near term and mental development of their infants (Chapter 5). However, it is not clear whether this was due to better caring capacity of mothers with better nutritional status [19] or to vitamin A status during pregnancy by itself. Studies that have reported positive effects of vitamin A supplementation on morbidity or growth, were carried out in children above the age of 6 months and were mostly conducted in areas where vitamin A deficiency was severe [20-24]. The children in the present study were younger and the population was 'only' marginally vitamin A deficient. In addition, although serum retinol concentrations of infants were increased after supplementation of their mothers during pregnancy. This may not have led to a sufficient increase in vitamin A status of infants to have a substantial impact on their growth and health. Supplementation with vitamin A during pregnancy also did not reduce infant mortality in the study in Nepal [1]. While the impact of vitamin A supplementation during pregnancy on other functional outcomes needs to be explored further, the large reduction in maternal mortality [25], night blindness [26] and anaemia [27,28] justifies vitamin A supplementation during pregnancy as long as such supplementation does not exceed the advised safe dosage [29].

6.3 Effects of iron supplementation during pregnancy on iron status, growth and health of infants

6.3.1 Iron status and intake of infants

Iron status of mothers during pregnancy or lactation in the present study was not associated with iron status of their infants (Chapter 2). Although half of the women were anaemic or iron deficient during pregnancy and one fifth had iron deficiency anaemia (Chapter 5), the range of the parameters of iron status was relatively small. Studies which found a relationship between infant iron status and that of their mothers during pregnancy, were those that included women with severe anaemia or with very low iron stores (serum ferritin concentrations <10 μg/L) [30,31], or compared supplementation with high doses of iron (100 mg per day) with placebo [32].

Iron levels in breast milk declined from about 5 days to 3 months postpartum and were low compared to populations of developed countries [4]. Although infants in the present study were not considered iron deficient at the time of the assessment at ~4 months of age (Chapter 2). The negative relationship between serum ferritin concentrations and age indicates that many infants may become iron deficient by 6 months of age, unless iron intake is increased. Increased intake of iron may affect growth not only by providing sufficient stores, but also by increasing appetite [33].

6.3.2 Growth and health of infants

Because it was considered unethical to include a placebo group in which mothers did not receive iron in our study, we cannot draw conclusions regarding the impact of iron supplementation on growth and health of infants. However, maternal iron status during pregnancy was not associated with infant growth or health in the present study (Chapters

2 & 5). The present study did not find any differences between weekly supervised iron supplementation and 'daily' iron supplementation in the national program. On the basis of infant outcomes (Chapters 2 & 5), there is no justification for recommending one regime above the other. This may not be surprising considering that the average amount of iron taken in the weekly groups was comparable to that in the 'daily' group, about 40 to 50 tablets of 60 mg iron. Although iron status of mothers in the 'daily' group who took less than 50 tablets decreased during pregnancy [28], this did not lead to differences in iron status or growth of infants (Chapter 2).

The interpretation of haemoglobin concentrations during pregnancy in relation to anaemia should be related to the period of gestation because of its association with plasma volume expansion. Taking that into account, the relative risk of babies being born with low birth weight or preterm tends to show a higher risk only for severe anaemia [34]. One study showed that at about 17 weeks of pregnancy iron deficiency anaemia, but not anaemia from other causes, was associated with lower birth weight and preterm delivery [35]. Folate is critical in foetal development especially of the neural tube and may affect pregnancy outcome as well [36]. Because both iron and folic acid are included in the supplements, we cannot assess the separate contribution of folic acid or iron on pregnancy outcome, but from a public health point of view both should be included.

The high proportion of mothers that were anaemic or had iron-deficiency anaemia postpartum [4] is worrying. Poor maternal nutritional status and especially anaemia may lead to less caring capacity and thus may affect development of infants (Chapter 5). As shown by others [37-40], iron deficiency anaemia during infancy has a negative effect on mental and motor development. However, the effects of iron deficiency during pregnancy on infant development have not yet been thoroughly investigated.

6.4 Growth retardation in infancy

One third of livebirth infants were born preterm (<37 weeks) but the proportion of low birth weights (<2500 g) was small (5.5%). Experienced midwives estimated the gestational age of the infants at enrolment and near term by palpation and by asking about the last menstruation date. From these assessments and the date of birth, we calculated the duration of gestation. Therefore, the estimated proportion of preterm births may not be very accurate. Neonatal weight and length were measured within 72 hours only for 20% of the infants, therefore, the proportion of low birth weight may be somewhat underestimated. However the proportion of infants that were stunted (7%) or underweight (5%) at the neonatal assessment was small. The present studies showed that growth of infants started to falter during the second half of infancy (Chapter 4) as is often reported in other Asian studies [41,42]. By 12 months of age 24% of the infants were stunted while 32% of the infants were underweight. The proportions had increased even more at 15 months of age (Chapter 4). The reference curves used to determine prevalence of stunting have been criticised because they do not reflect growth of breast-fed infants and do not include a sufficient sample size [43-45]. A new international reference curve for infant and child growth is being prepared currently by the World Health Organisation.

During infancy, prevalence of fever, running nose and coughing, was high and contributed to growth retardation. Immunisation coverage was found to be inadequate, by 1 year of age about half of the infants had completed BCG, DTP and polio vaccinations but only about 8% of the infants had completed their immunisation schedule which also includes measles and hepatitis. Because almost all infant were breast-fed up to 12 months of age but exclusive breast-feeding was rare, we did not find a relationship between breast-feeding and growth or morbidity of infants. However, intake of complementary foods contributed to infant growth. It has been suggested recently that not only infant food practices, but also feeding behaviour and stimulation may affect infant food intake and thus nutritional status and growth [46,47].

The most important determinants of infant growth and nutritional status were neonatal weight and length. This suggests that maternal nutrition during pregnancy is indeed an important determinant of infant nutritional status and growth. Hence, the gestation period or early infancy may be the best time to intervene in order to improve growth performance later in life [48]. However, as infant growth is influenced by many factors (Chapter 4), interventions directed at infants or their mothers during pregnancy may not be effective in improving infant growth. Therefore, the whole life cycle should be considered and intervention should start before pregnancy. In addition, deficiencies of other micronutrients, besides iron and vitamin A, which could negatively affect growth, may be prevalent. Therefore, improving growth of infants may not be achieved by increasing intake of only 1 or 2 nutrients during infancy. Existing child health and nutrition promotion programs need to be improved and intensified with special attention to immunisation coverage, feeding practices and behaviour, and intake of micronutrients.

6.5 Methodological considerations of the study[1]

The aim of this community-based study was to examine the effect of supplementation with vitamin A and iron on functional outcomes including pregnancy outcomes, breast milk quality, and infant growth and health. It is worth explaining some methodological aspects of the whole study [49,50].

6.5.1 Inclusion criteria

It has been found that the efficacy of iron supplementation is proportional to the dose, duration of the supplementation and the initial level of iron deficiency [51,52]. An earlier study on iron and vitamin A supplementation carried out among anaemic women, in which the supplements were administered daily but for a shorter duration, found a higher response on haemoglobin concentration [27]. We conducted the study as close as possible to the 'real life' setting in the community, therefore women were administered into the study irrespective of whether they were suffering from anaemia or deficiencies of iron or vitamin A. This would allow us to predict the likely effect of such supplementation at the programmatic level, although the intake of supplements was supervised.

[1] This and subsequent sections of this chapter have been written jointly with Siti Muslimatun and also appear in her thesis [49].

6.5.2 Absence of a placebo group and weekly versus 'daily' supplementation

It was not possible to include a placebo group in this study as this was considered as unethical because of government policy that pregnant women should receive 90-120 iron tablets throughout pregnancy. We started this study assuming that compliance to iron supplementation in the national iron supplementation program ('daily' group) would be very low [53]. As has been reported from other studies [53-55], indeed, the compliance was poor and the variation in iron tablet intake was large. Only 17% of the women ingested the amount as prescribed by the government (>90 tablets), although >95% of women received and took iron supplements.

By including this 'daily' group we aimed to investigate the impact of either low or high compliance on improving iron status of pregnant women and their infants. The first aim was to measure the comparative effectiveness of the daily and weekly regimens in improving iron status of pregnant women. However, it was not possible to address this aim fully because of differences in the degree of supervision, which has an effect on compliance. The compliance of iron tablet intake in the group allocated to the ongoing national iron program ('daily' group) was assessed by interview at the postnatal visit whereas the tablet intake in the weekly iron group was strictly supervised. The assessment of iron supplement intake in the 'daily' group by means of interview was considered to be sufficient as shown by the consistency of changes in iron parameters with the number of iron tablets taken during pregnancy. The second aim of the study was to assess the effect of low versus high iron tablet intake on maternal iron status and iron status of newborn infants. However, this aim could not be addressed satisfactorily. Although there was a wide variation in tablet intake, the number of subjects with either a high (≥90) or low (≤30) intake of tablets was insufficient. In order to have a reasonable number of subjects with different levels of compliance, the number of subjects in the 'daily' group should have been 5-6 times larger based on the observation that only one fifth of the pregnant women consumed between 90 and 120 tablets as is the policy.

6.5.3 Attendance rate and loss to follow-up

High attrition (20-45%) is commonly observed in prospective community-based studies of pregnant women. This can be due to either non-attendance or early delivery. In our study, 26% and 38%, in the weekly groups and 'daily' group respectively, of pregnant women did not come for the near term assessment, whereas the proportion of pregnant women having antenatal care ≤4 times (including 2 times for study assessment) were 38% and 35%, respectively. However, only 7.4% of women in the weekly groups withdrew during pregnancy. The difficulty of reaching pregnant women is also reflected in the relatively long delay in reporting birth of babies, particularly from the 'daily' group. Neonates in the 'daily' group were measured later than those in the weekly groups (6.9 ± 3.7 versus 5.2 ± 3.3 days, $P < 0.05$; mean ± SD). Continuous motivation and conducting the assessment closer to their place of residence improved the attendance, as shown by low prevalence of lost to follow-up during infancy, 9% and 8% in the weekly groups and 'daily' group, respectively.

6.5.4 Indonesian crises

The economic crisis in Indonesia and other Southeast Asian countries started 3 months prior to the start of the fieldwork in October 1997. Between October 1997 and January 1998 the exchange rate of the Indonesian Rupiah against the US Dollar increased from 3845 to 17000. In one year the per capita growth in Gross Domestic Product declined from +3.3% to –14.8% [56]. The crisis increased the number of people in poverty by about 13 million. Inevitably, the increased price of basic commodities and food had compromised the consumption of meat, eggs, cooking oil and sugar, although rice consumption did not decrease substantially [57]. Hence, iron status may have decreased due to the lower intake of animal foods. Fortunately, the design of our study was such that if the crisis had affected the nutritional status of the women or infants, this would not have influenced the comparisons made and the conclusions that can be drawn from the study. Unfortunately we have no data to show the impact of the crisis on the nutritional status of our study population or on coping strategies of households.

6.6 General conclusions

The present study [49,50] demonstrated that weekly supplementation with iron and vitamin A during the gestation period improved the serum retinol concentration of women during pregnancy and of their infants at about 4 months of age and retinol concentration in breast milk. However, supplementation with vitamin A and iron did not reduce the prevalence of anaemia during pregnancy and did not affect growth, iron status, morbidity, or mental or psychomotor development of infants.

The study also showed that neither the weekly iron supplementation nor the governmental iron supplementation program improved the iron status of the pregnant women. In addition, there were no differences between the 2 treatment groups with respect to iron status, nutritional status, or development of infants.

Almost half of the women were anaemic and had low iron stores during pregnancy and lactation indicating a problem of public health significance. Although infants in the present study were not considered iron deficient at about 4 months of age. Based on the negative correlation between age and serum ferritin concentrations and decreasing iron levels in breast milk, it may be expected that many infants had become iron deficient by 6 months of age. On the basis of the mean concentration of retinol in serum and in breast milk and the prevalence of marginal vitamin A status (serum retinol concentration <0.70 μmol/L), the vitamin A status of pregnant and lactating women could be considered as marginal. Hence, vitamin A intake from breast milk was probably too small to build up adequate stores in infants. In addition, a large proportion of infants had a serum retinol concentration <0.70 μmol/L. Criteria for defining inadequate vitamin A status however are based on children 6-71 months of age. Hence, the functional significance of low serum retinol concentrations in young infants is still unclear.

Although the neonatal weight and length were considered as satisfactory, growth faltering of infants started at about 6 months of age, and a large proportion had become stunted (24%) and underweight (32%) by 12 months of age. Gestational age and maternal weight were the predictors of neonatal weight and length, whereas neonatal weight and length were important determinants of infant growth and nutritional status. This suggests that maternal nutrition during pregnancy is indeed an important determinant of infant nutritional status and growth. However, as infant growth is influenced by many factors, interventions directed at infants or their mothers during pregnancy may not be effective in improving infant growth. Therefore, the whole life cycle should be considered and intervention should start before pregnancy.

6.7 Implications

Although the programmatic issues, such as availability of supplements, accessibility, and compliance of prenatal iron supplementation for the prevention of anaemia need to be resolved further, the inclusion of vitamin A in iron supplementation should be considered. In this study, pregnant women were supplemented with 4800 RE vitamin A together with 120 mg elemental iron and 500 μg folic acid weekly. However, the dose of vitamin A might be increased up to 7500 RE [29]. No congenital malformations were observed in any of the infants born during the study. In a study in Nepal [25] using a dose of 7000 RE, no malformations were reported neither. The delivery of vitamin A and iron supplements should be incorporated in a safe motherhood programme, which among other measures to improve the programme should aim at increasing the number of deliveries assisted by trained health workers. The inclusion of vitamin A in the iron supplement did not appear to influence iron bioavailability [Annex of 49]. However, the shelf life of vitamin A in the non-oil-based supplement needs to be addressed carefully because of its vulnerability to losses during storage.

The delivery of iron-folic acid supplements on a weekly basis for the prevention of anaemia among pregnant women is not an alternative solution to overcoming the low effectiveness of the current iron supplementation programme. In order to prevent anaemia and iron deficiency during pregnancy, daily iron supplementation during pregnancy needs to be continued beyond birth provided that the compliance is improved and side effects due to iron tablet ingestion are reduced. Iron supplements in different preparations such as those based on the gastric delivery system or on ferrous fumarate may reduce gastrointestinal problems. Besides efficacy and effectiveness of the supplementation, affordability in the country or area to implement the program needs to be tested. In addition, other causes of anaemia should be elucidated and quantified.

Immunisation coverage was found to be low and few infants were exclusively breast-fed. In addition, vitamin A and possibly iron intake of infants needs to be increased. Existing child health and nutrition promotion programs need to be improved and intensified with special attention to immunisation coverage, feeding practices, and intake of micronutrients.

While pharmanutrient supplementation is an effective way to improve the micronutrient status of a population, other solutions such as food based approaches, either with natural, enriched or fortified foods, need to be integrated in striving towards the elimination of micronutrient deficiency disorders.

6.8 Future research

In order to elucidate the role of iron and vitamin A in foetal development, including the neurological development, basic cellular and molecular research should be directed at the transfer mechanisms of iron and vitamin A from mothers to infants and factors associated with its mechanisms.

Three important factors for an effective nutrient supply are the availability of foods rich in the respective nutrient, the content of the respective nutrient in food, and bioavailability or bioefficacy of respective nutrient for the bodily function. On the short term, advocating increased intake of supplements for mothers and infants is necessary to improve their nutritional status. However, longer-term solutions, such as identifying easy accessible foods rich in iron and vitamin A, or food enrichment by plant breeding or fortification, need to be addressed.

References

1. Katz J, West KP-Jr, Khatry SK, et al. Maternal low-dose vitamin A or {beta}-carotene supplementation has no effect on fetal loss and early infant mortality: a randomized cluster trial in Nepal. Am J Clin Nutr 2000;71:1570-1576.
2. Azais-Braesco V, Pascal G. Vitamin A in pregnancy: requirements and safety limits. Am J Clin Nutr 2000;71:1325S-1333S.
3. Stoltzfus RJ, Underwood BA. Breast-milk vitamin A as an indicator of the vitamin A status of women and infants. Bull WHO 1995;73:703-711.
4. Muslimatun S, Schmidt MK, West CE, Schultink W, Hautvast JGAJ, Karyadi D. Weekly vitamin A and iron supplementation during pregnancy increases vitamin A concentration of breast milk but not iron status in Indonesian lactating women. J Nutr 2001;131:2664-2669.
5. Panth M, Shatrugna V, Yasodhara P, Sivakumar B. Effect of vitamin A supplementation on haemoglobin and vitamin A levels during pregnancy. Br J Nutr 1990;64:351-358.
6. Howells DW, Haste F, Rosenberg D, Brown IR , Brooke OG. Investigation of vitamin A nutrition in pregnant British Asians and their infants. Hum Nutr Clin Nutr 1986;40:43-50.
7. Olson JA, Gunning DB, Tilton RA. Liver concentrations of vitamin A and carotenoids, as a function of age and other parameters, of American children who died of various causes. Am J Clin Nutr 1984;39:903-910.
8. Brown KH. WHO/UNICEF review on complementary feeding and suggestions for future research: WHO/UNICEF guidelines on complementary feeding. Pediatrics 2000;106:1290-1291.
9. Nommsen LA, Lovelady CA, Heinig MJ, Lonnerdal B, Dewey KG. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING Study. Am J Clin Nutr 1991;53:457-465.
10. FAO/WHO. Requirements of vitamin A, iron, folate and vitamin B12. Joint Expert Consultation Report. Rome: Food and Agriculture Organization, 1988.
11. Kusin JA, Kardjati S, Houtkooper JM, Renqvist UH. Energy supplementation during pregnancy and postnatal growth. Lancet 1992;340:623-626.
12. West CE. Meeting requirements for vitamin A. Nutr Rev 2000;58:341-345.
13. WHO. Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. WHO/NUT/96.10. Geneva: WHO, 1996.
14. Lindblad BS, Patel M, Hamadeh M, et al. Age and sex are important factors in determining normal retinol levels. J Trop Pediatr 1998;44:96-99.
15. Rahman MM, Mahalanabis D, Alvarez JO, et al. Acute respiratory infections prevent improvement of vitamin A status in young infants supplemented with vitamin A. J Nutr 1996;126:628-633.

16. Hadi H, Stoltzfus RJ, Dibley MJ, et al. Vitamin A supplementation selectively improves the linear growth of indonesian preschool children: results from a randomized controlled trial. Am J Clin Nutr 2000;71:507-513.
17. Hautvast JL, Tolboom JJ, West CE, Kafwembe EM, Sauerwein RW, van Staveren WA. Malaria is associated with reduced serum retinol levels in rural Zambian children. Int J Vitam Nutr Res 1998;68:384-388.
18. Bhaskaram P, Balakrishna N, Nair KM, Sivakumar B. Vitamin A deficiency in infants: Effects of postnatal maternal vitamin A supplementation on the growth and vitamin A status. Nutr Res 2000;20:769-778.
19. Adair LS, Pollitt E. Outcome of maternal nutritional supplementation: a comprehensive review of the Bacon Chow study. Am J Clin Nutr 1985;41:948-978.
20. Allen LH. Nutritional influences on linear growth: a general review. Eur J Clin Nutr 1994;48:75S-89S.
21. Beaton GH. Iron needs during pregnancy: do we need to rethink our targets? Am J Clin Nutr 2000;72:265S-271S.
22. Glasziou PP, Mackerras DE. Vitamin A supplementation in infectious diseases: a meta-analysis. BMJ 1993;306:366-370.
23. Muhilal, Permeisih D, Idjradinata YR, Muherdiyantiningsih, Karyadi D. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. Am J Clin Nutr 1988;48:1271-1276.
24. West KP-Jr, LeClerq SC, Shrestha SR, et al. Effects of vitamin A on growth of vitamin A-deficient children: field studies in Nepal. J Nutr 1997;127:1957-1965.
25. West KP-Jr, Katz J, Khatry SK, et al. Double blind, cluster randomised trial of low dose supplementation with vitamin A or beta carotene on mortality related to pregnancy in Nepal. The NNIPS-2 Study Group. BMJ 1999;318:570-575.
26. Christian P, West KP-Jr, Khatry SK, et al. Vitamin A or beta-carotene supplementation reduces but does not eliminate maternal night blindness in Nepal. J Nutr 1998;128:1458-1463.
27. Suharno D, West CE, Muhilal, Karyadi D, Hautvast JG. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet 1993;342:1325-1328.
28. Muslimatun S, Schmidt MK, Schultink W, et al. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J Nutr 2001;131:85-90.
29. WHO. Safe vitamin a dosage during pregnancy and lactation. Recommendations and report of a consultation. Geneva:WHO/Ottawa: Micronutrient Initiative, 1998.
30. Singla PN, Tyagi M, Shankar R, Dash D, Kumar A. Fetal iron status in maternal anemia. Acta Paediatr 1996;85:1327-1330.
31. Hokama T, Takenaka S, Hirayama K, et al. Iron status of newborns born to iron deficient anaemic mothers. J Trop Pediatr 1996;42:75-77.
32. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997;66:1178-1182.
33. Lawless JW, Latham MC, Stephenson LS, Kinoti SN, Pertet AM. Iron supplementation improves appetite and growth in anemic Kenyan primary school children. J Nutr 1994;124:645-654.
34. Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr 2001;131:590S-601S.
35. Scholl TO, Hediger ML, Fischer RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr 1992;55:985-988.
36. Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy. Am J Clin Nutr 2000;71:1295S-1303S.
37. 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. A report of the International Nutritional Anemia Consultative Group. Washington, DC: International Life Science Institute, 1998.
38. Oski FA, Honig AS. The effects of therapy on the developmental scores of iron-deficient infants. J Pediatr 1978;92:21-25.

39. Lozoff B, Brittenham GM, Viteri FE, Wolf AW, Urrutia JJ. The effects of short-term oral iron therapy on developmental deficits in iron-deficient anemic infants. J Pediatr 1982;100:351-357.
40. Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. Lancet 1993;341:1-4.
41. Kolsteren PW, Kusin JA, Kardjati S. Pattern of linear growth velocities of infants from birth to 12 months in Madura, Indonesia. Trop Med Int Health 1997;2:291-301.
42. Shrimpton R, Victora CG, de Onis M, Lima RC, Blossner M, Clugston G. Worldwide timing of growth faltering: implications for nutritional interventions. Pediatrics 2001;107:e75.
43. Dewey KG, Peerson JM, Brown KH, et al. Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian, and European data sets. World Health Organization Working Group on Infant Growth. Pediatrics 1995;96:495-503.
44. Garza C, de-Onis M. A new international growth reference for young children. Am J Clin Nutr 1999;70:169S-172S.
45. de-Onis M, Garza C, Habicht JP. Time for a new growth reference. Pediatrics 1997;100:e8.
46. Mora JO, Nestel PS. Improving prenatal nutrition in developing countries: strategies, prospects, and challenges. Am J Clin Nutr 2000;71:1353S-1363S.
47. Pelto GH. Improving complementary feeding practices responsive parenting as a primary component of interventions to prevent malnutrition in infancy and early childhood. Pediatrics 2000;106:1300-1301.
48. Arifeen SE, Black RE, Caulfield LE, et al. Infant growth patterns in the slums of Dhaka in relation to birth weight, intrauterine growth retardation, and prematurity. Am J Clin Nutr 2000;72:1010-1017.
49. Muslimatun S. Nutrition of Indonesian women during pregnancy and lactation: a focus on vitamin A and iron. Thesis Wageningen University, 2001.
50. Schmidt MK. The role of maternal nutrition in growth and health of Indonesian infants: a focus on vitamin A and iron. Thesis Wageningen University, 2001.
51. Beard JL. Effectiveness and strategies of iron supplementation during pregnancy. Am J Clin Nutr 2000;71:1288S-1294S.
52. Zavaleta N, Caulfield LE, Garcia T. Changes in iron status during pregnancy in peruvian women receiving prenatal iron and folic acid supplements with or without zinc. Am J Clin Nutr 2000;71:956-961.
53. Schultink W, van-der-Ree M, Matulessi P, Gross R. Low compliance with an iron-supplementation program: a study among pregnant women in Jakarta, Indonesia. Am J Clin Nutr 1993;57:135-139.
54. Galloway R, McGuire J. Determinants of compliance with iron supplementation: supplies, side effects, or psychology? Soc Sci Med 1994;39:381-390.
55. Yip R. Iron supplementation during pregnancy: is it effective? [comment]. Am J Clin Nutr 1996;63:853-855.
56. ACC/SCN. Fourth report on the world nutrition situation. Nutrition throughout the life cycle. Geneva: ACC/SCN in collaboration with IFPRI, 2000.
57. UNICEF. Indonesia: National Report on Follow-up to the World Summit Children (1990-2000). UNICEF 2001;1-32.

Summary

In Indonesia, many infants and children are undernourished as is illustrated by the high prevalence of stunting and micronutrient deficiencies. Nutrition during pregnancy is important for women's health, pregnancy outcome, and infant growth and health. Vitamin A and iron are important nutrients for foetal development and for infant growth, immunity and development. Vitamin A plays a role in iron metabolism and has been shown to reduce anaemia in vitamin A deficient pregnant women. In Indonesia, half of the pregnant women suffer from iron deficiency anaemia whereas one third of pregnant and lactating women are marginally vitamin A deficient. In Indonesia iron supplementation during pregnancy is standard practice and thus it is not ethical to conduct a study in which pregnant women are not provided with iron. However, effectiveness of the national daily iron supplementation programme has been shown to be low. Weekly iron supplementation has been proposed as a method of choice for providing iron supplements.

The aim of the present study was to investigate whether weekly supplementation with iron and vitamin A of pregnant women improves growth and health of their infants. Improvement of health was defined as a reduction in morbidity and an improvement of mental and psychomotor development. The impact of supplementation with iron and vitamin A on nutritional status of women during pregnancy and lactation are described in the complementary thesis entitled 'Nutrition of Indonesian women during pregnancy and lactation: a focus on vitamin A and iron' by Siti Muslimatun.

From November 1997 until November 1999, we conducted a community-based study in 9 rural villages in Leuwiliang subdistrict, Bogor district, West Java, Indonesia, in which women were recruited who were aged 17-35 years, parity <6, 16-20 weeks pregnant and had haemoglobin concentrations 80-140 g/L. Women from 5 villages were randomly assigned on an individual basis to supervised, double-masked supplementation once weekly from enrolment until delivery. Supplementation comprised 2 tablets each containing 60 mg elemental iron as ferrous sulphate and 250 μg folic acid with or without 2400 RE vitamin A. Subjects from the other 4 villages were assigned to a third group, referred to as the 'daily' group. These subjects were participating in the ongoing national iron supplementation programme and received iron/folic acid tablets through medical services. These tablets were similar in appearance and in iron and folic acid composition to the tablets administered weekly. Intake of iron tablets was not supervised. Initially 366 women were enrolled. Their newborn infants were followed up at least until 1 year of age. Subjects were considered to have dropped out when they withdrew from the study, moved outside the research area, or had twins, stillbirth or infant death. Non attendants were not considered lost to follow up.

Firstly, we investigated whether vitamin A and iron supplementation during gestation affected vitamin A and iron status of infants. In Chapter 2, we present data from infants (n=222) from whom sufficient blood was drawn for the assessment of haemoglobin concentration at ~4 [3.7 (1.9-6.3), mean (range)] months of age. Infants in the weekly vitamin A plus iron group had significantly higher serum retinol concentrations than

infants in the weekly iron group (0.62 ± 0.02 versus 0.54 ± 0.02 μmol/L, mean ± SE). Serum retinol concentrations did not differ between the weekly iron and 'daily' groups. In all groups >70% of the infants had serum retinol concentrations <0.70 μmol/L. Iron status was similar in all groups. The proportion of infants with a haemoglobin concentration <100 g/L was 29% while only 3 infants had a serum ferritin concentration <12 μg/L. Iron status of infants did not differ between mothers in the 'daily' group who had taken <50 tablets and those who had taken >50 tablets during pregnancy. Serum retinol concentrations of infants were correlated with those of their mothers at ~4 months postpartum in all groups ($r = 0.374$, $P < 0.01$) and at enrolment in the weekly iron and 'daily' groups. None of the variables of iron status of mothers during pregnancy was associated with iron status of their infants. Serum retinol concentrations of mothers ~4 months postpartum, upper respiratory tract infection and vitamin A supplementation showed the strongest association with serum retinol concentrations in infants.

Secondly, we investigated whether vitamin A and iron supplementation during gestation had an impact on growth or morbidity of infants until 12 months of age. In Chapter 3, we present data from infants, in the weekly vitamin A and iron (n=107) and weekly iron (n=105) groups, who were eligible for follow up after birth, and who attended at least once the monthly anthropometric assessments at the health post. Length, weight and growth, and incidence and prevalence of selected symptoms of morbidity of infants, whose mothers had been supplemented with vitamin A during gestation, did not differ from that of their counterparts at any age during the first year of life. Breast-feeding prevalence and immunisation coverage were similar in both groups, but only 6.7% of the infants had already completed the immunisation schedule. During the first 6 months of life, infants with serum retinol concentrations >0.70 μmol/L increased their weight and length more and had higher z-scores for height-for-age than those with serum retinol concentrations ≤0.70 μmol/L, but had similar prevalence of morbidity.

In Chapter 4, growth patterns and determinants of growth and nutritional status of infants from the 3 treatment groups were investigated using data of 151 boys and 167 girls who were available for follow up after birth. Most of the increase in length and weight of infants occurred during the first 6 months of life. At the neonatal assessment only 7% of the infants were stunted, 5% were underweight and none were wasted. This situation was similar at 6 months of age. Thereafter growth started to falter. At 12 months of age, 24% were stunted, 32% underweight and 4% wasted, while in the subgroup that was followed up until 15 months of age these proportions had increased even more. Boys had higher weight and length than girls from 0 to 15 months of age, although nutritional status (z-scores) did not differ significantly between boys and girls. During the first month of life, 80% of the infants received breast milk only ('exclusively breast-fed'), however the intake of complementary foods increased rapidly with age. Almost all women continued breast-feeding their infants during the study follow up. Running nose, fever and coughing, which were the most reported symptoms of morbidity, were significantly associated with smaller weight increase and z-scores. Consumption of the food group of animal products, fruit and biscuits were significantly associated with higher increases in weight and length, and z-scores. Feeding practices such as prelacteal feeding, partly discharging first colostrum, or 'exclusive' breast-feeding for ≥4 months were not associated with growth or nutritional status. In multiple linear regression, neonatal weight and length, reflecting prenatal factors, were the strongest predictors of growth and nutritional status of infants at 12 months of age. In addition, maternal weight and height at ~18 weeks of pregnancy, housing, and postnatal variables such as intake of certain food groups and morbidity were predictors of growth and nutritional status of infants at 12 months of age.

We also investigated whether vitamin A and iron supplementation during gestation improved mental and psychomotor development of infants (Chapter 5). Infants (n=276) were tested using the Bayley test either at 6 or at 12 months of age, and these age groups were evenly distributed over the 3 treatment groups. Mental and psychomotor development of infants whose mothers were supplemented weekly with vitamin A plus iron during pregnancy was similar to those whose mothers were supplemented with weekly iron alone. In addition, infants of mothers supplemented with weekly iron during pregnancy performed similar to those whose mothers had participated in the national iron supplementation program. Mental development index scores were significantly (P <0.05) but weakly associated with maternal age (r =-0.139) and the number of times a mother had a check up during pregnancy (r =0.125). Correcting for these confounders, and for age and treatment group, infants of mothers with serum retinol concentrations ≥0.70 µmol/L at near term had significantly higher mental development index scores than those of mothers with serum retinol concentrations <0.70 µmol/L. However, it is not clear whether this was due to better caring capacity of mothers with better nutritional status or to vitamin A status during pregnancy by itself.

In conclusion, the present study showed that vitamin A supplementation during gestation improved vitamin A status of infants at ~4 months of age. The difference in serum retinol concentrations of infants of vitamin A supplemented mothers during pregnancy compared with their counterparts could have resulted from differences in the vitamin A stores built up during gestation, from differences in retinol concentrations in breast milk, or both. However, vitamin A supplementation had no impact on growth or health, as indicated by morbidity and mental or psychomotor development, of infants. Considering the association of vitamin A status with growth of infants and the high prevalence of marginal serum retinol concentrations, an increased intake of vitamin A during infancy may be beneficial. Infants whose mothers were supplemented weekly with iron during pregnancy did not differ in any of the mentioned parameters from those whose mothers participated in the national iron supplementation program. Thus on the basis of outcomes on the infants there is no justification for recommending one regime above the other. At ~4 months of age, none of the infants suffered from iron deficiency anaemia but infants may have become iron deficient at later age. Growth faltering of infants started around 6 months of age and led to high prevalence of underweight and stunting at 12 months of age. The most important determinants of infant growth and nutritional status were neonatal weight and length. Hence, the gestation period or early infancy may be the best time to intervene in order to improve growth performance later in life. Incorporation of vitamin A supplementation in the health care program for pregnant women in Indonesia should be considered. However, in order to optimise nutritional status and growth of infants the whole life cycle should be considered.

Samenvatting

Nog steeds zijn veel baby's en kinderen in Indonesië ondervoed, hetgeen blijkt uit de hoge prevalentie van groeiachterstand en micronutrientendeficiënties. Voeding tijdens de zwangerschap is niet alleen van belang voor de gezondheid van de moeder, maar ook voor een goede ontwikkeling van de foetus en een goede groei en gezondheid van de baby. Vitamine A en ijzer zijn twee van de belangrijkste nutriënten voor een goede ontwikkeling van de foetus en voor groei, immuniteit en ontwikkeling van de baby. Vitamine A heeft een functie in het ijzermetabolisme en er is aangetoond dat vitamine A-suppletie de prevalentie van anemie bij vitamine A-deficiënte, zwangere vrouwen kan reduceren. De helft van de zwangere vrouwen in Indonesië lijdt aan ijzerdeficiëntie anemie terwijl eenderde marginale vitamine A-deficiëntie heeft. In Indonesië is ijzersuppletie voor zwangere vrouwen reeds standaardpraktijk. Het zou daarom onethisch zijn geweest een studie uit te voeren met een ijzer-placebo groep. De effectiviteit van het nationale programma, waarin ijzersuppletie dagelijks genomen moet worden, is echter laag. Daarom werd wekelijkse ijzersuppletie gekozen als een voorkeursmethode om zwangere vrouwen van voldoende ijzer te voorzien.

Het doel van de huidige studie was te onderzoeken of wekelijkse ijzer- en vitamine A-suppletie van zwangere vrouwen de groei en gezondheid van hun baby's zou verbeteren. Een betere gezondheid werd gedefinieerd als een reductie in het voorkomen van ziekten en een betere mentale en motorische ontwikkeling. Het effect van ijzer- en vitamine A-suppletie op de voedingsstatus van de vrouwen gedurende de zwangerschap en lactatie zijn door Siti Muslimatun beschreven in een complementair proefschrift getiteld 'Nutrition of Indonesian women during pregnancy and lactation: a focus on vitamin A and iron'.

Van november 1997 tot november 1999 voerden we een studie uit in 9 rurale dorpen in Leuwiliang subdistrict, Bogor district, West Java, Indonesië. Voor deze studie werden vrouwen gerekruteerd die voldeden aan de volgende criteria: 17-35 jaar oud, pariteit <6, 16-20 weken zwanger en een hemoglobineconcentratie van 80-140 g/L. Vrouwen uit 5 dorpen kregen aselect supplementen voorgeschreven die bestonden uit 2 tabletten die elk 60 mg elementair ijzer als ijzersulfaat en 250 μg foliumzuur bevatten, met of zonder 2400 RE vitamine A. De suppletie moest één maal per week genomen worden vanaf het moment van inschrijving tot de bevalling. Om de inname van de supplementen te verzekeren, gebeurde dit onder supervisie. Een derde groep werd gevormd door vrouwen uit de andere 4 dorpen, die de 'dagelijkse' groep wordt genoemd. Deze vrouwen konden participeren in het nationale ijzersuppletieprogramma en ontvingen ijzer/foliumzuur-tabletten via de reguliere medische kanalen. Deze tabletten zagen er hetzelfde uit en hadden dezelfde ijzer- en foliumzuursamenstelling als de tabletten die genomen werden in de wekelijkse groepen. De inname van tabletten in de 'dagelijkse' groep gebeurde echter niet onder toezicht. Aanvankelijk schreven zich 366 vrouwen in voor de studie. De pasgeboren baby's van deze vrouwen werden gevolgd totdat ze tenminste 1 jaar oud waren. Wanneer vrouwen zich terugtrokken uit de studie, verhuisden naar buiten het onderzoeksgebied, een tweeling of een doodgeboren kind kregen of als de baby overleed, vielen zij af en konden niet meer met het onderzoek meedoen. Echter, vrouwen die afwezig waren bij één of meer metingen mochten blijven meedoen tot het einde van het onderzoek.

Als eerste hebben we onderzocht of vitamine A- en ijzersuppletie tijdens de zwangerschap invloed had op de vitamine A- en ijzerstatus van de baby's. In hoofdstuk 2 presenteren we daarvoor de gegevens van de baby's (n=222) waarvan voldoende bloed kon worden afgenomen voor een bepaling van de hemoglobineconcentratie op de leeftijd van ~4 maanden [3.7 (1.9-6.3), gemiddelde (spreiding)]. Baby's wiens moeders wekelijks pillen met vitamine A en ijzer hadden genomen, hadden een significant hogere retinolconcentratie in hun serum dan baby's wiens moeders wekelijks alleen ijzer had genomen (0.62 ± 0.02 versus 0.54 ± 0.02 µmol/L, gemiddelde ± standaardfout). De retinolconcentratie in serum verschilde niet tussen de groepen die wekelijks of 'dagelijks' alleen ijzer hadden genomen. In alle 3 de groepen had meer dan 70% van de baby's een retinolconcentratie in serum <0.70 µmol/L. IJzerstatus van de baby's was hetzelfde in alle groepen. Het percentage baby's met een hemoglobineconcentratie <100 g/L was 29%, terwijl slechts 3 baby's een ferritineconcentratie in serum <12 µg/L hadden. IJzerstatus van baby's in de 'dagelijkse' groep van wie de moeders <50 tabletten hadden geslikt verschilde niet van de baby's van wie de moeder >50 tabletten hadden geslikt tijdens hun zwangerschap. De retinolconcentratie in serum van de baby's was gecorreleerd met die van hun moeders ~4 maanden na de bevalling in alle groepen (r =0.374, P <0.01) en tevens met die van hun moeders tijdens de zwangerschap op het moment van inschrijving in de groepen die wekelijks en 'dagelijks' alleen ijzer hadden genomen. Er was geen correlatie tussen de ijzerstatus van de baby's en die van hun moeders tijdens de zwangerschap. Retinolconcentraties in serum van de baby's was het meest gerelateerd aan retinolconcentraties in serum van hun moeders ~4 maanden na de bevalling, bovenste luchtweginfecties bij de baby's en vitamine A-suppletie.

Ten tweede hebben we onderzocht of vitamine A- en ijzersuppletie tijdens de zwangerschap leidde tot betere groei en een reductie in het voorkomen van ziekte van de baby's tot 12 maanden oud. In hoofstuk 3 presenteren we de gegevens van de baby's, in de wekelijkse vitamine A en ijzer (n=107) en wekelijkse ijzer (n=105) groepen, die gevolgd konden worden vanaf de geboorte en die tenminste één keer aanwezig waren geweest bij de maandelijkse antropometriemeting op de gezondheidspost. Gedurende het eerste levensjaar waren er geen verschillen in lengte, gewicht en groei, en incidentie en prevalentie van geselecteerde ziektesymptomen tussen de baby's wiens moeders vitamine A-suppletie hadden genomen tijdens de zwangerschap en de baby's wiens moeders geen vitamine A hadden gekregen. Prevalentie van borstvoeding en immunisatiedekking waren vergelijkbaar tussen de groepen, maar slechts 6.7% van de baby's had reeds het volledige vaccinatieschema afgerond. Tijdens de eerste 6 levensmaanden hadden baby's met retinolconcentraties in serum >0.70 µmol/L meer gewicht- en lengtetoename en hogere z-scores voor lengte-voor-leeftijd dan degenen met retinolconcentraties in serum ≤0.70 µmol/L, maar de prevalentie van ziektesymptomen was vergelijkbaar.

In hoofdstuk 4 worden groeicurven en determinanten van groei en voedingsstatus van baby's in de 3 behandelingsgroepen gepresenteerd. Voor deze groeicurven en de bepaling van de determinanten werd gebruik gemaakt van de gegevens van 151 jongens en 167 meisjes die vanaf hun geboorte gevolgd waren. Lengte en gewicht van de baby's namen het meest toe tijdens de eerste 6 levensmaanden. De neonatale meting (de eerste meting na de geboorte) liet zien dat slechts 7% van de baby's een groeiachterstand had, 5% had ondergewicht en geen enkele baby was ondervoed. Bij de meting van de baby's toen ze 6 maanden oud waren was de situatie niet veranderd. Op de leeftijd van 6 tot 7 maanden begon de groei echter af te nemen. Bij de meting van de baby's toen ze 12 maanden oud waren had 24% een groeiachterstand, 32% ondergewicht en was 4% ondervoed, terwijl in de subgroep van baby's die tot 15 maanden oud gevolgd waren deze percentages zelfs nog waren toegenomen. Van 0 tot 15 maanden waren jongens zwaarder en langer dan meisjes, hoewel de voedingsstatus (z-scores) tussen beiden niet significant verschilde. Tijdens de eerste maand na de geboorte kreeg 80% van de baby's alleen borstmelk ('exclusieve' borstvoeding) maar met toenemende leeftijd nam het percentage dat complementaire voeding kreeg snel toe. Bijna alle vrouwen bleven hun baby borstvoeding geven gedurende de studie. Verkoudheid, koorts en hoest bij de baby's, de ziektesymptomen die het meest door hun moeders gerapporteerd werden, waren significant gerelateerd aan een kleinere gewichtstoename en lagere z-scores. Consumptie van de voedselgroepen van dierlijke producten, fruit en biscuit was significant gerelateerd aan een grotere gewichtstoename en hogere z-scores van de baby's. Voedingspraktijken zoals het geven van prelactale voeding, het gedeeltelijk weggooien van het eerste colostrum of 'exclusieve' borstvoeding gedurende $\geq$4 maanden waren niet geassocieerd met groei of voedingsstatus. Met behulp van multipele lineaire regressie werd gevonden dat neonataal gewicht en lengte, die indicatief zijn voor prenatale factoren, de belangrijkste voorspellers van groei en voedingsstatus van baby's op de leeftijd van 12 maanden waren. Daarnaast werd groei en voedingstatus van baby's van 12 maanden oud voorspeld door gewicht en lengte van de moeder tijdens de ~18de week van de zwangerschap, de woonsituatie en postnatale variabelen, zoals de consumptie van bepaalde voedselgroepen en het voorkomen van ziektesymptomen.

We hebben ook onderzocht of vitamine A- en ijzersuppletie tijdens de zwangerschap leidde tot een betere mentale en motorische ontwikkeling van de baby's (hoofdstuk 5). Op de leeftijd van 6 of 12 maanden werden de baby's (n=276) getest met de Bayley test. Beide leeftijdsgroepen waren gelijk verdeeld over de 3 behandelingsgroepen. De mentale en motorische ontwikkeling van de baby's wiens moeders wekelijks vitamine A- en ijzersuppletie hadden genomen, was vergelijkbaar met de baby's wiens moeders wekelijks alleen ijzer hadden genomen. Daarnaast was er geen verschil tussen de baby's wiens moeders wekelijks ijzer hadden genomen en de baby's wiens moeders hadden deelgenomen aan het nationale ijzersuppletieprogramma. Mentale ontwikkelingsindices waren significant ($P < 0.05$) maar zwak geassocieerd met de leeftijd van de moeder ($r = -0.139$) en het aantal keer dat de moeder een controleonderzoek had laten uitvoeren tijdens haar zwangerschap ($r = 0.125$). In de analyse werd gecorrigeerd voor deze variabelen en voor de leeftijd van de baby en de behandelingsgroep. Baby's wiens moeders aan het eind van de zwangerschap een retinolconcentratie in serum $\geq$0.70 µmol/L hadden, hadden significant hogere mentale ontwikkelingsindices dan baby's wiens moeders een retinolconcentratie in serum <0.70 μmol/L hadden. Het is echter niet duidelijk of dit verschil te danken is aan een verschil in zorgcapaciteit van de moeders of aan de vitamine A-status tijdens de zwangerschap zelf.

Concluderend kunnen we stellen dat de huidige studie heeft aangetoond dat vitamine A-suppletie tijdens de zwangerschap kan leiden tot een verbeterde vitamine A-status van baby's op een leeftijd van 4 maanden. Het verschil tussen de retinolconcentratie in serum van de baby's wiens moeders wel en niet vitamine A hadden genomen tijdens de zwangerschap, kan een gevolg zijn van een verschil in vitamine A-voorraden opgebouwd tijdens de zwangerschap, van een verschil in retinolconcentratie in borstmelk, of van beiden. Vitamine A-suppletie had echter geen invloed op de groei van de baby's of op hun gezondheid, die werd gemeten als het voorkomen van ziektesymptomen en de mentale en motorische ontwikkeling. De associatie tussen vitamine A-status en groei van baby's en de hoge prevalentie van marginale vitamine A-concentraties in serum in aanmerking nemend, zou een verhoogde inname van vitamine A tijdens het eerste levensjaar gunstig voor de gezondheid kunnen zijn. Baby's wiens moeders wekelijks ijzersuppletie hadden genomen tijdens de zwangerschap verschilden in geen van de genoemde parameters van de baby's wiens moeders hadden deelgenomen aan het nationale ijzersuppletieprogramma. Op basis van de gegevens over de groei en gezondheid van de baby's kan dan ook geen voorkeur worden uitgesproken voor één van de 2 regimes. Op de leeftijd van ~4 maanden oud had geen van de baby's ijzerdeficiëntie maar dit was mogelijk wel het geval op latere leeftijd. Op de leeftijd van 6 maanden begon de groei van de baby's te stagneren hetgeen leidde tot een hoog percentage baby's op de leeftijd van 12 maanden met ondergewicht en groeiachterstand. De belangrijkste voorspellers van groei en voedingstatus van de baby's waren hun neonatale gewicht en lengte. Daarom zou de zwangerschap of het begin van het eerste levensjaar de beste periode zijn voor een interventie die kan leiden tot betere groei op latere leeftijd. Het zou overwogen moeten worden om vitamine A-suppletie in te bedden in het gezondheidszorgprogramma voor zwangere vrouwen in Indonesië. Om echter een optimale voedingstatus en groei van baby's te bereiken zou de hele levenscyclus in beschouwing genomen moeten worden.

Ringkasan

Masih banyak bayi dan anak-anak yang kekurangan gizi seperti tampak dari tingginya prevalensi *stunting* (kurang tinggi menurut umur) dan kekurangan gizi mikro di Indonesia. Gizi selama kehamilan penting bagi kesehatan ibu, kualitas kehamilan, dan pertumbuhan dan kesehatan anak. Vitamin A dan besi adalah zat gizi yang penting untuk pertumbuhan janin serta pertumbuhan, imunitas, dan perkembangan bayi. Vitamin A berperanan dalam metabolisme besi dan telah dibuktikan dapat menurunkan anemia pada ibu hamil yang kekurangan vitamin A. Di Indonesia, separuh dari ibu hamil menderita anemia defisiensi besi sedangkan sepertiga dari ibu hamil dan menyusui menderita defisiensi vitamin A. Suplementasi besi selama kehamilan di Indonesia merupakan standar kebijaksanaan sehingga tidaklah etis untuk melaksanakan penelitian yang ibu-ibu hamilnya tidak diberi tablet besi. Akan tetapi, efektivitas program suplementasi besi adalah rendah. Suplementasi besi secara mingguan telah diusulkan sebagai metode pilihan dalam pemberian suplemen besi.

Tujuan dari penelitian ini adalah untuk mengetahui apakah suplementasi mingguan dengan besi dan vitamin A pada ibu-ibu hamil meningkatkan pertumbuhan dan kesehatan, yang diindikasikan dengan penurunan kesakitan dan peningkatan perkembangan mental dan psikomotor, pada bayi-bayi yang dilahirkan. Hasil suplementasi besi dan vitamin A terhadap status gizi ibu-ibu hamil dan menyusui ditampilkan dalam tesis yang berjudul 'Nutrition of Indonesian women during pregnancy and lactation: a focus on vitamin A and iron' oleh Siti Muslimatun.

Dari bulan November 1997 hingga November 1999, telah dilaksanakan penelitian pada masyarakat di 9 desa di kecamatan Leuwiliang, kabupaten Bogor, Jawa Barat, Indonesia, yang merekrut ibu-ibu berusia 17-35 tahun, pernah melahirkan <6 kali, hamil 16-20 minggu dan mempunyai konsentrasi hemoglobin 80-140 g/L. Ibu-ibu dari 5 desa dibagi secara acak per orang untuk mendapatkan suplementasi buta-ganda satu kali setiap minggu, dalam pengawasan, mulai saat pendaftaran hingga melahirkan. Suplementasi terdiri dari 2 tablet yang setiap tabletnya mengandung 60 mg elemental besi dalam bentuk besi sulfat dan 250 μg asam folat dengan atau tanpa 2400 RE vitamin A. Subyek dari 4 desa lain dikelompokkan ke dalam group ketiga, yang disebut sebagai group 'harian'. Subyek dalam group ini berpartisipasi dalam program nasional suplementasi besi dan mendapatkan tablet besi/asam folat dari pusat pelayanan kesehatan. Tablet besi/asam folat tersebut mempunyai bentuk fisik dan kandungan besi dan asam folat yang sama dengan tablet yang diberikan secara mingguan. Konsumsi tablet tidak diawasi. Pada awalnya, 366 ibu-ibu terdaftar. Bayi-bayi yang dilahirkan diikuti perkembangannya sampai paling tidak berumur 1 tahun. Subyek dianggap drop out bila mereka mengundurkan diri dari penelitian, pindah dari lokasi penelitian, melahirkan bayi kembar, bayi mati atau bayinya meninggal. Subyek yang tidak hadir dalam pemeriksaan tidak dianggap drop out.

Pertama, kami meneliti apakah suplementasi vitamin A dan besi selama kehamilan mempengaruhi status vitamin A dan besi pada bayi. Di Bab 2, kami menampilkan data bayi (n=222) yang mempunyai data biokimia darah konsentrasi hemoglobin pada umur ~4 bulan [3.7 (1.9-6.3), rerata (kisaran)]. Bayi-bayi dalam kelompok mingguan vitamin A plus besi mempunyai konsentrasi retinol yang lebih tinggi secara bermakna daripada bayi-bayi dalam kelompok mingguan besi (0.62 ± 0.02 versus 0.54 ± 0.02 µmol/L, rerata ± galat baku). Konsentrasi serum retinol tidak berbeda antara kelompok mingguan besi dan kelompok 'harian'. Pada semua kelompok, lebih dari 70% bayi-bayi mempunyai konsentrasi serum retinol <0.70 µmol/L. Semua kelompok mempunyai status besi yang tidak berbeda secara bermakna. Proporsi bayi-bayi yang mempunyai konsentrasi hemoglobin <100 g/L sebanyak 29%, sementara hanya ada 3 bayi yang mempunyai konsentrasi serum ferritin <12 µg/L. Status besi pada bayi dalam kelompok 'harian' yang ibunya minum <50 tablet besi selama kehamilan tidak berbeda dari kelompok yang minum >50 tablet besi. Konsentrasi serum retinol pada bayi berhubungan dengan konsentrasi serum retinol pada ibu-ibu ~4 bulan pasca melahirkan pada semua kelompok ($r = 0.374$, $P < 0.01$) dan pada saat awal kehamilan di kelompok mingguan besi dan kelompok 'harian'. Variabel status besi pada ibu sewaktu hamil tidak ada yang berhubungan dengan variabel status besi pada bayi. Konsentrasi serum retinol pada ibu ~4 bulan pasa melahirkan, infeksi saluran pernafasan atas dan suplementasi vitamin A menunjukkan hubungan yang kuat dengan konsentrasi serum retinol pada bayi.

Kedua, kami meneliti apakah suplementasi vitamin A dan besi selama kehamilan mempunyai dampak terhadap pertumbuhan dan kesakitan bayi hingga umur 12 bulan. Dalam Bab 3, kami menampilkan data bayi dalam kelompok mingguan vitamin A dan besi (n=107) dan mingguan besi (n=105), yang layak untuk diikuti sejak lahir, dan yang mengikuti pengukuran anthropometri paling tidak satu kali di Posyandu. Panjang, berat dan pertumbuhan, dan insiden dan prevalensi beberapa jenis penyakit bayi dalam kelompok yang diberi suplementasi vitamin A selama kehamilan, tidak berbeda nyata dari kelompok lainnya pada tiap-tiap bulan selama satu tahun pertama. Prevalensi bayi yang mendapat ASI (air susu ibu) dan imunisasi tidak berbeda di kedua kelompok, akan tetapi hanya 6.7% bayi yang mendapat imunisasi lengkap. Selama 6 bulan pertama, bayi-bayi yang mempunyai konsentrasi serum retinol >0.70 µmol/L pertambahan berat dan panjang lebih banyak dan mempunyai *z-score* tinggi-untuk-umur lebih tinggi daripada bayi-bayi yang mempunyai konsentrasi serum retinol ≤0.70 µmol/L, akan tetapi mempunyai prevalensi kesakitan yang sama.

Dalam Bab 4, pola pertumbuhan, faktor-faktor penentu pertumbuhan dan status gizi bayi dari tiga kelompok diteliti berdasarkan data dari 151 bayi laki-laki dan 167 bayi perempuan yang mengikuti pemeriksaan lanjutan sejak lahir. Pertambahan berat dan panjang sebagian besar terjadi pada usia 6 bulan pertama. Pada pemeriksaan neonatus, hanya 7% bayi yang *stunting*, 5% kurang berat, dan tidak ada yang *wasting* (kurang berat untuk tinggi). Keadaan tersebut sama pada umur 6 bulan. Pertumbuhan mulai menurun pada umur 6 hingga 7 bulan. Akan tetapi, pada umur 12 bulan, ada 24% bayi *stunting*, 32% kurang berat, dan 4% *wasting*, sementara di sub kelompok yang diikuti hingga umur 15 bulan, angka tersebut lebih tinggi. Bayi laki-laki mepunyai berat dan panjang yang lebih tinggi dari pada bayi-bayi perempuan dari umur 0 hingga 15 bulan, meskipun status gizi (*z-score*) tidak berbeda secara bermakna. Selama satu bulan pertama, 80% bayi mendapatkan ASI ekslusif, tetapi asupan makanan tambahan meningkat bersamaan dengan bertambahnya usia. Hampir semua ibu tetap menyusui bayi mereka selama penelitian. Pilek, panas dan batuk, yang merupakan gejala penyakit yang paling sering dilaporkan, berhubungan secara bermakna dengan penambahan berat dan *z-score* yang lebih rendah. Konsumsi kelompok makanan hewani, buah-buahan dan biskuit berhubungan bermakna dengan penambahan berat dan panjang dan *z-score* yang lebih tinggi. Pola makan seperti makanan '*prelacteal*', pembuangan sebagian kolostrum, atau ASI eksklusif ≥4 bulan tidak berhubungan dengan pertumbuhan atau status gizi. Dalam regresi linier ganda, berat dan panjang neonatus sebagai faktor yang berperanan sebelum kelahiran, merupakan faktor penentu yang kuat dalam pertumbuhan dan status gizi pada bayi usia 12 bulan. Selain itu, berat dan tinggi ibu ~18 minggu kehamilan, jenis rumah, dan variabel sesudah kelahiran seperti asupan makanan tertentu dan penyakit merupakan faktor penentu pertumbuhan dan status gizi bayi pada usia 12 bulan.

Kami juga meneliti apakah suplementasi vitamin A dan besi selama kehamilan memperbaiki perkembangan mental dan psikomotor bayi (Bab 5). Bayi-bayi (n=276) diperiksa dengan menggunakan test Bayley pada usia 6 atau 12 bulan, dan kelompok umur ini terdistribusi secara merata diantara tiga kelompok perlakuan. Bayi-bayi yang ibunya diberi suplementasi besi dan vitamin A selama kehamilan mempunyai perkembangan mental dan psikomotor yang sama dengan bayi-bayi yang ibunya diberi suplementasi besi saja selama kehamilan. Selain itu, bayi-bayi yang ibunya diberi suplementasi mingguan besi selama kehamilan mempunyai perkembangan mental dan psikomotor yang sama dengan bayi-bayi yang ibunya berpartisipasi dalam program nasional suplementasi besi. Nilai indeks perkembangan mental berhubungan secara bermakna ($P < 0.05$) tetapi berasosiasi lemah dengan umur ibu ($r = -0.139$) dan jumlah pemeriksaan kehamilan ($r = -0.125$). Dengan mengoreksi faktor tersebut diatas dan juga umur dan kelompok perlakuan, bayi-bayi yang ibunya mempunyai konsentrasi serum retinol ≥0.70 μmol/L pada saat menjelang melahirkan mempunyai nilai indeks perkembangan mental yang lebih tinggi dari pada bayi-bayi yang ibunya mempunyai konsentrasi serum retinol <0.70 μmol/L. Akan tetapi, tidaklah jelas apakah hal tersebut disebabkan oleh pengasuhan yang baik oleh ibu yang mempunyai status gizi yang lebih baik atau karena status vitamin A selama kehamilan itu sendiri.

Sebagai kesimpulan, suplementasi vitamin A selama kehamilan meningkatkan status vitamin A pada bayi umur ~4 bulan. Perbedaan konsentrasi serum retinol pada bayi-bayi yang ibunya diberi suplementasi vitamin A selama kehamilan dapat terjadi karena perbedaan vitamin A yang disimpan selama proses pertumbuhan di dalam kandungan, dari perbedaan konsentrasi vitamin A di dalam ASI, atau dari kedua-duanya. Akan tetapi, suplementasi vitamin A tidak berdampak terhadap pertumbuhan atau kesehatan, seperti ditunjukkan dengan penurunan kesakitan dan peningkatan perkembangan mental dan psikomotor pada bayi-bayi yang dilahirkan. Berdasarkan hubungan antara status vitamin A dengan pertumbuhan dan tingginya prevalensi kekurangan vitamin A, penambahan asupan vitamin A pada bayi-bayi mungkin akan bermanfaat. Bayi-bayi yang ibunya diberi suplementasi mingguan besi selama kehamilan tidak berbeda dalam parameter yang telah disebutkan tersebut diatas dengan bayi-bayi yang ibunya berpartisipasi dalam program nasional suplementasi besi. Sehingga berdasarkan hasil-hasil pada bayi, tidak ada alasan untuk merekomendasikan satu metode adalah lebih baik dari yang lain. Pada umur

~4 bulan, tidak ada bayi yang menderita anemia kurang besi, akan tetapi bayi-bayi tersebut mungkin akan kekurangan besi sejalan dengan bertambahnya umur. Penurunan pertumbuhan bayi dimulai pada umur sekitar 6 bulan dan menyebabkan tingginya prevalensi kurang berat dan *stunting* pada umur 12 bulan. Faktor-faktor penentu yang penting dalam pertumbuhan dan status gizi bayi adalah berat dan panjang neonatus. Oleh karena itu, kemungkinan masa kehamilan atau awal pasca kelahiran adalah waktu yang tepat untuk intervensi perbaikan pertumbuhan di masa selanjutnya. Disarkan untuk menambahkan vitamin A dalam program kesehatan untuk ibu hamil di Indonesia. Akan tetapi, agar status gizi dan pertumbuhan bayi dapat dicapai secara optimal, seluruh aspek lingkaran kehidupan harus dipertimbangkan.

Acknowledgements

The writing of this thesis and herewith most of the work concerning my PhD study has come to an end. I would like to take this opportunity to write a personal note and to show my appreciation to the persons who have helped me, directly or indirectly, and from whom I learned during the past 5 years.

First of all, I want to mention my counterpart Siti Muslimatun. Dearest Siti, you have been so much more than a colleague and a friend to me. Without you I would never have felt so much at home in Indonesia. Your support, care and believe in me were indispensable. Terima kasih dari sepenuh hati saya.

Werner Schultink, je was de initiatiefnemer van dit project, bedankt voor je aanmoediging en enthousiasme vooral tijdens het werk in Indonesië. Prof. Hautvast, bedankt voor uw steun en vertrouwen in mijn kunnen vanaf het begin en voor uw motiverende woorden en aantekeningen op mijn artikelen. Clive, ik was zeer verheugd dat jij mij en ons project in mei 1999 wilde adopteren. Ondanks je drukke werkzaamheden heb je tijd gevonden om je te verdiepen in ons onderzoek, dank je wel dat je me gestimuleerd hebt om te proberen het beste eruit te halen. Jouw begeleiding bij het schrijven van de artikelen is bijzonder waardevol geweest, ik heb er veel van geleerd.

I have many good memories of Indonesia and I would like to thank everyone who has made my stay comfortable and the research possible. I would like to acknowledge the Indonesian Ministry of Health, LIPI and Dinas Kesehatan Bogor for accepting my study. I am indebted to former director Prof. Darwin Karyadi of SEAMEO TROPMED and his staff who have helped me in many ways. Erika, I really appreciated your help, thank you so much for conducting the pilot study on breast milk and the bioavailability study of iron in the tablets. I would like to thank Asih and Ndari for the analyses of serum ferritin concentrations and their help with arranging our laboratory and fieldwork equipment. Thanks also to Pak Embong and Maulana, to the administrative staff for getting me all the permits that I needed (which were many) and to Ibu Evita and Mia for their administrative support in many ways.

Dr. Muhilal, I especially want to thank you for your advice, continuing interest and support for our project. Furthermore I sincerely thank your staff at the Nutrition Research and Development Center, Bogor, among others Dr. Susilowati Herman, Ibu Ina, Ibu Srigati, Mbak Yanti and Pak Edi, for their help in the laboratory and obtaining the necessary permits.

Without the co-operation of the 366 pregnant women, this research would not have been possible. Terima kasih untuk semua ibu-ibu dan anak-anak dari Barengkok, Karacak, Karyasari, Puraseda, Purasari, Cibeber I, Sibanteng, Kalong II dan Karehkel yang menikuti penelitian saya. Our project heavily depended on the help of the health staff, especially from Puskesmas Leuwiliang, Puskesmas Puraseda, Puskesmas Kalong and Posko Karehkel. Dr. Sri Murawati, Dr. Chrisming Wiraja, Dr. Linda Chandranata, Dr. Annette Mariza, Dr. Farida Sulistyowati, Dr. Fusia Mediawaty and Dr. Agus S (†) are remembered for their co-operation and the physical examination of the infants studied.

Bidan Asnelli, Eti, Yanti, Yayat, Dewi, Farida, Wiwik, Puspa, Darnih and Peni, thank you for the assessment of the gestational age, the blood drawing and your kind co-operation at the monthly Posyandu. Pak Nurdin Hadiat, I am grateful for your excellent and careful drawing of blood from the infants and lactating women. I also want to thank you and Ibu Eti for letting us use your house for a goodbye for all participants. I am very grateful to all kaders (voluntary health workers) who recruited the pregnant women, accompanied them to the assessments and took care of the weekly distribution of the supplements and supervision of intake. Terima kasih kepada semua kader-kader, tanpa bantuan kalian saya sulit untuk mengadakan penelitian ini. In addition, I would like to thank all ibu-ibu paraji (traditional birth attendants) for their co-operation and explanation of the handling of delivery, the kepala desa of all mentioned villages, and especially Pak Asnan and Ibu Mimim from Barengkok. I gratefully thank Black Nanang for safely driving us everywhere.

We had help of many fieldworkers and without them it would not have been possible to achieve the follow up of the infants, thus I am very grateful for their hard work. Diana, Enung, Tami, Restu, Ina, Rika, Wiwit, Umi, Elin and Rita, thank you for your contribution to the study, your dedication and your friendliness. Furthermore I would like to express my thankfulness to Dr. Siti Dhyanti Wishnuwardhani from the Obstetric and Gynaecology Department, Medical Faculty, University of Indonesia for her advice on colostrum and breast milk sampling and for giving us the possibility to do an observation at the maternity ward and to Dr. Fadjar Satrija and his staff from the Helminthology Laboratory, Veterinary Faculty, Bogor Agricultural University for the analysis of parasites in faeces. Ibu Lies Karyadi and Ibu Hanny S. Sunggi, thank you for conducting the Bayley test and for your explanations of the test.

Ik wil graag Wageningen Universiteit, NWO-WOTRO en de Neys-van Hoogstraten Foundation bedankten voor hun financiële steun aan dit onderzoek. I also would like to acknowledge GTZ for financial contribution, Numico for analysing the breast milk samples, especially Prof. Jacques Bindels (the Netherlands) and Dr. Günther Raffler (Germany), and Kimia Farma (Indonesia) for providing the supplements.

Bij deze wil ik alle medewerkers van de Divisie Humane Voeding en Epidemiologie van Wageningen Universiteit bedanken, met name Jan Harryvan, Paul Hulshof, Joke Barendse, Pieter Versloot en Peter van de Bovenkamp voor het analyseren van onze pillen, de vitamine A analyses en verdere hulp en Jan Buream voor statistische adviezen. Dirk Joghems en Ben Scholte, ontzettend bedankt voor jullie onmisbare IT ondersteuning en het altijd klaarstaan om problemen op te lossen. Eric van Munster en Riekie Janssen, gelukkig dat jullie er waren om geldsommen naar Indonesië te sturen. Dick van Rumpt en Martijn van Steenbergen van Stichting Huisartsenlaboratorium Oost in Velp, ik dank jullie hartelijk voor het analyseren van de transferrine receptor en jullie uitleg over de analyse.

When I just arrived in Indonesia, the friendliness of Machteld Voerman, Yudhi Widyantoro, Andrea, and Josi helped me feel at home. I would like to thank my neighbours in Barengkok and Ciomas for their warm interest and giving me all nice foods to try, and especially the volleyball team in Ciomas, Bogor for a lot of fun. Marjoleine en Frank, onze etentjes waren zeer geslaagd. Tante and oom 'Lenteng Agung' (Kadarno), thank you so much for your kind hospitality and offering me a home in Jakarta where I could always come whenever I needed a place to sleep.

Lieve kamergenote in Nederland en bijna buurvrouw in Indonesië, Judith, ik zal je missen en de eekhoorntjes ook, het was een gave tijd op het John Snow. Astrid, bedankt voor je gezelschap. Hilda, leuk dat we de laatste twee jaar samen in Nederland zaten, bedankt voor je steun op vele fronten, en fijn dat je mijn paranimf wilt zijn. Verder wil ik alle AIO's bedanken voor de discussies, uitstapjes, lol en zeer geslaagde excursies naar Scandinavië en Zuid Afrika. Alle John Snowers, Machteld, Tiny en Mariska, bedankt voor jullie gezelschap en gezelligheid. To all 'foreign' PhD students, Elvina, Jane, Mr. Kahn, Dewan, Jesus, Marie-Françoise, Romain, Alice, Monika, Andre and others, thank you for all the good times and nice diners and for bringing an international flavour into the Department which otherwise I would have terribly missed.

Lieve meiden: Sonja, Marjon, Femke, Diana, Corien, Cornelly, Brechtje, bedankt voor jullie belangstelling, steun, gezellige uitjes, brieven en veel meer. Op naar ons 10-jarig jubileum! Cornelly, leuk dat je mijn paranimf wilt zijn, Triviant zullen we nooit leren, maar we hebben dan toch maar mooi een promotieonderzoek (bijna) afgerond. Ike, ik vind het heel bijzonder dat we na 28 jaar nog steeds contact hebben. Joost, Greet en Flip, bedankt voor jullie lieve belangstelling en voor alle goede tijden. Ardy Kielmann and Nandita, thank you for warmly welcoming me in France. Lieve familie Koster, bedankt voor jullie steun en belangstelling.

Taco, grote broer, ver weg blijft gelukkig dichtbij, nogmaals bedankt voor je hulp met de kaft van mijn proefschrift. Toch gaaf dat ik nu wat dichter bij jou en Cindy in de buurt woon. Oma To, tsja...eigenwijsheid is erfelijk, bedankt dat je zo'n toffe oma bent. Ik weet hoe trots opa geweest zou zijn.

Lieve mam en pap, aan het einde van het proefschrift maar aan de basis van alles, zonder jullie had ik het nooit gered. Dank jullie wel dat jullie je reislustige dochter zo steunen, het is fijn om een warme plek te hebben waar ik altijd naar terug kan komen. Jullie bezoek aan Indonesië was heel bijzonder voor mij. Zelfs in de meest spannende tijden wisten jullie het hoofd koel en vertrouwen in mijn beslissingen te houden, ik ben trots op jullie.

Liefste Werner, dit boekje is aan jou opgedragen voor meer redenen dan ik hier beschrijven kan. Gelukkig maar dat het niet alleen wetenschappelijk interessant is dat vlakken dicht tegen elkaar aanliggen en dat er bloemen zijn die de grens kunnen overschrijden. Jouw liefde en steun in de afgelopen 2 jaar waren onmisbaar voor mij. En zoals jij het zo mooi kan zeggen: laten we verder verven aan ons prachtige schilderij, ik weet zeker dat het nog veel kleurrijker gaat worden!

About the author

Marjanka Kathelina Schmidt was born on the first of October 1972 in Castricum, the Netherlands. In 1991 she finished secondary school (VWO) at the Bonhoeffer College in Castricum. In 1992 she went to Spain for 3 months to study Spanish, before that she worked and took Spanish language courses in the Netherlands. In September 1992 she started her MSc study in Human Nutrition at Wageningen University. She specialised in nutrition and health with special interest in nutrition deficiencies and the tropics. In 1996 she went to Guatemala and in co-operation with CeSSIAM institute she conducted a study on the relationship of iodine, parasitic infestation and nutritional status of schoolchildren and their mothers. Through this project her interest in doing research in developing countries became fully awakened. In January 1997 she finished her MSc study because she was appointed as a PhD student at the Division of Human Nutrition and Epidemiology at Wageningen University. She carried out the work described in this thesis within the framework of a co-operation between Wageningen University and SEAMEO TROPMED Regional Centre for Community Nutrition in Indonesia.

Research papers

Schmidt MK, Muslimatun S, West CE, Schultink W, Gross R, Hautvast JGAJ. Vitamin A and iron supplementation of Indonesian pregnant women benefits vitamin A status of their infants. Br J Nutr 2001;86: in press.

Schmidt MK, Muslimatun S, Schultink W, West CE, Hautvast JGAJ. Randomised double-blind trial of the effect of vitamin A supplementation of Indonesian pregnant women on morbidity and growth of their infants during the first year of life. Eur J Clin Nutr 2002: in press.

Schmidt MK, Muslimatun S, West CE, Schultink W, Gross R, Hautvast JGAJ. Patterns and determinants of growth faltering and nutritional status in Indonesian infants. Submitted.

Schmidt MK, Muslimatun S, Schultink W, West CE, Hautvast JGAJ. Mental and psychomotor development of infants of mothers supplemented with iron and vitamin A during pregnancy in Indonesia. Submitted.

Muslimatun S, **Schmidt MK**, Schultink W, West CE, Hautvast JGAJ, Gross R, Muhilal. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J Nutr 2001;131:85-90.

Muslimatun S, **Schmidt MK**, West CE, Schultink W, Hautvast JGAJ, Karyadi D. Weekly vitamin A and iron supplementation during pregnancy increases vitamin A concentration of breast milk but not iron status in Indonesian lactating women. J Nutr 2001;131:2664-2669.

Muslimatun S, **Schmidt MK**, West CE, Schultink W, Gross R, Hautvast JGAJ. Determinants of weight and length of Indonesian neonates. Submitted.

Muslimatun S, **Schmidt MK**, West CE, Schultink W, Hautvast JGAJ. Weekly vitamin A and iron supplementation of Indonesian pregnant women – the association between nutritional status, food intake and household characteristics. Presented at the Neys-van Hoogstraten International workshop on Socio-economic research as a tool for improving household food security and nutrition, July 8-12, 2001, Bogor, Indonesia.

Charlton KE, **Schmidt M**. Structured, Holistic Approach for Research Planning (SHARP). S Afr J Clin Nutr 2000;13:S45-S48.

Abstracts

Schmidt MK, Muslimatun S, West CE, Schultink W, Hautvast JGAJ. Vitamin A and iron supplementation during the gestation period and nutritional status of Indonesian infants. XX IVACG Meeting, Hanoi, 12-15 February 2001.

Schmidt MK, Muslimatun S, West CE, Schultink W, Hautvast JGAJ. Iron and vitamin A supplementation during gestation and growth of Indonesian infants. INACG Symposium, Hanoi, 15-16 February 2001.

Schmidt MK, Muslimatun S, West CE, Schultink W, Hautvast JGAJ. Vitamin A in conjunction with iron supplementation during gestation improves vitamin A status of infants in West Java, Indonesia. XVII International Congress in Nutrition, IUNS, Vienna, 27-31 August 2001.

Muslimatun S, **Schmidt MK**, Schultink W, Karyadi D, Hautvast JGAJ. The effect of weekly iron and vitamin A supplementation during pregnancy on infant growth. INACG Symposium, Durban, 12 March 1999.

Muslimatun S, **Schmidt MK**, West CE, Schultink W, Hautvast JGAJ. Iron and vitamin A status during pregnancy and lactation determine iron and retinol concentrations in breast milk. INACG Symposium, Hanoi, 15-16 February 2001.

Muslimatun S, **Schmidt MK**, West CE, Schultink W, Hautvast JGAJ, Gross R. Weekly supplementation with iron and vitamin A during pregnancy does not improve iron status of Indonesian women 4 months postpartum. XVII International Congress in Nutrition, IUNS, Vienna, 27-31 August 2001.

GRADUATE SCHOOL

This PhD-project was part of the research program of the Graduate School VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences).

FINANCIAL SUPPORT

Financial support by the Netherlands Foundation for Scientific Research-Netherlands Foundation for the Advancement of Tropical Research (The Netherlands), the Neys-van-Hoogstraten Foundation (The Netherlands), SEAMEO TROPMED Regional Center for Community Nutrition and German Agency for Technical Cooperation (Indonesia) and Wageningen University is gratefully acknowledged.

COVER

Marjanka K. Schmidt and Taco H. Schmidt

PRINTING

Grafisch bedrijf Ponsen & Looijen bv Wageningen