Nutrition of Indonesian women during pregnancy and lactation:
a focus on vitamin A and iron

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Propositions

1. Weekly iron supplementation during pregnancy is as effective as daily iron supplementation in improving the hemoglobin concentration of pregnant women at term provided compliance is assured (*this thesis*).

2. Supplementation with vitamin A and iron during pregnancy prevents an increase in the prevalence of marginal vitamin A deficiency beyond parturition, but does not decrease the prevalence of anemia and iron deficiency (*this thesis*).

3. At the present rate of progress, it will take yet another hundred years before the scourge of iron deficiency will be eliminated.

4. We learn mistakes from others because we do not live long enough to make all mistakes ourselves.

5. Preventable death and disability among mothers and expectant mothers is an all-encompassing tragedy: for families, for communities, for societies, and most of all, for children. *Carol Bellamy, Executive Director, UNICEF, World Health Day, 1998*

6. Working with a Dutch counterpart gives a richer taste to a Sandwich Ph.D.

7. Wageningen is the melting pot of The Netherlands.

Propositions pertaining to the thesis "Nutrition of Indonesian women during pregnancy and lactation: a focus on vitamin A and iron".

Siti Muslimatun
Wageningen, 17 December 2001

Abstract

Nutrition of Indonesian women during pregnancy and lactation: a focus on vitamin A and iron
PhD thesis by Siti Muslimatun, Division of Human Nutrition and Epidemiology,
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Nutrition during pregnancy is important for women's health, outcome of pregnancy and child survival. A community-based study was conducted in a rural area of West Java, Indonesia to investigate 1) the effect of weekly vitamin A and iron supplementation during pregnancy on iron and vitamin A status of women near term and on postpartum and pregnancy outcomes, 2) whether weekly iron supplementation was as effective as the ongoing national iron supplementation program in improving iron status. Women from 5 villages, 16 – 20 weeks pregnant, aged 17 – 35 years, parity <6, and with hemoglobin concentrations 80 – 140 g/L, were randomly allocated on an individual basis to receive a weekly supplement either with 120 mg iron as Fe_2SO_4 and 500 µg folic acid (n = 121) or the same amount of iron and folic acid plus 4,800 RE vitamin A (n = 122). A third group participating in the ongoing national iron supplementation program in which women are advised to take iron tablets daily during pregnancy ("daily" group) was recruited at the same time from 4 neighboring villages (n = 123). At near term, the iron status of pregnant women in the group supplemented weekly with iron (n = 66) was not different from the "daily" group (n = 53). However, iron status decreased with daily iron supplementation if <50 iron tablets were ingested. Hemoglobin concentrations in the group supplemented weekly with iron and vitamin A (n = 71) increased but serum ferritin concentrations decreased significantly, suggesting that vitamin A improved utilization of iron for hematopoiesis. Concentrations of serum transferrin receptor increased significantly in all groups. Serum retinol concentrations remained constant in the weekly iron and vitamin A group but decreased significantly in the other two groups. At ~4 months postpartum, compared with the weekly iron group (n = 88), the weekly iron and vitamin A group (n = 82) had significantly fewer subjects with serum retinol concentrations ≤0.70 µmol/L. The iron status of women in the weekly iron and vitamin A group did not differ from that of women in the weekly iron group. The concentrations of iron and retinol in transitional milk (4 – 7 days postpartum) was almost double than that in mature milk (3 months postpartum). Compared with the weekly iron group, the weekly iron and vitamin A group had significantly higher concentrations of retinol in transitional milk (as µmol/L) and in mature milk (as µmol/g fat). Neonatal weight (3094 ± 440 g) and length (49.1 ± 2.0 cm) did not differ among the three groups (n = 296). Iron and vitamin A status during pregnancy did not influence neonatal weight and length. Gestational age, maternal weight at the beginning of the second trimester and infant gender were the main predictors of neonatal weight and length. The proportion of women with a body mass index ≤21.0 kg/m² was 37% at the beginning of the second trimester of pregnancy and 52% at ~4 months postpartum. Low nutritional status of the women was associated with household characteristics reflecting a lower socioeconomic status. In conclusion, compared with weekly supplementation with iron alone, supplementation with vitamin A and iron given at the time when women entered their second trimester of pregnancy prevented the deterioration of vitamin A status near term and ~4 month postpartum, and increased retinol concentration in breast milk. The performance of weekly iron supplementation did not differ from the ongoing daily iron supplementation program in improving the iron status during pregnancy and lactation. Intervention did not influence weight and length of the neonates. It is recommended to include vitamin A in the iron supplementation program.

Contents

1

Introduction

THE IMPORTANCE OF MATERNAL NUTRITION

Nutrition during pregnancy is important for women's health, outcome of pregnancy and child survival [1; 2]. Therefore improvement of nutritional status of women during pregnancy will not only benefit them, but also optimize the growth in utero and postnatally of their infants. Approximately 21 million infants in developing countries are born each year with low birth weight (< 2,500 g). Of those, 75% are born in Asia – mostly in South Asia. The prevalence of intrauterine growth retardation (weight <10^{th} percentile of a birth-weight-for-gestational-age reference curve) is even higher [3]. A fetus with intrauterine growth retardation is more prone to morbidity and death in the perinatal period [4; 5], subsequent growth stunting [6; 7] and chronic diseases in adulthood [8].

Undernutrition is the result of a complex interrelationship among food supply, disease and care with geographical, historical, social, economic and political factors as basal causes [9]. Various diseases may result in undernutrition as such diseases reduce appetite, decrease absorption of nutrients, increase nutrient losses and increase nutrient requirements. Care, particularly health care and feeding practices, is also involved in the etiology of undernutrition. It is important to note that food supplies not only energy and protein but also minerals and micronutrients.

In most cases, micronutrient deficiencies share the same etiology. Thus deficiency of one micronutrient is often associated with deficiencies of others. In addition, the deficiency of one micronutrient can exacerbate the deficiency of another. These factors explain why it is common for there to be concomitant deficiencies of more than one micronutrient [10-14]. Using the approach of nutrition challenges throughout the life cycle, the risk of micronutient deficiencies is often greatest when a woman is pregnant and when a child is young and growing (**Fig. 1**). As such, micronutrient deficiencies today constitute a major constraint to future human development. Nutritional anemia, particularly due to iron deficiency and deficiencies of vitamin A and folate are common in pregnant women [15; 16]. Deficiencies of other micronutrients such as of iodine, zinc, and various B vitamins have also been commonly reported [17; 18].

IRON NUTRITION AND ANEMIA

Anemia, defined according to age- and sex-related "cut-off points" of hemoglobin concentration [19], is caused not only by nutritional but also non-nutritional factors. Nutritional causes include deficiencies of iron, folic acid, vitamin B12, vitamin B2, copper and vitamin A, whereas non-nutritional causes include chronic infectious diseases due to intestinal parasites, malaria or HIV and also hemoglobinopathies, such as sickle cell disease and thalassemia. Anemia of chronic infection is due not only to blood loss but also to disturbances in the synthesis and breakdown of red cells. Blood loss can also be a result of donating blood.

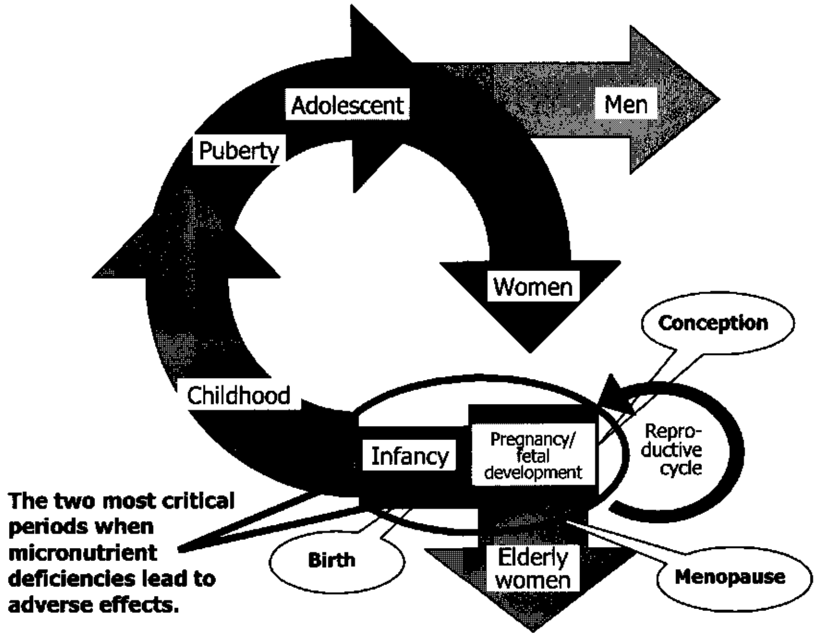

Figure 1. Critical physiological stages of micronutrient deficiencies throughout the life cycle (modified from ref. [20]).

Half of the anemia cases during pregnancy have been attributed to iron deficiency [21; 22]. Therefore, it is generally assumed that where prevalence of anemia is high, an iron deficiency problem exists [21]. Iron deficiency anemia is the most common form of malnutrition affecting about 1.3 billion people, 75% of them are in developing countries. Because iron requirements in pregnant women are higher than in other women and in men and children, the risk of anemia in this group is high. Iron requirements exceed average intake of absorbable iron substantially [23]. Inadequate iron status during pregnancy, particularly in early pregnancy, is associated with perinatal mortality, low birth weight and preterm birth [24]. It is speculated that infants born prematurely or with low weight have lower stores of iron and other nutrients [16].

Iron is required during pregnancy for the fetus, the placenta, the increase in maternal red cell mass, and to cover basal losses. Iron requirements increase throughout pregnancy so that only half of the iron requirements are met even though iron absorption increases during the second half of pregnancy and up until the first month after parturition. Thus half of the iron requirements for iron need to be met either from iron stores that need to be adequate for this purpose or from iron supplements [23]. One of the reasons why iron absorption cannot meet iron requirements is that both the content and bioavailability of iron in diets are low [23]. Typical diets in developing countries comprise cereals, vegetables and limited amounts of animal foods containing only small amount of available iron. This is because of the low heme-iron content and the low bioavailability of non-heme iron due to the presence of inhibitors of iron absorption (e.g. phytic acid) and the complete absence of enhancers of iron absorption (e.g. ascorbic acid). Chronic infections as a result of poor sanitation facilities and living conditions may aggravate the need for iron. In order to meet the challenge of anemia, supplementation of pregnant women with iron is common practice. Folic acid is commonly included in iron supplements since iron and folate deficiencies are usually concomitant and because folate deficiency causes megaloblastic anemia [15].

Unlike during pregnancy, women are in positive iron balance during lactation because of amenorrhea and the maintenance of high iron absorption [25]. Therefore, if lactating women are anemic, they were also anemic during pregnancy. The low concentration of iron in breast milk arising from the relatively low transfer of iron to breast milk may explain the lack of effect of iron deficiency or iron supplementation on iron concentration in breast milk [26-28]. However, healthy full term breast-fed infants are unlikely to become iron deficient before 6 months of age [27] because of the high bioavailability of the iron in breast milk [29].

Clinical trials have provided evidence that the efficacy of iron supplementation is proportional to the dose, duration of the supplementation and to the initial level of iron deficiency [22; 30]. However the effectiveness of iron supplementation programs through primary health care are often low [31]. One of the reasons for this discrepancy between efficacy and effectiveness is the low compliance to the iron supplements prescribed due mainly to the undesirable side effects caused by ingesting iron supplements [32]. Other causes of low compliance are low motivation both of the patients and health personnel, inadequate supplies of supplement tablets, and poor access to the health services [30; 31].

Given the similar efficacy between daily and weekly iron supplementation in preschool children [33-36], non-pregnant women [37; 38] and pregnant women [39], weekly iron supplementation has been proposed as a method of choice to provide iron supplements. Initial studies on this subject are based on the mucosal block theory [40; 41], but this theory has been shown not to be consistent with the facts in humans [42-44]. Weekly or intermittent iron supplementation offers advantages on the reduction of side effects [37; 45], easier distribution, lower cost [46], and prevention of iron overload [22; 37]. However, a recent analysis by Beaton and McCabe [47] concluded that both weekly and daily iron supplementation are efficacious, but daily iron supplementation is consistently more efficacious than weekly iron supplementation across different age groups and with different levels of supervision. These authors also concluded that weekly iron supplementation should not be recommended during pregnancy, regardless of the degree of supervision. However, it should be noted that the number of studies reviewed was small [47]. Moreover, the difference in mean hemoglobin concentration as one of the outcome measures between groups supplemented weekly and that supplemented daily was relatively small. Because it is not yet clear whether there is a difference in effectiveness between daily and weekly supplementation with iron, further studies are required.

VITAMIN A NUTRITION

As with iron deficiency and anemia, children and pregnant women are also affected by vitamin A deficiency. Worldwide, it is estimated that 2.8 million children have clinical eye signs due to vitamin A deficiency and 250 million children have low serum retinol concentrations, respectively [48]. As early as at 6 months of age, both clinical and subclinical vitamin A deficiencies are related with an excess of morbidity and mortality [49-52]. Vitamin A supplementation among preschool children has reduced morbidity, particularly of measles, respiratory and diarrheal diseases, and mortality by around 23% [53; 54]. The problem of vitamin A deficiency among pregnant women was not addressed until only recently. Early reports suggest that vitamin A deficiency is prevalent in Asian pregnant and lactating women, such as in Nepal [55] and Indonesia [56-58]. Nightblindness during pregnancy is associated with higher risk of hyporetinemia, severe anemia, infectious morbidity, and maternal and infant mortality [59-61]. Vitamin A supplementation beginning prior to and continuing throughout pregnancy reduces maternal deaths related to pregnancy by 40% [62], but does not improve fetal or early infant survival [63]. The term "vitamin A deficiency disorders" (VADD) has been endorsed recently to cover the broad range of adverse effects associated with vitamin A deficiency and of population groups affected.

Vitamin A has an essential role in immune function, maintenance of epithelial cellular integrity, growth and development, vision and reproduction [64]. Vitamin A transfer from mother to offspring occurs in two ways: via the placenta during gestation and via breast milk during lactation [65; 66]. While transfer of vitamin A during gestation is limited as can be seen from the very low vitamin A stores in infants, transfer via breast milk is quantitatively more important with 60 times more vitamin A being transferred via breast milk during 6 months lactation than via the placenta during 9 months gestation [66]. Assurance of adequate maternal vitamin A status is important since the retinol concentration in breast milk is affected by vitamin A intake and status during the third trimester of pregnancy [67] and lactation [57].

Vitamin A deficiency occurs when diets supply insufficient vitamin A required for growth, development and physiological functions, or in periods of added stress due to illness which cause vitamin A losses [68; 69]. Effective vitamin A supply from the diets depends on the type and amount of food consumed as well as the provitamin A/vitamin A content and the bioavailability. In developing countries where intake of animal foods and vitamin A-fortified products are limited, the main sources of vitamin A in the diet are plant foods, such as dark green vegetables and yellow/orange-colored vegetables and fruit. Bioavailability of vitamin A (mostly from animal foods) is generally more than 80%, whereas bioefficacy of provitamin A (mostly from plant foods) is much lower and affected by various factors that have been incorporated into the mnemonic SLAMENGHI. This refers to **S**pecies, molecular **L**inkage, **A**mount of carotene absorbed in a meal, **M**atrix in which the carotene is incorporated, **E**ffectors of absorption and bioconversion, **N**utrient status of the host, **G**enetic factors, **H**ost-related factors, and mathematic **I**nteractions [70]. Current evidence shows that the bioefficacy of provitamin A is lower than that previously thought and a factor of 21 µg ß-carotene to provide 1 µg retinol from a mixed diet is proposed [71]. The Institute of Medicine recently revised the conversion factor in the new Dietary Reference Intakes and proposed that 12 µg of ß-carotene in a mixed diet is equivalent to 1 µg retinol [72]. The consequence of this revised rate of conversion of ß-carotene to retinol results in a substantial increase in the proportion of the population in developing countries who do not meet their daily requirements of vitamin A [71].

High-dose vitamin A supplementation is a proven means of controlling xerophthalmia, preventing nutritional blindness, and among deficient populations, reducing the severity and case fatality rate of certain childhood infections, particularly measles and diarrhea [73]. It is also an effective means of improving the vitamin A status of lactating mothers and also their breast-fed infants following delivery [74-76]. Women should not be supplemented with more than 10,000 IU (10.5 µmol) daily or 25,000 IU (25.25 µmol) weekly [77] unless there is absolutely no chance of them becoming pregnant. This is because of the risk of teratogenicity [65].

INTERRELATIONSHIP BETWEEN VITAMIN A AND IRON METABOLISM

Deficiencies of iron and vitamin A share the same etiology. In addition, the metabolisms of both nutrients are interrelated. Observational studies in children [78; 79] and in pregnant women [56] showed an association between indices of iron and vitamin A status: poor vitamin A status is associated with low hemoglobin concentration. Community intervention studies showed that vitamin A fortification of sugar in Guatemala [80] and of monosodium glutamate in Indonesia [81] improved concentrations of both serum retinol and of hemoglobin of the population. Compared with supplementation with iron alone, combination of iron and vitamin A supplementation in children [82], pregnant women [83; 84], and adolescent girls [85; 86] result in a greater improvement of the hemoglobin concentration. However, vitamin A supplementation alone cannot overcome anemia not caused by vitamin A deficiency [11].

Hodges et al. in 1978 [87] concluded that vitamin A is essential for normal hematopoiesis on the basis of studies in human subjects and in experimental animals, although the mechanisms are poorly understood up until now. The reduction of hemoglobin synthesis despite an unaltered iron absorption in rats fed a vitamin A-deficient diet suggesting the impairment of either mobilization iron from stores or erythropoiesis in vitamin A deficiency [12; 88]. A recent study in humans indicates that the inhibiting effect of polyphenols and phytates on iron absorption is reduced by the presence of vitamin A in the diet [13].

MATERNAL AND INFANT NUTRITIONAL STATUS IN INDONESIA

Indonesia is the largest archipelago in the world. It is very rich in geographic diversity and is the home of 210 million inhabitants [89]. The country experienced high economic growth (6 – 8%) before the economic crisis hit in July 1997 but the economic crisis has put a quarter of Indonesian population into poverty. In 1999, growth began to improve and it is predicted that in 2001 economic growth will be 4.0 – 4.5%.

Like many other countries in transition, Indonesia faces problems of undernutrition and overnutrition both at the same time. The four most undernutrition problems recognized are chronic energy deficiency (protein energy malnutrition), iron deficiency anemia, vitamin A deficiency and iodine deficiency disorders, whereas overnutrition-related problems are obesity, coronary heart disease, and diabetes mellitus [90]. Indonesia has an infant mortality rate of 38 per 1,000 live births, maternal mortality ratio of 450 per 100,000 live births, life expectancy at birth of 66 years, and annual population growth of 1.6% [89]. The absence of positive trends in height of women, particularly rural women, over the last few generations suggests that environmental conditions have not improved [91]. Scattered studies among women showed that 38% are chronically energy deficient [91; 92], 50% are anemic and 30% are marginally vitamin A deficient [56; 58; 74]. The proportion of infants with low birth weight is 8%. The nutritional situation of the children is not better than that of the women. The proportion of children <5 years of age who are stunted or wasted is 42% [89] and who have iron deficiency anemia 40% [90]. Although clinical manifestations of vitamin A deficiency have decreased substantially compared to in 1977 [93], >60% of preschoolers are marginally vitamin A deficient [94].

MOTIVATION OF THE STUDY

Many preschoolers in Indonesia are stunted and the process starts as early as 3 months after birth [95] or even in utero [96]. Stunting is associated with postnatal factors such as the quality of care and living condition and maternal nutrition rather than with genetic background [97; 98]. Improvement of maternal nutritional status before and during pregnancy is crucial to maximize intrauterine growth as it amenable to direct intervention to break the vicious cycle of malnutrition. Energy and protein supplementation during pregnancy have been reported to benefit pregnancy outcomes, albeit moderately, in some studies. Implementation of such supplementation poses difficulties with respect to logistics, costs, targeting and leakage. Micronutrient deficiencies, particularly of iron and vitamin A, are prevalent among pregnant and lactating women as well as in children. Both iron and vitamin A deficiencies may have a negative influence on pregnancy outcomes and on child growth. In addition, vitamin A deficiency can contribute to anemia. Bearing in mind that iron and vitamin A deficiencies widely affect pregnant women, that maternal nutritional status affects child nutritional status and growth, and that micronutrient status influences child growth, we hypothesized that iron and vitamin A supplementation during pregnancy will positively influence not only pre- and post-natal growth of infants but also nutritional status of women. These women were administered each week, from 16 – 20 weeks pregnancy until delivery, a supplement containing iron or a supplement containing iron plus vitamin A. Both supplements containing folic acid. The dose of vitamin A chosen was 20,000 IU (6,000 Retinol Equivalent, RE or 21 μmol) which was regarded as sufficient to increase the vitamin A intake to meet the recommended daily allowance of vitamin A for pregnant women in Indonesia. The amount provided was less than maximum recommended level [77]. Because the policy of the Indonesian Government is to provide iron to all women during pregnancy, it was not considered ethical to withhold iron supplements from women in the study. The study also included a group in which women received iron plus folic acid daily through the government nutrition program.

OBJECTIVES, HYPOTHESES AND OUTLINE OF THE THESIS

From November 1997 until November 1999, the study was carried out under controlled condition in 9 rural villages in Leuwiliang subdistrict, Bogor district, West Java province, Indonesia where it has been reported frequently that nutritional status of both the women and children is low. This thesis is part of a research project with the theme ***The role of maternal nutrition in infant stunting in Indonesia*** with the aim of investigating the effect of weekly vitamin A and iron supplementation during pregnancy on infant growth in the first year of life. It reports the outcomes on pregnant and postpartum mothers and neonates. The thesis by Marjanka K. Schmidt entitled "***The role of maternal nutrition in growth and health of***

Indonesian infants: a focus on vitamin A and iron" [99] complements this thesis.

The objectives with hypotheses in *italics* of the thesis are to examine:

1. Whether weekly supplementation with vitamin A and iron during pregnancy improves iron status at near term and whether weekly iron supplementation is as effective as the ongoing national iron supplementation program in improving iron status at near term (***Chapter 2***). The hypotheses related to these objectives are:
   - *Weekly supplementation with vitamin A and iron during pregnancy improves iron status at near term better than supplementation with iron provided daily or weekly.*
   - *Weekly supplementation with iron during pregnancy will improve iron status at near term as effectively as the ongoing national iron supplementation program.*
   - *Weekly supplementation with vitamin A and iron during pregnancy will improve vitamin A status at near term more than will weekly supplementation with iron alone.*
2. Whether weekly supplementation with vitamin A and iron during pregnancy improves the vitamin A and iron concentrations in breast milk and in serum at ~4 months postpartum (***Chapter 3***). The associated hypothesis is:
   - *Iron and vitamin A concentrations in breast milk and in serum at ~4 months postpartum are higher in women who are supplemented weekly with vitamin A and iron during pregnancy than those of whom are supplemented weekly with iron only.*
3. Determinants of neonatal weight and length with respect to maternal nutritional status and socioeconomic factors where the women had been supplemented during pregnancy with iron or iron and vitamin A weekly, or iron "daily" (***Chapter 4***). The associated hypothesis is:
   - *The maternal nutritional status during pregnancy is a stronger predictor of neonatal weight and length than socioeconomic factors.*

4. Characteristics of the household associated with the food intake and nutritional status of women during pregnancy and lactation (***Chapter 5***). The associated hypothesis is:

- *Household characteristics reflecting lower socioeconomic status are associated with lower energy intake and nutritional status of women during pregnancy and lactation.*

Chapter 6 discusses the main findings of the studies described in this thesis, and draws conclusions. The methodological aspects of the study, general conclusions, implications and future research written jointly with Marjanka K. Schmidt are also presented in ***Chapter 6***. A complimentary article with the objective to evaluate the bioavailability of iron in the tablet containing iron, vitamin A and folic acid relative to an aqueous iron solution is attached in the ***Annex***.

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ABSTRACT

We investigated whether weekly iron supplementation was as effective as the national daily iron supplementation program in Indonesia in improving iron status at near term in pregnancy. In addition, we examined whether weekly vitamin A and iron supplementation was more efficacious than weekly supplementation with iron alone. One group of pregnant women (*n* = 122) was supplemented weekly with iron (120 mg Fe as $FeSO_4$) and folic acid (500 µg); another group (*n* = 121) received the same amount of iron and folic acid plus vitamin A [4,800 retinol equivalent (RE)]. A third ("daily") group (*n* = 123), participating in the national iron plus folic acid supplementation program, was also recruited. Data on subjects with complete biochemical data are reported (*n* = 190). At near-term, hemoglobin concentrations increased, whereas serum ferritin concentrations decreased significantly in the weekly vitamin A and iron group, suggesting that vitamin A improved utilization of iron for hematopoiesis. Iron status in the weekly iron group was not different from that of the "daily" group. However, iron status decreased with daily supplementation if <50 iron tablets were ingested. Serum transferrin receptor concentrations increased in all groups ($P < 0.01$). Serum retinol concentrations were maintained in the weekly vitamin A and iron group, but decreased in the other two groups ($P < 0.01$). Thus, delivery of iron supplements on a weekly basis can be as effective as on a daily basis if compliance can be ensured. Addition of vitamin A to the supplement improved hemoglobin concentration.

More than half of the pregnant women in Indonesia suffer from iron deficiency anemia [1]. Adequate iron status during pregnancy, particularly in early pregnancy, is crucial for reducing the risk of perinatal mortality, low birth weight, and preterm birth [2]. Therefore, iron supplementation programs are common in areas in which the prevalence of iron deficiency anemia is high, particularly in developing countries.

Weekly iron supplementation has been shown to be as effective as daily iron supplementation with respect to the improvement of iron status in preschool children [3-5], pregnant women [6], and nonpregnant women [7; 8]. Therefore, weekly supplementation has been proposed as the method of choice for providing iron as a supplement. It has been argued that by reducing the frequency of iron tablet ingestion, side effects will be less and compliance will improve [7; 9].

A recent analysis by Beaton and McCabe [10] concluded that both weekly and daily iron supplementation are efficacious, but daily iron supplementation is consistently more efficacious than weekly iron supplementation across different age groups and with different levels of supervision. These authors concluded that weekly iron supplementation should not be recommended during pregnancy, regardless of the degree of supervision. However, it should be noted that the number of studies reviewed was small. Moreover, the difference in mean hemoglobin concentration as one of the outcome measures between groups supplemented weekly and those supplemented daily was relatively small. Because it is not yet clear whether there is a practical difference in effectiveness between daily and weekly supplementation with iron, further studies are required.

The relatively high prevalence of marginal vitamin A status among pregnant and lactating women has raised concerns about its contribution to morbidity and mortality and to the etiology of anemia among women [11; 12]. West et al. [13] showed a 40% reduction in maternal deaths related to pregnancy after 1.5 y of weekly supplementation with 7,000 μg RE vitamin A or 42 mg (7,000 RE) β-carotene beginning before and continuing throughout pregnancy. Combining iron and vitamin A supplementation in pregnant women has been shown to improve both vitamin A and iron status [14; 15].

We conducted a community-based study to investigate the effect of weekly vitamin A and iron supplementation during pregnancy on infant growth in y 1 of life. This paper aimed to answer two questions with respect to the improvement of iron status at near term in pregnancy. The first question was whether weekly iron supplementation was as effective as the ongoing national iron supplementation program; the second question was whether weekly supplementation with vitamin A and iron was more efficacious than supplementation with iron alone, daily or weekly. Results on infant growth will be presented elsewhere.

MATERIALS AND METHODS

A randomized double-blind community-based trial was conducted from November 1997 until November 1999 to investigate the effect of weekly vitamin A and iron supplementation during pregnancy on infant growth in y 1 of life. A sample size of 63 per group was calculated on the bases of a 5% significance level (two-tailed test), a power of 80%, a difference in hemoglobin concentration of 5 g/L and a standard deviation of 10 g/L. For the infant, a sample size of 51 per group was calculated on the bases of a 5% significance level (two-tailed test), a power of 80%, a difference in length at 1 y of age of 1.5 cm and a standard deviation of 2.7 cm. A sample size of 120 women per group was chosen to allow for 50% drop out until their children reached 1 y of age.

Women who were 16 – 20 wk pregnant, aged 17 – 35 y and parity <6 were recruited from nine villages in Leuwiliang subdistrict, Bogor district, West Java, Indonesia. The nine villages, each with ~6,500 inhabitants, had similar socioeconomic characteristics. The maximum distance between study villages was 20 km. The area was rural and hilly. Only the main road had asphalt; the remaining roads were made from stone or soil.

Allocation of group and tablet intake. Subjects from five villages were assigned randomly to two weekly groups on an individual basis. They were supplemented each week from enrolment until delivery with two tablets each containing 60 mg elemental iron as ferrous sulfate and 250 μg folic acid, or with two tablets each of which contained 3,000 RE vitamin A in addition to the ferrous sulfate and folic acid. PT Kimia Farma, Indonesia, prepared the supplements and both types of tablets were similar in physical appearance.

The pregnant women received the supplement once a week between 0900 and 1200 h from voluntary health workers who ensured that the women swallowed the tablets in their presence. These volunteers also recorded the date tablets were taken, reasons why women did not take tablets, and whether any other supplement or medication was ingested during the previous week. Every 4 wk, two of the authors (S.M. and M.K.S.) distributed the supplement to the voluntary health workers. Random supervision of tablet intake was carried out by two assistants who recorded any complaints of side effects in the preceding 4 wk. Compliance of tablet intake throughout pregnancy was assessed again through interview during the postnatal home visit.

Subjects from the other four villages were assigned to a third group referred to as the "daily" group. These subjects were participating in the ongoing national iron supplementation program. Government policy is that pregnant women receive 90 – 120 iron plus folic acid tablets throughout pregnancy distributed through medical services. PT Kimia Farma, Indonesia also produced the tablets for the governmental program. These tablets were similar in appearance and in iron and folic acid content to the tablets administered weekly but were packed in aluminum foil sachets each of 30 tablets. Adherence of iron tablet intake in the "daily" group was assessed through interview during the postnatal home visit.

There were three villages in the service area of the main health center and six villages in the service area of two smaller health centers supervised by the main health center. Groups were evenly distributed across the health centers.

Pregnancy and anthropometry assessment. At baseline, demographic characteristics and pregnancy history of the subjects were assessed through interview by using a precoded questionnaire. Village midwives estimated gestational age by palpation and from the last menstruation date, which was later verified against the date of delivery. Body weight, mid-upper arm circumference (MUAC), and gestational age were measured at baseline (wk 16 – 20 of pregnancy) and at near term assessments (wk 34 – 36 of pregnancy). Body weight was measured using a UNICEF electronic SECA 890 weighing scale (Hamburg, Germany) to the nearest 0.1 kg; MUAC was measured using a plastic measuring tape to the nearest 0.1 cm; and height was measured to the nearest 0.1 cm (only at enrolment) using a standing height measurement microtoise.

Iron and vitamin A status assessments. Two blood samples were taken to evaluate the treatment, immediately before the intervention at 16 – 20 wk of pregnancy and at 34 – 36 wk of pregnancy. The days on which blood samples were taken were chosen to maximize the period of supplementation as well as to ensure that the second blood sample was taken before delivery. Venous blood samples (~ 5 mL) were collected in a tube without anticoagulant at 0900 – 1200 h. Hemoglobin was determined using the cyanmethemoglobin method (Merck test 3317; Merck, Darmstadt, Germany) at the Nutrition Research and Development Center laboratory, Bogor. For preparation of serum, blood samples were allowed to clot before they were placed in a cool box with cooling elements for transport to the laboratory. After arrival at the laboratory, blood samples were centrifuged at 3000 x *g* for 10 min at room temperature and serum distributed among three vials. Serum samples were kept for 1 mo at -20°C and subsequently at -79°C. All analyses were carried out within 1 y of blood collection.

Serum ferritin was analyzed by enzyme immunoassay using a commercial kit (IMX System, Abbott, Abbott Park, IL) at the laboratory of the South East Asian Ministers of Education Organization/Tropical Medicine, Jakarta, Indonesia. Duplicate analyses were performed on one eighth of the samples and the estimated variability was 0.9 µg/L. Three control serum samples with low (20 µg/L), medium (150 µg/L) and high (400 µg/L) concentrations of serum ferritin were provided by the assay manufacturer. The between-day CV for low, medium, and high concentrations were 4.5%, 4.4% and 7.1%, respectively. Serum soluble transferrin receptor was measured by immunoturbidimetric assay (IDeA® sTfR-IT, Orion Diagnostica, Espoo, Finland) as described by Suominen et al. [16] at "Stichting Huisartsenlaboratorium Oost" in Velp, The Netherlands. Duplicate analyses were performed for 10% of the samples and the estimated variability was 0.06 mg/L. Between-day CV for low (1.38 mg/L) and high (5.66 mg/L) serum controls were 2.5% and 3.6%, respectively. Serum retinol was analyzed using HPLC at the Division of Human Nutrition and Epidemiology, Wageningen University. Ten percent of the analyses were carried out in duplicate and the estimated variability was 0.05 µmol/L. The between-day CV was 7.4%.

Parasite infestation and dietary assessment. Subjects were requested to provide a stool sample for examination of intestinal parasites at 30 wk of pregnancy. Stool samples were collected in small plastic containers, and kept refrigerated at 4°C until examination. The intestinal parasites looked for were *Ascaris lumbricoides, Trichuris trichiura,* and hookworm using a modified Kato-Katz method at the Parasitology Department, Agricultural Institute, Bogor. Dietary assessment was carried out using a single 24-h recall in 50% of the subjects at 30 wk of pregnancy.

Statistics. The normality of data distribution was checked using the Kolmogorov-Smirnov test. Serum ferritin and soluble transferrin receptor concentrations were not normally distributed; therefore, these data were transformed logarithmically and reported as geometric mean and 95% confidence interval (CI). Normally distributed data are reported as mean and SD or SEM. To test the difference between baseline and near term examination, the paired t-test was used for continuous data, whereas McNemar test was used for dichotomous variables.

To answer the two research questions, each weekly group was compared with the "daily" group, and then the two weekly groups were compared. The independent t-test was employed to test the difference between groups taking into account the equality of variance using the Lavene test. The change of hemoglobin was correlated with its baseline concentration; therefore, baseline hemoglobin concentration was included as a covariate in the analysis. Pearson correlation coefficients were calculated for relationship between continuous variables.

Energy and nutrient intake data are presented as median and 25^{th} - 75^{th} percentile, and Mann-Whitney U test was employed to test the difference between groups. Energy and nutrient intake was calculated on the basis of Indonesian food composition tables. The SPSS software package (Windows version 7.5.2. SPSS Inc., Chicago, IL) was used for all statistical analyses and a *P* value <0.05 was considered significant.

Ethical consent. One of the authors (S.M.) explained the objectives and procedures of the study to the women in Bahasa Indonesia, which the women understood. Only women who gave written informed consent were allowed to participate in the study. Before the study commenced, the Medical Ethical Committees of the Medical Faculty of the University of Indonesia, the

Indonesian Ministry of Health and Wageningen University had approved the research proposal.

RESULTS

Initially, 366 pregnant women were enrolled in the study (**Fig. 1**) with age, 24.2 ± 4.6 y; parity, 1.4 ± 1.3 (30% were primiparous); height, 149.2 ± 4.8 cm; weight, 48.9 ± 6.4 kg; and body mass index, 21.9 ± 2.5 kg/m^2. We present data for 190 subjects on whom complete biochemical data at the baseline and near term examinations are available (**Fig. 1**). These subjects did not differ from the other subjects with respect to anthropometric and other biochemical variables at baseline. Compared with the other subjects, those included in the data analysis had ($P < 0.05$) lower serum soluble transferrin receptor concentrations [1.47 (95%CI: 1.41 - 1.54) versus 1.64 (1.54 - 1.74) mg/L] and higher parity (1.6 versus 1.3) at baseline.

Supplement composition and intake. The supplements were produced in July 1997 and in February 1998. The tablets were analyzed at the Division of Human Nutrition and Epidemiology, Wageningen University in October 1998. The vitamin A content of the tablets was 2,340 RE and 2,475 RE as retinyl acetate in the first and second batches, respectively. The iron content of both tablets was 60 mg Fe as $FeSO_4$. The first batch of tablets was used from November 1997 until March 1998 and the second batch from April until October 1998.

The average duration of supplementation in the weekly groups was 20 wk: 63% of subjects took all tablets, whereas the remaining subjects reported that they took no supplements on one or two occasions. Nausea and dizziness were experienced by 25% and 13% of the subjects, respectively. Supplement compliance had no influence on hemoglobin, serum ferritin and soluble transferrin receptor concentrations.

Iron tablet intake in the "daily" group was assessed through interview. All subjects received iron tablets from one of the health services such as health centers, midwives, or general practitioners. The median iron tablet intake was 50. Only 17% of the subjects took ≥90 tablets, and 43% took <30 tablets.

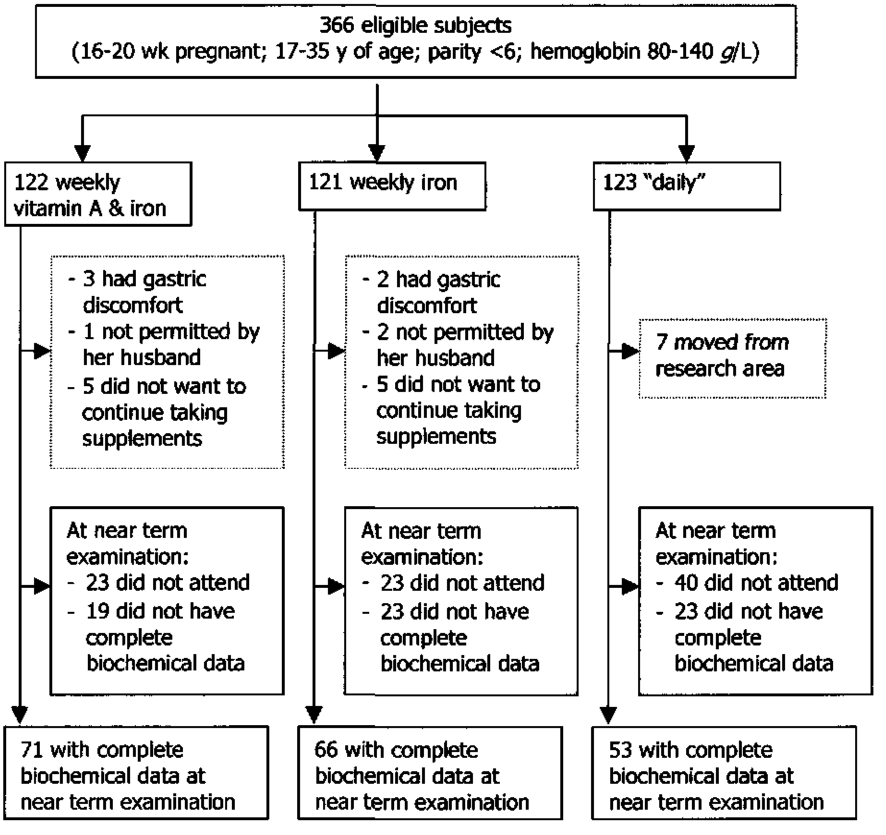

FIGURE 1 Selection and retention of eligible subjects in a randomized double-blind community-based trial to investigate the effect of vitamin A and/or iron supplementation during pregnancy.

Observations on the mothers at baseline and near term. Gestation, body weight and MUAC did not differ among groups either at baseline or at near term examination (**Table 1**). The period between the two examinations was 17.3 ± 2.6 wk. The mean weight gain was 0.3 kg/wk. All three groups had similar energy, protein, fat, iron, and vitamin A intake ($P > 0.05$). Median (25^{th} - 75^{th} percentile) daily intake of energy and nutrient were as follows: energy, 5.1 (3.7 – 6.5) MJ; protein, 39 (30 – 57) g; fat, 35 (22 – 55) g; iron, 6.5 (3.8 – 8.6) mg; and vitamin A, 274 (76 – 648) RE. Heme iron intake was only 0.6 (0.3 – 0.9) mg/d and vitamin A intake from animal sources was 5 (0 – 36) RE/d.

Iron status did not differ between each of the weekly groups and between the two weekly groups and the "daily" group either at baseline or at near term examination (Table 1). At baseline, the proportion of subjects with hemoglobin concentration <100 g/L, <110 g/L and <120 g/L were 19, 46 and 83%, respectively. Therefore, the mean hemoglobin concentration was slightly above the threshold for anemia (110.4 ± 10.8 g/L). At the near-term examination, only the weekly vitamin A and iron group had significantly higher hemoglobin concentration compared with baseline. The hemoglobin concentration of anemic subjects (hemoglobin <110 g/L) increased from baseline by 10.7 ± 2.3 g/L (mean ± SEM) in the weekly vitamin A and iron group, by 6.6 ± 2.3 g/L in the weekly iron group ($P < 0.01$), and by 3.4 ± 2.6 g/L in the "daily" group ($P > 0.05$). The increase of hemoglobin concentration in anemic subjects in the weekly vitamin A and iron group was significantly higher than in the "daily" group ($P < 0.05$). The hemoglobin concentration in nonanemic subjects in the weekly vitamin A and iron group and in the weekly iron group did not change, and decreased from baseline by 4.1 ± 1.9 g/L in the "daily" group ($P < 0.05$).

The mean serum ferritin concentration decreased significantly in the weekly vitamin A and iron group and in the "daily" group at near term (Table 1). The changes in serum ferritin were related to the baseline concentration ($r = -0.67$, $P < 0.01$). The proportion of subjects with low iron stores (serum ferritin concentration <12 µg/L) increased significantly in the "daily" group (**Table 2**). In all three groups, serum soluble transferrin receptor concentration increased significantly from baseline to the near term examination by about one third ($P < 0.01$). At baseline, subjects with serum ferritin concentration <12 µg/L had a mean serum soluble transferrin receptor concentration of 1.74 mg/L, whereas subjects with serum ferritin concentration ≥12 µg/L had a mean serum soluble transferrin receptor concentration of 1.33 mg/L ($P < 0.01$). Compared with subjects with serum ferritin concentration <12 µg/L at both baseline and at near term, subjects with serum ferritin concentration ≥12 g/L on both occasions had significantly lower ($P < 0.01$) mean serum serum soluble transferrin receptor concentrations at near term (1.61 vs. 2.44 mg/L).

TABLE 1

Anthropometric and biochemical characteristics in the weekly vitamin A and iron group, the weekly iron group, and the "daily" group at baseline and at near term[1]

	Vitamin A & Iron	Iron	"Daily"
N	71	66	53
Gestational stage, *wk*			
Baseline	17.5 ± 0.2	17.9 ± 0.7	17.7 ± 0.3
Near term	35.0 ± 0.2	34.9 ± 0.2	35.4 ± 0.2
Duration with intervention	17.6 ± 0.3	16.9 ± 0.3	17.6 ± 0.4
Height, *cm*			
Baseline	148.9 ± 0.6	149.0 ± 0.6	148.8 ± 0.7
Weight, *kg*			
Baseline	48.3 ± 0.8	48.5 ± 0.6	48.7 ± 0.9
Near term	53.0 ± 0.9	53.6 ± 0.7	53.7 ± 0.9
Change	4.7 ± 0.3**	5.1 ± 0.3**	5.0 ± 0.3**
MUAC, *cm*			
Baseline	24.4 ± 0.3	24.7 ± 0.2	24.9 ± 0.3
Near term	23.9 ± 0.3	24.5 ± 0.2	24.3 ± 0.3
Change	-0.4 ± 0.1**	-0.3 ± 0.1	-0.5 ± 0.2**
Hemoglobin, *g/L*			
Baseline	110.1 ± 1.4	110.0 ± 1.3	111.3 ± 1.4
Near term	114.0 ± 1.4	112.1 ± 1.4	110.6 ± 1.5
Change	3.7 ± 1.7*	2.1 ± 1.4	-0.7 ± 1.6
Serum ferritin[2], *µg/L*			
Baseline	14.7 (12.3 – 19.1)	13.7 (11.3 – 16.5)	13.8 (11.0 – 17.3)
Near term	10.7 (9.1 – 13.0)	12.1 (10.2 – 14.4)	9.9 (8.0 – 12.3)
Change[3]	-7.1 (-11.2 – -3.1)**	-3.0 (-6.6 – 0.6)	-5.3 (-9.4 – -1.2)**
Serum soluble transferrin receptor[2], *mg/L*			
Baseline	1.50 (1.39 – 1.61)	1.50 (1.39 – 1.63)	1.44 (1.34 – 1.56)
Near term	1.91 (1.78 – 2.05)	1.96 (1.78 – 2.12)	1.92 (1.74 – 2.12)
Change[3)]	0.43 (0.31 – 0.55)**	0.47 (0.37 – 0.57)**	0.56 (0.31– 0.82)**
Serum retinol, *µmol/L*			
Baseline	0.96 ± 0.03	1.01 ± 0.03	1.00 ± 0.04
Near term	0.97 ± 0.03	0.88 ± 0.04	0.79 ± 0.07
Change†	0.01 ± 0.24[a]	-0.12 ± 0.29**[b]	-0.21 ± 0.33**[b]

[1] data are means ± SEM, unless otherwise stated. MUAC, mid upper arm circumference

[2] geometric mean (95% confidence interval)

[3] changes are normally distributed and presented as an arithmetic mean (95% confidence interval)

* significantly different from baseline, $P < 0.05$, ** $P < 0.01$ (paired *t* test)

† different superscript letters are significantly different, $P < 0.05$ (*t* test)

TABLE 2

Proportion of subjects with anemia, low iron stores and marginal vitamin A deficiency in the weekly vitamin A and iron group, the weekly iron group, and the "daily" group

	Vitamin A & Iron	Iron	"Daily"
n	71	66	53
		%	
Hemoglobin <110 g/L			
Baseline	50	44	45
Near term	36	44	43
Serum ferritin <12 µg/L			
Baseline	43	45	36
Near term	55	48	57*
Serum retinol <0.70 µmol/L			
Baseline	13	17	17
Near term	15	27	43**

* significantly different from baseline, $P < 0.05$, ** $P < 0.01$ (McNemar test)

All three groups had similar mean serum retinol concentrations at baseline. However, in the weekly vitamin A and iron group, serum retinol concentrations remained constant, whereas mean concentrations in the other two groups decreased significantly at near term (Table 1). In the "daily" group, the proportion of subjects with marginal vitamin A status (serum retinol concentration <0.70 µmol/L) increased significantly (Table 2).

In the subjects in the "daily" group who consumed <50 iron tablets during pregnancy, iron status decreased from baseline to near term as indicated by decreased hemoglobin and serum ferritin concentrations ($P < 0.05$) and increased serum soluble transferrin receptor concentrations ($P < 0.01$) (**Table 3**). On the basis of the same parameters, iron status did not differ between the weekly iron group and those women in the "daily" group who consumed ≥50 iron tablets. However, the weekly iron group performed better than those women in the "daily" group who consumed <50 iron tablets, on the basis of changes in concentrations of hemoglobin ($P < 0.05$) and serum soluble transferrin receptor ($P < 0.05$), but not serum ferritin.

TABLE 3

Iron status variables in the "daily" group according to the number of iron tablets taken during pregnancy

	<50 tablets	≥50 tablets
n	27	26
Number of tablets taken[1]	25 (0 – 48)	70 (50 – 120)
Hemoglobin[2], *g/L*		
Baseline	113.7 ± 1.9	108.8 ± 2.0
Near term	108.9 ± 1.9	112.4 ± 2.3
Change	-4.8 ± 2.0*	3.6 ± 2.4
Serum ferritin[3], *μg/L*		
Baseline	11.6 (8.7 – 15.5)	16.6 (11.6 – 23.7)
Near term	7.9 (5.9 – 10.7)	12.5 (9.2 – 16.9)
Change[4]	-3.7 (-8.1 – 0.8)*	-7.0 (-14.4 – 0.3)
Serum soluble transferrin receptor[3], *mg/L*		
Baseline	1.44 (1.30 – 1.60)	1.45 (1.28 – 1.64)
Near term	2.16 (1.87 – 2.48)	1.70 (1.51 – 1.96)
Change[4]	0.83 (0.38 – 1.28)**, †	0.29 (0.06 – 0.52)*

[1] median (min. – max.)
[2] arithmetic mean ± SEM
[3] geometric mean (95% confidence interval)
[4] changes are normally distributed and presented as an arithmetic mean (95% confidence interval)
* significantly different from baseline, $P < 0.05$, ** $P < 0.01$ (paired t-test)
† significantly different between tablet intake group, $P < 0.05$ (t-test)

Fecal samples were obtained from 169 of the 190 subjects (88%). The number of subjects infected by *Ascaris lumbricoides* and *Trichuris trichiura* was 24% and 30%, respectively, whereas hookworm was present in only 5% of the subjects. The intensity was light, as the highest egg count for *Ascaris lumbricoides* and *Trichuris trichiura* was only 348 and 125/g feces, respectively. No association was found between parasite infestation and iron status variables or serum retinol concentration.

DISCUSSION

With respect to the improvement of iron status at near term in pregnancy, our study showed that supervised weekly iron supplementation did not differ in outcome from daily supplementation. However, further analysis revealed that iron status decreased in the daily supplementation group if <50 iron tablets were ingested. In addition, vitamin A and iron supplementation on a weekly basis increased hemoglobin concentrations but decreased serum ferritin concentrations, although the mean increase in hemoglobin concentration was not different from that with iron alone. The loss to follow-up at near term examination was relatively high. However, we would not expect this to affect the results because the number of subjects in each group was still above that initially regarded as being required, except for the "daily" group. Moreover, characteristics at baseline were similar in subjects included in the analysis compared with those not included, except for serum soluble transferrin receptor concentration and parity.

In our study, the weekly dose of iron gave results that did not differ from those of the daily dose of iron when ≥50 tablets (median 70 tablets) were ingested. By comparison, the mean iron tablet intake in the weekly group was 34. If 20 mg iron was absorbed from 120 mg iron provided each week [17], 340 mg of iron would have been absorbed over 17 wk of supplementation in the weekly iron group. Those in our "daily" group with iron tablet intake ≥50 would have absorbed higher amounts of iron compared with the weekly iron group. Hence it may be concluded that it is the regularity of iron tablet ingestion rather than the total number of tablets consumed that is important. This observation has been described earlier by others [7; 8; 18].

Ridwan et al. [6] showed that loosely supervised weekly iron supplementation for 8 – 20 wk during pregnancy increased hemoglobin (by 6 g/L) but not serum ferritin concentrations. The increment in hemoglobin concentration was similar to that observed in our anemic subjects (initial hemoglobin concentration, 101.3 ± 6.9 g/L), considering that their initial hemoglobin concentration was higher. A recent study from a shanty town in Peru [19] revealed that daily supplementation with 60 mg iron and 250 µg folic acid from 10 to 24 wk gestation through 4 wk postpartum resulted in hemoglobin changes similar to those in our weekly iron group. In our weekly iron group, the fact that hemoglobin concentration did not improve significantly despite iron supplementation was due to the relatively high initial hemoglobin concentration.

It has been suggested that low serum soluble transferrin receptor concentration in early pregnancy reflects decreased red cell production, whereas the increase in serum soluble transferrin receptor concentration from early to late gestation reflects increased red cell production and also depleted tissue iron stores when the increase is above the reference interval [20-22]. The one third increase in serum soluble transferrin receptor concentration at near term in our population might reflect both increased red cell production and tissue iron depletion as shown by a consistently higher serum soluble transferrin receptor concentrations in subjects with low iron stores. In contrast to our findings, Carriaga et al. [23] did not see a significant increase of serum soluble transferrin receptor concentration as gestation proceeded. The serum soluble transferrin receptor concentration in our population was slightly lower than that reported by Choi et al. [20], but this can be attributed to the different methods of analysis used.

Referring to the study carried out by Suharno et al. [15], daily supplementation of 60 mg iron and 2.4 mg retinol (8,000 IU) to anemic pregnant women increased hemoglobin concentration by 12.8 g/l and serum ferritin concentration by 1.8 µg/L. One third of the increment in hemoglobin concentration could be attributed to vitamin A supplementation. Similar results are found in our study, with the increment of hemoglobin concentration 42% higher in the anemic vitamin A-supplemented subjects compared with subjects supplemented with iron alone. However, this increment was not significant ($P = 0.211$). Hodges et al. [24] concluded that vitamin A is essential for normal hematopoiesis on the basis of studies in human subjects and in experimental animals. It is suggested that mobilization of iron from body stores into the circulation and into hematopoietic tissues is impaired in vitamin A deficiency [25]. Rats fed a vitamin A-deficient diet increased iron absorption, which was associated with increased tissue iron concentration, suggesting therefore that vitamin A deficiency is possibly associated with impaired erythropoiesis [26]. Another study indicates that the inhibiting effect of polyphenols and phytates on iron absorption is reduced by vitamin A [27]. In our study, the improvement of iron mobilization from body stores into the circulation and the increase of erythropoiesis with vitamin A supplementation were indicated by a sharp decline of serum ferritin concentration.

Although only 15% of our subjects had serum retinol concentrations <0.70 μmol/L, vitamin A status was considered to be low. In a study among poor pregnant women in India, similar results were found showing that plasma retinol concentration declined in the third trimester and daily 1,800 RE vitamin A supplementation for 12 wk prevented this decline [28]. On the other hand, Sapin et al. [29] found that healthy, well-nourished pregnant women at term had the same absolute quantities of retinol as non-pregnant women, whereas the lower concentration of serum retinol in pregnant women at term was due to expansion in plasma volume.

The tablets used in this study had ~20% less vitamin A than intended, which can be expected after 1.5 y storage. Although this loss was not sufficient to affect our results, care should be taken in programs not to store such supplements for too long.

This study was conducted as close as possible to the "real life" setting in the community. All pregnant women in the "daily" group had free access to iron tablets from the health services. In the weekly groups, the voluntary health workers distributed the supplements and supervised their intake. All pregnant women who were 16 – 20 wk pregnant were recruited without considering whether they were anemic or not. In our population, the proportion of pregnant women who were anemic was 46%. Because there is a program that provides iron supplements to pregnant women in Indonesia, it is not ethical to conduct a study in which pregnant women do not receive iron. Only 17% of the subjects took ≥90 iron tablets suggesting that the compliance of iron tablet intake by pregnant women was low in the "daily" group.

In this setting, weekly iron supplementation during pregnancy is as effective as daily iron supplementation in improving the hemoglobin concentrations of pregnant women at term provided compliance can be ensured. Supplementation with vitamin A together with iron improved hemoglobin concentrations, whereas iron supplementation alone did not. In addition, the vitamin A supplement prevented a decrease in serum retinol concentrations. This effect of vitamin A supplementation on improving hemoglobin concentration and decreasing serum ferritin concentration may be due to increased iron mobilization from body stores and increased erythropoiesis.

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3

Weekly Vitamin A and Iron Supplementation during Pregnancy Increases Vitamin A Concentration of Breast Milk but not Iron Status in Indonesian Lactating Women

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ABSTRACT

Studies on the effect of vitamin A and iron supplementation during pregnancy on maternal iron and vitamin A status postpartum are scarce. We investigated whether retinol and iron variables in breast milk and in serum postpartum were enhanced more with weekly vitamin A and iron supplementation during pregnancy than with weekly iron supplementation. During pregnancy, subjects were randomly allocated to two groups and received either (n = 88) a weekly supplement of iron (120 mg Fe as $FeSO_4$) and folic acid (500 µg) or (n = 82) the same amount of iron and folic acid plus vitamin A [4,800 retinol equivalents (RE)]. Transitional *milk* (4 – 7 d postpartum) had higher ($P <$ 0.001) concentrations of retinol and iron than mature milk (3 mo postpartum). Compared with the weekly iron group, the weekly vitamin A and iron group had a greater ($P < 0.05$) concentration of retinol in transitional milk (as µmol/L) and in mature milk (as µmol/g fat). Although serum retinol concentrations ~4 mo postpartum did not differ significantly, the weekly vitamin A and iron group had significantly fewer ($P < 0.01$) subjects with serum retinol concentrations ≤0.70 µmol/L than the weekly iron group. Iron status and concentrations of iron in transitional and mature milk did not differ between groups. We have shown that weekly vitamin A and iron supplementation during pregnancy enhanced concentrations of retinol in breast milk although not in serum by ~4 mo postpartum. However, no positive effects were observed on iron status and iron concentration in breast milk.

Approximately half of the women and preschool children in developing countries suffer from iron deficiency anemia [1]. The consequences of iron deficiency anemia during pregnancy include increased risk of perinatal mortality, low birth weight and preterm birth [2]. Low maternal iron nutrition during pregnancy has been shown to cause low iron stores in infants [3; 4]. Although iron intake and status during pregnancy and lactation have not been shown to affect the iron concentration in breast milk significantly [5-7], healthy full-term breast-fed infants are unlikely to become iron deficient before 6 mo of age [5].

Vitamin A deficient lactating mothers may not have enough vitamin A in breast milk to maintain and build body reserves in their rapidly growing infants [8]. Also, vitamin A intake and status during the third trimester of pregnancy have been shown to affect the retinol concentration in breast milk [9]. In Indonesia, one third of pregnant and lactating women are marginally vitamin A deficient [10; 11]. Among lactating women, supplementation with a single high dose of vitamin A [12; 13], a small daily dose of vitamin A [14], or a small daily dose of β-carotene [11; 12] has been shown to increase retinol concentrations in breast milk and in serum.

Vitamin A is essential for normal hematopoiesis [15]. Combining supplementation of vitamin A with that of iron during pregnancy has been shown to increase the hemoglobin concentration by 40% near term [10; 16]; however, the outcome on breast milk and maternal nutritional status postpartum have not been studied and reported.

Daily iron supplementation of pregnant women is an approach used universally to reduce anemia. With the rationale to increase compliance, reduce costs, and avoid iron overload, weekly supplementation has been proposed as a method of choice to provide iron [17]. We have shown that weekly iron supplementation was as effective as daily iron supplementation in improving the iron status of pregnant women if compliance is ensured [16]. We investigated whether retinol and iron variables in breast milk and in serum postpartum were enhanced more with weekly vitamin A and iron supplementation during pregnancy than with weekly iron supplementation alone.

SUBJECTS AND METHODS

A randomized double-blind, community-based trial among pregnant women was carried out from November 1997 until November 1999 in the rural subdistrict of Leuwiliang, West Java, Indonesia. This paper presents data on mothers postpartum. During pregnancy, these subjects had received supplements, i.e., either iron and folic acid alone, or together with vitamin A. Details of subject selection and supplementation procedures have been described elsewhere [16]. In brief, women who were 16 – 20 wk pregnant, aged 17 – 35 y, and parity <6 were assigned randomly to two groups on an individual basis. They were supplemented once weekly from enrollment until delivery with two tablets each containing 60 mg iron as ferrous sulfate and 250 µg folic acid or with two tablets each containing 2,400 retinol equivalents (RE) vitamin A in addition to the same amount of ferrous sulfate and folic acid. Both of the supplements given were identical in physical appearance to the iron-folic acid tablets used for the national iron supplementation program and were provided by the same company, PT Kimia Farma, Indonesia. The subjects were instructed not to take iron-folic acid tablets from the national iron supplementation program, and village midwives were also advised not to give the tablets from the governmental program to the subjects.

Out of 243 pregnant women initially enrolled, 18 dropped out during pregnancy, 5 gave birth to a stillborn child, 1 had twins (only 1 survived), 7 had infants who died before reaching 3 mo of age and 11 moved from the research area. Among the remaining 201 eligible subjects, 182 subjects attended the postpartum examination. Data are presented for 170 subjects who had complete sets of biochemical and anthropometric data (**Fig. 1**). At the time of enrollment (16 – 20 wk pregnant), age, body weight, height, body mass index, parity, gestational age and iron status of these women did not differ from all subjects initially enrolled (data not shown).

Breast milk collection. Breast milk was collected in a standardized way 3 mo postpartum (referred to as mature milk) from 50% of the mothers chosen randomly. Breast milk 4 – 7 d postpartum (referred to as transitional milk) was also collected by means of a convenience sample from mothers whom we knew had given birth to a child within the previous week. Due to financial constraints, breast milk was collected only from a sub-sample. Between 0800 and 1100 h, all milk from the right breast, which had not been used to feed the child during the previous hour, was collected using a breast milk pump (White River Concepts, San Clemente, CA). The breast milk was stored in dark brown glass bottles and transported to the laboratory in a cool box with cooling elements. In the laboratory, two aliquots of 10 mL each were frozen at -79°C until analysis. Analyses were carried out within 1.5 y of breast milk collection.

Fat, iron, and vitamin A status assessments in breast milk.

Breast milk was analyzed at the Central Laboratories Friedrichsdorf GmbH, Friedrichsdorf, Germany. Before analysis, breast milk was brought to room temperature (~20°C), warmed in a water bath up to 40°C and homogenized. The temperature of breast milk at the time of analysis was 20°C. Fat was determined according to the Roese-Gottlieb method [18]. Water (8 mL) was added to 1- to 2-mL sample plus 1.5 mL NH_4OH and 10 mL ethanol; 25 mL diethyl ether and 25 mL light petroleum were subsequently added with vigorous shaking. The organic layer was separated by centrifugation (700 x *g*, 1 min). The extraction was done twice, each with 5 mL ethanol, 15 mL diethylether, and 15 mL light petroleum. The combined organic layers were evaporated and the remaining fat was weighed. The fat extraction was done in a Mojonnier extraction pipe (Funke-Gerber, Berlin). Iron was measured using atomic absorption spectrophotometry after dilution of samples (50 µL) with water (950 µL). Retinol was analyzed using HPLC after alkaline saponification with potassium hydroxide, ascorbic acid, ethanol and water, with retinyl acetate as internal standard. The between-run CV was 10%. All analyses in breast milk were performed singly.

Iron and vitamin A status assessments in serum. Venous blood samples (~5 mL) were collected ~4 mo postpartum in a tube without anticoagulant between 0900 and 1200 h. Hemoglobin was determined using the cyanmethemoglobin method (Merck test 3317; Merck, Darmstadt, Germany) at the laboratory of the Nutrition Research and Development Center, Bogor. The within-assay variability, based on duplicate measurements performed on ~5% of the samples, was 5.7 g/L. For preparation of serum, blood samples were allowed to clot before they were placed in a cool box with cooling elements for transport to the laboratory. Blood samples were centrifuged at 3000 x *g* for 10 min at room temperature and serum separated into three vials. Serum samples were kept for 1 mo at -20°C and subsequently at -79°C. All analyses were carried out within 1 y of blood collection.

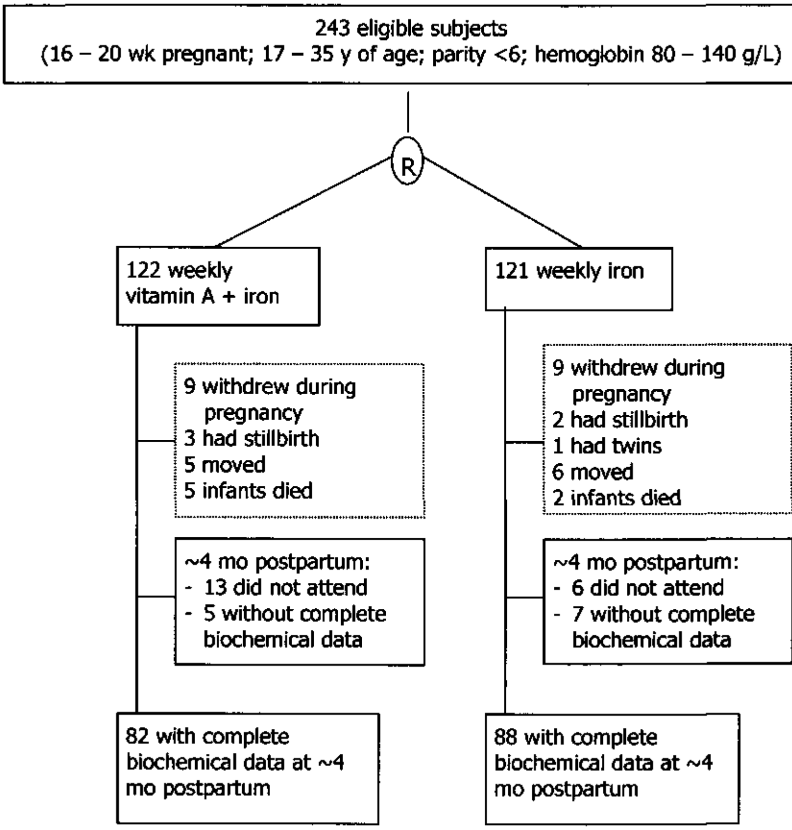

FIGURE 1 Eligible subject at enrollment and retention in a randomized double-blind, community-based trial to investigate the effect of vitamin A and/or iron supplementation during pregnancy on breast milk composition and maternal nutritional status postpartum.

Serum ferritin was analyzed by enzyme immunoassay using a commercial kit (IMX System, Abbott, Abbott Park, IL) at the SEAMEO TROPMED laboratory, Jakarta. On the basis of duplicate analyses performed on 15% of the samples, within-assay variability was 0.8 µg/L. Three control serum samples with low (20 µg/L), medium (150 µg/L), and high (400 µg/L) concentrations of serum ferritin were provided by the assay manufacturer. The between-day CV for low, medium, and high concentrations were 4.4, 4.7 and 4.9%, respectively. Serum soluble transferrin receptor was measured by immunoturbidimetric assay [19] (IDeA® sTfR-IT, Orion Diagnostica, Espoo, Finland) at Stichting Huisartsenlaboratorium Oost in Velp, the Netherlands. The within-assay variability, based on duplicate analyses on 10% of the samples, was 0.06 mg/L. Between-day CV for low (1.38 mg/L) and high (5.66 mg/L) serum controls were 2.5 and 3.6%, respectively. Serum retinol was analyzed using HPLC at the Division of Human Nutrition and Epidemiology, Wageningen University. Ten percent of the analyses were carried out in duplicate and the within-assay variability was 0.05 µmol/L. The between-day CV was 7.4%.

Anthropometric assessment. At the same time as blood was collected, body weight was measured using a UNICEF electronic weighing scale (SECA 890, Hamburg, Germany) to the nearest 0.1 kg, mid-upper arm circumference was measured using a plastic measuring tape to the nearest 0.1 cm, and height was measured at enrollment (16 – 20 wk pregnant) using a standing height measurement microtoise to the nearest 0.1 cm.

Statistics. The normality of data distribution was checked using the Kolmogorov-Smirnov test. Serum ferritin and soluble transferrin receptor concentrations were not normally distributed; therefore, these data were logarithmically transformed and reported as geometric mean and 95% confidence intervals (CI). Normally distributed data are reported as mean and SD or SEM. Iron and retinol concentrations in breast milk are reported as mean and 95% CI. The differences in concentration between transitional milk and mature milk were tested using Wilcoxon Signed Ranks test, except for fat, which was normally distributed, and thus tested using a paired *t* test. The differences between groups were tested using independent *t* test for normally distributed data or the Mann-Whitney U test for not normally distributed data. When control for possible confounding variables was necessary in testing the differences between two groups, ANOVA was employed instead of an independent *t* test. Differences in proportions were tested with a χ^2 test.

Determination of vitamin A status based on retinol concentrations in serum and in breast milk followed WHO recommendations [20], whereas determination of iron status based on hemoglobin and serum ferritin concentrations followed International Nutritional Anemia Consultative Group recommendations [21].

Correlation coefficients were calculated using Pearson correlation if both variables were normally distributed (such as the correlation between serum retinol and hemoglobin concentrations) or Spearman's rank correlation if one or both variables were not normally distributed (such as the correlation between retinol concentrations in serum and in breast milk). Calculation of correlation coefficients was carried out independently in each group.

The SPSS software package (Windows version 7.5.2. SPSS, Chicago, IL) was used for all statistical analyses and a *P* value of < 0.05 was considered as significant.

Ethical consent. One of the authors (S.M.) explained the objectives and procedures of the study to the women in Bahasa Indonesia, which the women understood. Only women who gave written informed consent were allowed to participate in the study. Before the study commenced, the Medical Ethical Committees of the Medical Faculty of the University of Indonesia, the Indonesian Ministry of Health and Wageningen University had approved the research proposal.

RESULTS

Samples of blood were taken and anthropometric measurements made between 1.9 and 5.9 mo postpartum, with an average of 3.7 ± 0.6 (mean ± SD) mo. None of the subjects were pregnant. The general characteristics of the subjects did not differ between groups (data not shown). The proportions of subjects having body mass index <18.5 kg/m^2 and < 21 kg/m^2 were 10 and 52% respectively.

Breast milk composition. Transitional milk (collected at 5.6 ± 1.2 d, mean ± SD) was available from 78 subjects and mature milk (collected at 3.0

± 0.1 mo) from 92 subjects. However, we report data on transitional milk from 73 subjects and on mature milk from 85 subjects for whom all analytical data are available. In both groups, the concentrations of fat, iron, retinol and retinol per g fat in mature milk were not correlated with its concentrations in transitional milk (data not shown).

In both transitional and mature milk, iron and fat concentrations were similar in the two groups (**Table 1**). Compared with the weekly iron group, the weekly vitamin A and iron group had significantly higher ($P < 0.05$) concentrations of retinol in transitional milk (as µmol/L) and in mature milk (as µmol/g fat).

When data were analyzed in a cross sectional manner, concentrations of iron and retinol (as µmol/L and µmol/g fat) in transitional milk were almost double those in mature milk ($P < 0.001$), whereas fat concentration did not differ (Table 1). Consistent results were found when data were analyzed in a longitudinal manner (data not shown).

The proportions of all subjects having concentrations of retinol in mature milk ≤1.05 µmol/L and ≤0.028 µmol/g fat were 51 and 21%, respectively. On the other hand, 28% of subjects had retinol concentration in mature milk >1.40 µmol/L. The weekly vitamin A and iron group had a lower ($P < 0.01$) proportion of subjects with a mature milk retinol concentration ≤0.028 µmol/g fat (**Fig. 2**).

Parity was slightly associated with fat concentration in mature milk but not in transitional milk. Primiparous mothers tended to have a higher ($P = 0.071$, controlling for age and treatment group) fat concentration in mature milk (29.1 ± 2.3 g/L; mean ± SEM) than their multiparous counterparts (25.6 ± 1.6 g/L). Body mass index, body weight and mid-upper arm circumference did not influence the transitional or mature milk composition (data not shown).

Hematological variables. Hemoglobin, serum ferritin and serum soluble transferrin receptor concentrations did not differ between the two groups (data not shown). The proportion of anemic subjects (hemoglobin concentration <120 g/L) was 48%, whereas the proportion of subjects with low iron stores (serum ferritin concentration <12 µg/L) was 47%. The proportions of subjects with anemia and low iron stores did not differ between groups (data not shown).

TABLE 1

Compositions of transitional (4 – 7 d postpartum) and mature (3 mo postpartum) milk from women supplemented weekly with vitamin A and iron or iron alone during pregnancy[1]

	Vitamin A + iron	Iron
	Transitional milk[2]	
n	31	42
Iron, *μmol/L*	8.13 (6.45 – 9.80)*	7.63 (6.61 – 8.64)*
Fat, *g/L*	29.4 ± 2.5	25.8 ± 2.0
Retinol,		
μmol/L	3.37 (2.47 – 4.26)*#	2.29 (1.80 – 2.79)*
μmol/g fat	0.113 (0.093 – 0.132)*	0.097 (0.078 – 0.115)*
	Mature milk[2]	
n	39	46
Iron, *μmol/L*	5.35 (3.84 – 6.86)	4.27 (3.50 – 5.00)
Fat, *g/L*	26.3 ± 2.1	27.2 ± 1.7
Retinol,		
μmol/L	1.24 (1.03 – 1.45)	1.06 (0.89 – 1.22)
μmol/g fat	0.053 (0.044 – 0.063)#	0.044 (0.037 – 0.052)

[1] Data expressed as mean (95% confidence interval), except for fat, mean ± SEM. Differences between groups were tested by the Mann-Whitney U test, except for fat by independent *t*-test. * $P < 0.001$ for transitional milk vs. mature milk. # $P < 0.05$ for weekly vitamin A + iron group vs. weekly iron group.
[2] Samples of transitional milk were obtained from a convenience samples of subjects, whereas samples of mature milk were obtained from a 50% random sample (see text).

Serum retinol concentrations in the weekly vitamin A and iron group were not significantly different from those in the weekly iron group (data not shown). However, compared with the weekly iron group, the weekly vitamin A and iron group had significantly fewer ($P < 0.01$) subjects with serum retinol concentration ≤0.70 μmol/L (18 vs. 5%) and tended to have fewer (P = 0.066) subjects with serum retinol concentration ≤1.05 μmol/L (52 vs. 38%; Fig. 2).

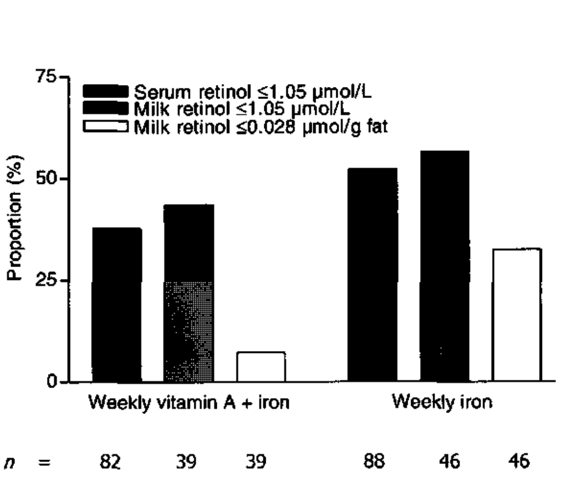

FIGURE 2 Proportion of women with low vitamin A status based on serum (~4 mo postpartum) and mature milk (3 mo postpartum) measurements in the weekly vitamin A and iron group and the weekly iron group. Differences between groups were tested by χ^2 test. * $P < 0.01$ for weekly vitamin A + iron group vs. weekly iron group.

Serum retinol concentrations were significantly correlated with hemoglobin concentrations only in the weekly iron group (r = 0.256, $P < 0.05$). In both groups, serum ferritin concentrations were positively correlated with hemoglobin concentrations (r = 0.448 and 0.455, $P < 0.01$), but negatively with serum transferrin receptor concentrations (r = −0.628 and −0.434, $P < 0.01$). Compared with subjects with serum retinol concentrations ≤1.05 µmol/L, subjects with retinol concentrations >1.05 µmol/L had higher ($P < 0.01$, controlling for treatment group) hemoglobin concentrations (122.2 ± 1.2 vs. 116.7 ± 1.6 g/L; mean ± SEM) and higher ($P < 0.05$) serum ferritin concentrations [14.3 (11.6 – 17.7) vs. 9.9 (7.8 – 12.5) µg/L; mean (95% CI)].

None of the anthropometric indices (body weight, height, body mass index, and mid-upper arm circumference) correlated with iron status or serum retinol concentrations. Parity was not associated with iron status nor with serum retinol concentrations (data not shown).

Correlations between retinol and iron concentrations in serum and in mature milk. In the weekly iron group, serum retinol concentrations were significantly correlated with retinol concentrations in mature milk when expressed in term of volume or per g fat (r = 0.304 and 0.487, respectively, $P < 0.01$). In the weekly vitamin A and iron group, iron concentrations in mature milk were correlated with hemoglobin concentrations (r = 0.443, $P < 0.01$), with serum ferritin concentrations (r = 0.359, $P < 0.05$) and with serum soluble transferrin receptor concentrations (r = -0.325, $P < 0.05$).

DISCUSSION

In this study, weekly supplementation of pregnant women with 4,800 RE vitamin A and 120 mg iron (and 500 µg folic acid) during pregnancy enhanced the retinol concentration in breast milk 4 –7 d postpartum, retinol concentration per gram of fat in breast milk 3 mo postpartum, but not serum retinol ~4 mo postpartum. It has been calculated [8] that a retinol concentration of 1.05 µmol/L in a mother's breast milk during the first 6 mo postpartum provides enough vitamin A to meet the metabolic needs of her baby but provides insufficient vitamin A to build up liver stores. In vitamin A-sufficient populations, average retinol concentrations in breast milk range from 1.75 to 2.45 µmol/L [20]. The mean retinol concentration in the breast milk of the women we studied was <1.4 µmol/L, typical of vitamin A-deficient populations. The relatively high retinol concentration in transitional milk was also within the range of values reported from developing countries [22]. The 47% difference of retinol concentrations in transitional with vitamin A and iron supplementation administered weekly during pregnancy was reduced to 17% in 3 mo period. A study carried out in Indonesia [13] showed that a single high dose supplementation with vitamin A (312 µmol retinyl palmitate) within 1 mo of parturition resulted in 35% higher retinol concentration in breast milk at 3 mo, which was maintained until 8 mo compared with placebo. A 3.5 mg β-carotene supplements from vegetables given daily for 3 mo did not increase the retinol concentration in breast milk compared with an increase of 67% with a similar amount of β-carotene in a wafer [11]. It should be noted that our subjects were supplemented during pregnancy only, whereas the subjects in the other studies mentioned were supplemented during lactation. Although retinol concentrations in breast milk of women supplemented weekly during pregnancy were not as high and not retained longer as with a single high dose supplement, weekly supplementation of vitamin A during pregnancy has an additional advantage of preventing a decline of maternal vitamin A status during pregnancy as had been reported previously [16]. In addition, the effects were sustained until postpartum as shown by a lower proportion of women with serum retinol concentrations ≤0.70 µmol/L. However, we did not observe the positive effect of vitamin A supplementation during pregnancy on mean serum retinol concentration ~4 mo postpartum. Three reasons may be proposed. First, the supplementation needs to be extended beyond pregnancy or the dose given was too small considering that the serum retinol concentration near term remained constant [16]. Second, the positive effect might be observed if it were measured at the same time as breast milk measurement or earlier. Third, serum retinol concentration was not a responsive indicator of vitamin A status [23]. Provision of weekly vitamin A supplements to pregnant women can be channeled through existing systems, such as local health posts or women's groups with which pregnant women have frequent contact.

The retinol concentration in breast milk is associated with that of in serum in populations with relatively low vitamin A status [24]. Retinol concentration in breast milk was related to that in serum only in the group that did not receive supplementary vitamin A.

Vitamin A in breast milk is found almost exclusively in fat; thus, factors that affect breast milk fat concentration may affect the vitamin A concentration as well [24]. Fat is the macronutrient in milk that varies most in concentration. Ruel et al. [25] showed that variability within individuals is influenced by the time of sampling through the day and the time elapsed since the last feeding. In our study, all breast milk samples were taken between 0800 and 1100 h from the right breast from which the baby had not been fed during the previous hour. This was done to reduce variation in nutrient concentrations of breast milk, particularly of fat. Fat concentration is higher in mature milk than in colostrum and somewhat higher in affluent than in poor societies [26]. Our population had lower fat concentrations in breast milk than those reported by Nommsen et al. [27] from the United States and

Ruel et al. [25] from Guatemala but similar to those reported by Brown et al. [28] from Bangladesh. Fat concentration of breast milk has been shown to be higher in mothers with triceps skinfold thickness ≥11 mm or mid-upper arm circumference ≥22 cm than in their thinner counterparts [28]. We did not find such an association between fat concentration in breast milk and maternal nutritional status, probably because 90% of our population had mid-upper arm circumference ≥22 cm. Among well-nourished lactating women, Nommsen et al. [27] showed that maternal triceps skinfold thickness was related to the fat concentration of breast milk only at the later stage of lactation. Parity tended to be negatively related (P = 0.07) to the fat concentration in mature milk, as shown by others that lipid concentrations in breast milk are higher in primiparous women [27; 28].

Weekly iron (and folic acid) together with vitamin A supplementation during pregnancy did not increase the iron status postpartum and iron concentration in breast milk compared with supplementation with iron (and folic acid) alone. The improvement of hemoglobin concentration observed at near term with weekly iron (and folic acid) and vitamin A supplementation [16] was much less than that reported previously [10]. Therefore it is not surprising that the benefit of iron (and folic acid) and vitamin A supplementation during pregnancy was not sustained until the postpartum period. In the previous study [10], daily administration of 60 mg elemental Fe and 2.4 mg (2,400 RE or 8,000 IU) retinol was used and the effects postpartum were not measured.

Studies reported so far do not indicate any relationship between maternal iron status and iron concentration in breast milk [6; 7]. Similarly, provision of iron supplements during lactation does not increase the volume or iron concentration of breast milk [5]. We observed a significant correlation between maternal iron status and iron concentration in breast milk only in the group supplemented with vitamin A, which might be due to chance or to an increased iron absorption because of the better vitamin A status during pregnancy. The decrease in iron concentrations in breast milk as lactation proceeded, from 7.63 – 8.31 µmol/L (425 – 453 µg/L) at 4 – 7 d to 4.27 – 5.35 µmol/L (238 – 299 µg/L) at 3 mo postpartum, was similar to that reported earlier [29]. Although the concentration of iron in breast milk is low, its bioavailability is up to 70% compared with 30% for iron from cow's milk and only 10% for iron from breast milk substitutes [30]. Lactoferrin, an iron binding protein of bacteriostatic importance, which is present in high concentrations in human milk, has been proposed to account for the high iron bioavailability [31].

In conclusion, we have shown that weekly supplementation during pregnancy with vitamin A and iron (and folic acid) compared with iron (and folic acid) alone increased the concentration of retinol in breast milk even though retinol concentrations in serum ~4 mo postpartum did not increase. Postpartum maternal iron status and iron concentration in breast milk of those supplemented weekly during pregnancy with vitamin A and iron (and folic acid) did not differ from those supplemented weekly with iron (and folic acid) alone. Considering that the iron and vitamin A status of the women in all groups was poor, iron and vitamin A intake should be increased beyond pregnancy. Provision of additional iron and vitamin A sources can be obtained from pharmanutrients or food-based approaches with natural or enriched foods.

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4

Determinants of Weight and Length of Indonesian Neonates

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ABSTRACT

Objective: To investigate the determinants of neonatal weight and length.

Design: From 16 – 20 wk pregnancy, 366 mothers of the neonates had participated in the community-based study to investigate the effect of weekly supplementation during pregnancy with iron and vitamin A on infant growth. Women from 5 villages were allocated randomly to receive either two tablets each containing 60 mg iron as ferrous sulfate and 250 μg folic acid (n=121) or two tablets each containing 2,400 RE vitamin A in addition to the same amount of ferrous sulfate and folic acid (n=122). A third ("daily") group (n=123) participating in the national iron supplementation program was recruited from 4 neighboring villages.

Results: Neonatal weight and length did not differ between the two weekly groups and between the weekly iron group and the "daily" group. Iron and vitamin A status during pregnancy did not influence neonatal weight and length significantly. Boys were 100 g heavier and 0.53 cm longer than girls ($P < 0.05$). First born neonates were lighter ($P < 0.01$) and tended to be shorter ($P = 0.070$) than neonates of higher birth order. Maternal age and education as well as other socioeconomic determinants were not associated with neonatal weight and length. Neonatal weight was 32% explained by gestational age, maternal weight, postnatal measurement, gender, and parity while neonatal length was 28% explained by gestational age, maternal weight, postnatal measurement, gender and maternal height.

Conclusions: Gestational age, maternal weight at second trimester of pregnancy and infant gender were the main predictors of neonatal weight and length.

Barker [1] has hypothesized that fetal undernutrition leads to various adult chronic diseases. In developing countries, one sixth of babies have a low birth weight (<2,500 g) [2]. Weight and length of a newborn infant are influenced to a certain extent by maternal nutritional status both before and during pregnancy [3] while the effect of socioeconomic condition on birth weight is mediated through effects on the mother [4; 5].

Low weight gain particularly during second trimester elevates the risk of intrauterine growth retardation [6-8]. However, efforts to control fetal nutrition through improvement of maternal dietary intake have given contradictory results. In the Gambia, energy and protein supplementation resulted in an increased birth weight only when provided to pregnant women during a season of hunger and intense agriculture work [9]. Provision of additional energy during the last trimester to mothers in Madura in East Java did not improve the birth weight of their infants [10].

Although many pregnant women, particularly in developing countries, are deficient in several micronutrients, iron deficiency is the most widespread problem. Risk of preterm delivery and low birth weight are associated with iron deficiency anemia during pregnancy [11]. The addition of vitamin A to routine iron supplementation has been shown to increase hemoglobin concentrations during pregnancy [12; 13].

In addition to the intake of energy and nutrients, socioeconomic factors are associated with nutritional status during pregnancy and determine, in part, neonatal weight and length. In planning effective supplementation, it is of particular importance to identify these crucial socioeconomic factors. Our first objective was to investigate the association between socioeconomic and maternal nutritional factors while our second objective was to investigate the determinants of neonatal weight and length. This study was within the framework of a larger study of which the main aim was to investigate the effect of weekly iron and vitamin A supplementation during pregnancy on infant growth in the first year of life. Results on the effect of iron and vitamin A supplementation on the iron and vitamin A status near term [13] has been reported elsewhere.

MATERIALS AND METHODS

Description of the study population. The study was conducted in 9 out of 19 rural villages in Leuwiliang sub-district, West Java, Indonesia from November 1997 until November 1999. Three villages were in the service area of the main health center and six other villages were in two other smaller health centers, which were under supervision of the main health center. Leuwiliang, 80 km south of Jakarta, has an area of 158 km^2 and a population of about 140,000. The maximum distance between study villages was 20 km. The area was rural and hilly. Only the main road had asphalt and the remaining roads were made from stone or soil. Public transportation was available throughout the day.

Study design has been described earlier [13]. In brief, women who were 16 – 20 wk pregnant, aged 17 – 35 y and parity <6 were assigned randomly to two weekly groups on an individual basis. They were supplemented once weekly from enrolment until delivery with two tablets each containing 60 mg iron as ferrous sulfate and 250 µg folic acid or with two tablets each containing 2,400 retinol equivalents (RE) vitamin A in addition to the same amount of ferrous sulfate and folic acid. A third ("daily") group participating in the national iron supplementation program was recruited from neighboring villages at the same time. According to government policy, pregnant women receive 90 – 120 tablets containing iron plus folic acid throughout pregnancy distributed through medical services. Therefore it was regarded as unethical to have a placebo group in which women did not receive iron supplements. The median number of iron tablets consumed by women in the "daily" iron group was 50.

Socioeconomic and pregnancy history indicators. Demographic and socio-economic characteristics and pregnancy history were assessed through interview using a pre-coded questionnaire at enrolment following the recommendations of Gross, *et al,* [14]. Socioeconomic variables included were occupation, educational level, household possessions, and hygiene facilities. Parity and the number of miscarriages were also reported. Village midwives estimated the gestation time by palpation and from the date of last menstruation period and was later verified against the date of actual childbirth.

Anthropometry assessment. Maternal anthropometric measurements were assessed at enrolment (~18 wk pregnancy) and near term (35 wk pregnancy). Body weight was measured to the nearest 0.1 kg using a UNICEF electronic weighing scale (SECA 890, Hamburg, Germany), mid-upper arm circumference (MUAC) was measured to the nearest 0.1 cm using a plastic measuring tape, and height was measured to the nearest 0.1 cm using a standing height measurement 'microtoise' (UNICEF, Copenhagen, Denmark). Neonatal weight and length were measured during a postnatal home visit. Neonatal weight was measured to the nearest 50 g using a baby weighing scale (Misaki, Japan) and length was measured to the nearest 0.1 cm using a wooden length board [14] by two of the authors (S.M. and M.K.S.).

Iron and vitamin A status assessments in serum. On the same day as anthropometric assessment was carried out, venous blood samples (~5 mL) were collected in a tube without anticoagulant between 0900 and 1200 h. Hemoglobin was determined using the cyanmethaemoglobin method (Merck test 3317; Merck, Darmstadt, Germany) at the laboratory of the Nutrition Research and Development Center, Bogor. Serum ferritin was analyzed by enzyme immunoassay using a commercial kit (IMX System, Abbott, Abbott Park, USA) at the SEAMEO TROPMED laboratory, Jakarta and duplicate analysis were performed on 12% of the samples. Serum retinol was analyzed using high-pressure liquid chromatography (HPLC) at the Division of Human Nutrition and Epidemiology, Wageningen University. Ten percent of the analyses were carried out in duplicate. All analysis had been described in more detail earlier [13].

Data analysis. The normality of data distribution was checked using the Kolmogorov-Smirnov test. Normally distributed data are reported as mean and SD or SEM. Serum ferritin concentrations were not normally distributed, therefore these data were logarithmically transformed and reported as geometric mean and 95% confidence interval. ANOVA and t-test were used to examine the relationship between maternal nutritional status and socioeconomic and other individual characteristics and to compare the weight and length of neonates born to mothers varying in nutritional status. Analysis by stepwise multiple linear regression was used to examine the determinant factors of neonatal weight and length. Only variables that had significant association in univariate analysis were included in the model. MUAC was not included in the model because it was highly correlated with weight ($r = 0.812$). The SPSS software package (Windows version 7.5.2. SPSS Inc., Chicago, USA) was used for all statistical analyses and a *P* value of <0.05 was considered as significant.

RESULTS

All women were Moslems, 95% originated from one single ethnic group (Sunda), and 95% did not earn any money. Pregnant women with primary school education had significantly lower body weight and MUAC than their peers with secondary school education (**Table 1**). Lower age, primiparity, and owning land, set of chairs, television, radio and/or cassette player were all associated with a higher MUAC. Height was not associated with age, education, occupation, possession of household goods, environmental conditions, and parity. As expected, primiparous women (30% of the total) were significantly younger than multiparous women [19.9 ± 2.2 vs. 26.1 ± 4.1 y, respectively; $P < 0.01$ (mean ± SD)]. The proportion of subjects who had miscarried was 11% (n = 36). Parity and age were not associated with the number of miscarriages.

At enrolment, the vitamin A and iron status of the pregnant women did not differ across the groups (**Table 2**). At near term, the prevalence of marginal vitamin A deficiency (serum retinol concentrations <0.70 µmol/L) remained constant in the weekly vitamin A and iron group and significantly lower than the weekly iron group (15% vs. 32%, $P < 0.01$). Also, the prevalence of anemia (hemoglobin concentrations <110 g/L) tended to be lower ($P = 0.061$) in the weekly vitamin A and iron group, while persisted in the other two groups. In all groups, the prevalence of iron deficiency anemia (hemoglobin concentrations <110 g/L and serum ferritin concentrations <12 µg/L) did not change. The anthropometric indices of the pregnant women did not differ across the groups both at enrolment and near term (data not shown). On average, women gained 5.0 ± 2.5 kg (0.29 ± 0.14 kg/wk) from ~18 wk until 35 wk pregnancy (n = 255).

TABLE 1

General characteristics of women at the time of enrolment[1]

Maternal characteristics	*n*	Weight (kg)	Height (cm)	MUAC (cm)
Age:				
17 – 21 y	122	48.1 ± 5.1	149.1 ± 4.2	24.1 ± 2.1**
22 – 26 y	126	49.2 ± 6.6	149.1 ± 4.9	24.7 ± 2.3
>26 y	118	49.4 ± 7.4	149.4 ± 5.3	25.1 ± 2.6
Educational level:				
Primary school	269	48.4 ± 6.3**	149.1 ± 4.8	24.4 ± 2.3*
Secondary school	97	50.4 ± 6.6	149.7 ± 4.7	25.2 ± 2.3
Husband's occupation[2]:				
Daily paid worker	165	48.4 ± 6.3	149.1 ± 4.9	24.5 ± 2.3
Monthly paid worker	128	49.5 ± 6.1	149.4 ± 4.8	24.9 ± 2.4
Trader	73	49.0 ± 7.2	149.3 ± 4.8	24.5 ± 2.6
Land:				
Yes	99	49.2 ± 6.9	149.1 ± 5.0	25.1 ± 2.7*
No	267	48.8 ± 6.2	149.2 ± 4.7	24.4 ± 2.2
Television:				
Yes	210	49.2 ± 6.8	149.2 ± 4.8	24.9 ± 2.6*
No	156	48.5 ± 5.9	149.3 ± 4.8	24.3 ± 1.9
Set of chairs:				
Yes	235	49.5 ± 6.6*	149.4 ± 4.8	24.9 ± 2.4**
No	131	47.9 ± 5.9	148.8 ± 4.8	24.1 ± 2.2
Radio and/or cassette player:				
Yes	258	49.2 ± 6.4	149.3 ± 4.8	24.8 ± 2.5*
No	108	48.3 ± 6.4	148.9 ± 4.7	24.1 ± 2.0
Bicycle:				
Yes	112	49.9 ± 7.1*	149.1 ± 4.7	25.2 ± 2.5**
No	254	48.4 ± 6.0	149.3 ± 4.8	24.3 ± 2.3
Place of defecation:				
Latrine	169	49.5 ± 6.5*	149.6 ± 4.8	24.9 ± 2.5*
River/pond	197	48.2 ± 6.2	148.8 ± 4.8	24.3 ± 2.2
Sources of drinking water:				
Well	290	48.9 ± 6.0	149.0 ± 4.8	24.7 ± 2.3
Spring water	76	48.8 ± 7.8	149.8 ± 4.7	24.3 ± 2.7
Parity:				
0	110	48.2 ± 5.1	149.0 ± 4.4	24.0 ± 2.0**
1 – 2	181	49.3 ± 6.8	149.4 ± 5.1	24.8 ± 2.4
3 – 5	75	49.0 ± 7.3	149.1 ± 4.6	25.0 ± 2.5

[1] Mean ± SD

[2] Daily paid workers include farmers, laborers, drivers; Monthly paid workers include teachers, government and private employees

* $P < 0.05$; ** $P < 0.01$; ANOVA controlling for time of measurement during pregnancy

The multiple regression analysis revealed that MUAC, height, maternal education, and parity explained 12% of weight gain (data not shown).

Out of the 366 pregnant women initially enrolled, 25 dropped out during pregnancy, 10 gave birth to a stillborn child, 3 had twins (only 1 survived), and 2 moved. The weight, height, and MUAC of mothers having stillborn or twin babies did not differ from mothers having live born babies (data not shown). The majority of births took place at home (89%) and with the assistance of traditional birth attendants (80%). Only few births were assisted by midwives (15%) or medical doctors (3%).

TABLE 2

The proportion of subjects with anemia, low iron stores, and vitamin A deficiency in the weekly vitamin A and iron group, the weekly iron group, and the "daily" group at enrolment

	Number of women (%)			
	Weekly vitamin A and iron	Weekly iron	"Daily" iron	Total
n total	122	121	123	366
Hemoglobin:				
<110 g/L	56 (46)	51 (42)	60 (49)	167 (46)
<105 g/L	33 (27)	35 (29)	35 (28)	103 (28)
<100 g/L	22 (18)	24 (20)	21 (17)	65 (18)
Serum ferritin[1]:				
<20 μg/L	80 (68)	81 (68)	68 (64)	229 (67)
<12 μg/L	54 (46)	53 (45)	47 (44)	154 (45)
Iron deficiency anemia[1,2]	25 (21)	24 (20)	27 (25)	76 (22)
Serum retinol[3]:				
<1.05 μmol/L	71 (60)	70 (60)	66 (59)	207 (60)
<0.70 μmol/L	18 (15)	18 (16)	21 (19)	57 (17)

[1] n = 118, 119, 106, and 343
[2] defined as hemoglobin concentrations <110 g/L and serum ferritin concentrations <12 μg/L
[3] n = 118, 116, 111, and 345

Weight and length were reported from 296 (91%) neonates based on measurement within 15 d of birth. Maternal anthropometric parameters at enrolment of these neonates did not differ significantly from the other neonates (data not shown). Neonates in the "daily" group were measured later than those in the weekly groups (6.9 ± 3.7 vs. 5.2 ± 3.3 d; $P < 0.01$). The period between birth and the postnatal home visit varied largely due to the difficulties in communication and reaching the subjects. Length of four neonates was not measured. Boys were 100 g heavier and 0.53 cm longer than girls ($P < 0.05$, controlling for age at measurement, **Table 3**).

Neonatal weight (3,094 ± 440 g) and length (49.1 ± 2.0 cm) did not differ between the two weekly groups and between the weekly iron group and the "daily" group ($P > 0.05$, after controlling for age at measurement and gender). Within the "daily" group, neonates from mothers who consumed ≥50 iron tablets during pregnancy had similar weights compared to those who consumed <50 iron tablets. Also, iron and vitamin A status during pregnancy did not significantly influence neonatal weight and length (data not shown). The duration of pregnancy of women with anemia at enrolment was shorter than those without anemia (37.4 ± 2.7 vs. 38.0 ± 2.8 wk, $P < 0.05$).

We pooled neonatal weight and length from the three treatment groups to investigate factors associated with neonatal weight and length. Compared to the lowest tertile, neonates from mothers in the highest tertile of maternal weight, MUAC, and height at ~18 wk pregnancy were heavier and longer ($P < 0.01$, controlling for postnatal measurement and gender, Table 3). Preterm neonates (gestational age <37 completed weeks) were significantly ($P < 0.001$) lighter and shorter than term neonates. First born neonates were lighter ($P < 0.01$) and tended to be shorter ($P = 0.07$) than neonates of higher birth order.

Maternal age and education as well as other socioeconomic determinants were not associated with neonatal weight and length. From multiple regression analysis, gestational age, maternal weight, postnatal measurement and gender determined both neonatal weight and length (**Table 4**). In addition, parity determined neonatal weight while maternal height determined neonatal length. Of the 296 neonates, data on maternal weight gain during pregnancy were available from 226 (76%) subjects.

TABLE 3

Factors associated with neonatal weight and length[1]

	n	Neonatal weight	*n*	Neonatal length
Treatment group:				
Weekly vitamin A and iron	103	3,094 ± 455	101	49.1 ± 2.1
Weekly iron	102	3,096 ± 396	101	49.1 ± 2.0
"Daily" iron	91	3,089 ± 475	90	49.1 ± 2.0
Gender:				
Boys	142	3,146 ± 436*	140	49.4 ± 2.1*
Girls	154	3,045 ± 441	152	48.8 ± 2.0
Age at measurement:				
0 – 3 d	71	3,028 ± 333*	70	48.6 ± 1.7**
3 – 7 d	131	3,059 ± 424	131	48.9 ± 2.1
7 – 15 d	94	3,191 ± 515	91	49.8 ± 2.0
Gestational age:				
<37 wk	58	2,835 ± 496**	91	48.1 ± 2.2**
≥37 wk	238	3,155 ± 403	201	49.3 ± 1.9
Maternal weight:				
<45.9 kg	100	2,968 ± 392**	99	48.7 ± 1.9**
45.9 – 50.7 kg	101	3,038 ± 427	98	48.9 ± 2.1
≥50.7 kg	95	3,285 ± 443	95	49.7 ± 1.9
Maternal MUAC:				
<23.4 cm	97	2,968 ± 417**	94	48.7 ± 2.0*
23.4 – 25.5 cm	106	3,096 ± 408	105	49.1 ± 2.0
≥25.5 cm	93	3,221 ± 467	93	49.5 ± 2.0
Maternal height:				
<147.4 cm	99	2,992 ± 406**	98	48.6 ± 1.8**
147.7 – 150.9 cm	95	3,093 ± 410	92	49.1 ± 1.7
≥150.9 cm	102	3,193 ± 480	102	49.6 ± 2.3
Maternal age:				
17 – 21 y	98	3,030 ± 432	94	49.1 ± 1.9
22 – 26 y	107	3,128 ± 440	107	49.1 ± 2.1
26 – 35 y	91	3,121 ± 448	91	49.1 ± 2.1
Maternal educational level:				
Primary school	216	3,099 ± 433	214	49.1 ± 1.9
Secondary school	80	3,079 ± 464	78	49.0 ± 2.1
Paternal occupation[2]:				
Daily paid worker	140	3,127 ± 439	139	49.2 ± 2.0
Monthly paid worker	98	3,071 ± 409	97	49.1 ± 1.9
Trader	58	3,050 ± 494	56	49.0 ± 2.2
Maternal parity:				
0	84	2,920 ± 392**	80	48.6 ± 1.8
1 – 2	148	3,156 ± 436	148	49.2 ± 2.1
3 – 5	64	3,177 ± 454	64	49.3 ± 2.1

[1] Mean ± SD

[2] Daily paid workers include farmers, laborers, drivers; Monthly paid workers include teachers, government and private employees

* $P < 0.05$; ** $P < 0.01$; ANOVA, controlling for age at measurement

When we included this variable into the model, 37% of neonatal weight and 30% of neonatal length were explained. Determinants of neonatal weight did not change, but gestational age and maternal height were no longer significant determinants of neonatal length (data not shown).

DISCUSSION

In this study, we showed that predictors of neonatal weight and length were gestational age, maternal weight at second trimester and infant gender. Weekly supplementation of vitamin A and iron during pregnancy did not influence neonatal weight and length. Socioeconomic variables were associated with maternal nutritional status but not with neonatal weight and length. The influence of education and possession of household goods on maternal weight reflects the role of socioeconomic and environmental conditions.

Maternal weight prior to pregnancy is an important determinant of pregnancy outcomes, such as low birth weight, intrauterine growth retardation and preterm birth [3; 15]. Also, weight gain, particularly during the second trimester, has been known to influence birth weight positively [6-8]. In practice, weight gain monitoring or assessment of total weight gain during pregnancy is implemented only rarely in developing countries where early prenatal care is very weak or non-existent. Only in very advanced stages of pregnancy, women attend health services. It is recommended that women should have a weight gain of at least 6 kg (1 kg per mo) during the second and third trimesters [16]. On average, our population had met this recommendation.

Supplementation with iron and vitamin A once weekly increased hemoglobin concentration moderately [13]. We did not find an association between maternal iron status on neonatal weight and length, but anemia was associated with shorter gestation. However, the difference of 0.6 wk might not be biologically significant. Reviews on observational studies show that maternal hemoglobin concentration has a U-shaped relationship with birth weight and preterm delivery [11; 17]. However, most of the studies did not take into account the gestation time at which hemoglobin concentrations were measured. In contrast to findings from observational studies, controlled trials on iron supplementation do not provide evidence for benefits on preventing intrauterine growth retardation [18]. Preziosi *et al,* [19] showed that infants of iron supplemented mothers were longer, but not heavier, at birth than those of non-supplemented mothers.

TABLE 4

Multiple regression analysis on the determinants of neonatal weigh and length

	Standardised Coefficient	Adjusted R^2
Neonatal weight (n = 296)		0.321
Gestational age	0.367	
Maternal weight at enrolment	0.347	
Age at measurement	0.231	
Gender (0 = boy, 1 = girl)	-0.158	
Parity	0.147	
Neonatal length (n = 292)		0.276
Gestational age	0.321	
Age at measurement	0.310	
Maternal weight at enrolment	0.220	
Gender (0 = boy, 1 = girl)	-0.176	
Maternal height at enrolment	0.125	

Poor maternal vitamin A status was found to be associated with prematurity and intrauterine growth retardation in a low income group, but not in a high income group, of an Indian population in Baroda [20]. The mean neonatal weight and maternal serum retinol concentrations of subjects in our study were comparable to those from high income groups, which explains why maternal vitamin A status and neonatal weight and length were not associated. In Tanzania, supplementation with 30 mg β-carotene and 5,000 IU vitamin A daily starting from between 12 and 27 wk pregnancy among HIV-infected women did not significantly affect the risk of low birth weight, severe preterm birth or small-for-gestational age at birth [21]. An earlier study revealed that despite an improvement of serum retinol concentrations after daily supplementation with 8,000 IU vitamin A among Indo-Pakistani women living in South London, the anthropometric indices of the baby were not affected [22].

In West Java, socioeconomic factors have been found to be associated with nutritional status of pregnant women but not with the neonatal weight and length [23]. In Thailand [5], *family income was the only factor found to* be strongly associated with birth weight. In our population, maternal age was not associated with birth weight as shown also by other studies [6; 23]. Primiparous women, who were younger than multiparous women, gave birth to babies with lower birth weight [6].

In conclusion, gestational age, maternal weight at second trimester of pregnancy and infant gender were the main predictors of neonatal weight and length in our population. The importance of socioeconomic and environmental conditions on maternal nutritional status suggests that effort to improve maternal nutritional status before and during pregnancy should be encouraged. Although iron and vitamin A supplementation during pregnancy did not affect neonatal weight and length, it may influence subsequent nutritional status and growth, as well as mental and motor development.

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5

Weekly Vitamin A and Iron Supplementation of Indonesian Pregnant Women: The Association between Nutritional Status, Food Intake and Household Characteristics

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ABSTRACT

In Indonesia many pregnant and lactating women suffer from iron deficiency anemia and vitamin A deficiency. Women with marginal food intake and low socioeconomic status are at particular risk of micronutrient deficiencies. This article reports the association between characteristics of the household and food intake and nutritional status of Indonesian women. In addition the impact of vitamin A and iron supplementation during pregnancy on prevalence of micronutrient deficiencies during pregnancy and at ~4 mo postpartum is reported. Women who were 16 – 20 wk pregnant from 5 villages were randomly allocated on an individual basis to receive two tablets once weekly. Each tablet contained both iron and folic acid or the same amounts of iron and folic acid plus vitamin A. Pregnant women from 4 neighboring villages with similar characteristics were recruited at the same time to serve as a control ("daily") group. These women had free access to iron tablets from government health services. At enrollment, household characteristics and pregnancy history were assessed. Anthropometric assessments and blood taking for the assessment of vitamin A and iron status were done at enrollment, near term and at ~4 mo postpartum. Food intake was assessed by 24-h recall at 30 wk of pregnancy. A large proportion of the women in our study had marginal nutritional status during pregnancy and at ~4 mo postpartum while energy and nutrient intakes were low. Low nutritional status and energy intake were associated with household characteristics reflecting a lower socioeconomic status. Women in households with a monthly income had a higher energy intake. Vitamin A supplementation during pregnancy prevented an increase in the prevalence of marginal vitamin A deficiency until ~4 mo postpartum. However no positive impact was observed with respect to iron status. In order to have a greater impact of intervention programs aimed at improving nutritional status of women, the role of household characteristics reflecting the socioeconomic condition should be taken into account. Considering the inadequate nutritional status of the women in the present study, intervention programs aimed at improving income or increasing food intake together with integration of vitamin A and iron supplementation would be beneficial.

Health and nutrition of women reflects the economic livelihood of a population. Mothers with low body mass index have been found to have a lower work capacity, limited social activity and lower income, as well as a greater proportion of low birth weight babies [1]. In Asia, more than 30% of women are underweight [2] while in an Indonesian study, 38% women were found to have a body mass index <18.5 kg/m² [3].

Nutritional status of women during pregnancy and lactation is of concern because of its relationship with nutritional status of their infants [4]. Adequate supply of micronutrients during pregnancy and lactation is important for both women's health and development of their infants [2]. More than half of pregnant women in developing countries suffer from anemia, mainly caused by iron deficiency. Also, marginal vitamin A deficiency among pregnant and lactating women is frequently observed [5; 6].

The negative impact of iron deficiency anemia on maternal and infant health has led to the implementation of iron supplementation programs for pregnant women [7; 8]. Since 1990, there is an ongoing discussion about the use of intermittent, instead of daily, dose of iron supplementation to reduce the prevalence of iron deficiency anemia [9; 10]. Our study found that supervised weekly iron supplementation performed similarly to the daily iron supplementation program of the Indonesian government in improving the iron status of pregnant women [11].

Vitamin A supplementation in conjunction with iron supplementation has been shown to benefit hemoglobin and serum retinol concentrations of Indonesian women during pregnancy [11; 12]. The importance of an adequate vitamin A supply during pregnancy was demonstrated by 40% reduction of maternal mortality rate with weekly vitamin A supplementation [13]. Women with marginal food intake and low socioeconomic status are particularly at risk of micronutrient deficiencies. Low levels of intake of vitamin A and iron were associated with lower intakes of energy, which was related to household food security as assessed by hunger feelings of women [14].

In this study, we investigate the characteristics of the household associated with the food intake and nutritional status of Indonesian women during pregnancy and lactation. In addition we report the impact of vitamin A and iron supplementation during pregnancy on the prevalence of iron and vitamin

A deficiencies during pregnancy and lactation. This study is part of a larger study which investigates the effect of iron and vitamin A supplementation during pregnancy on growth of Indonesian infants. Other findings had been presented elsewhere [11; 15].

METHODS

Study area and population under study. The study was carried out among pregnant women living in 9 out of 19 villages in Leuwiliang subdistrict, Bogor, West Java, Indonesia from November 1997 until November 1999. Leuwiliang is situated 80 km south of Jakarta and covers an area of 158 km^2 and has ~1400,000 inhabitants. The area is rural and hilly. Only the main road is asphalt and the remaining roads are made from stone or soil. Public transportation is available throughout the day.

Midwives and village women volunteers registered pregnant women and brought them to the examination points (mostly the village offices) on the appointed dates. Pregnant women received detailed explanation concerning the objectives and procedures of the study prior to inclusion. Only those who submitted written informed consent were eligible for the study. The medical ethical committees from the University of Indonesia, the Indonesian Ministry of Health, and Wageningen University approved the study protocol prior to the conduct of the study.

Allocation to treatment. Women who were 16 – 20 wk pregnant, aged 17 – 35 y, and parity <6 from 5 villages were randomly allocated on an individual basis to receive two tablets once weekly (**Fig. 1**). Each tablet contained both iron (60 mg elemental iron) and folic acid (250 µg) or the same amount of iron and folic acid plus vitamin A (8,000 IU or 2,400 RE). The two types of tablet had identical physical appearance (PT Kimia Farma, Indonesia). The pregnant women received the supplement once a week from voluntary health workers who ensured that the women swallowed the tablets in their presence.

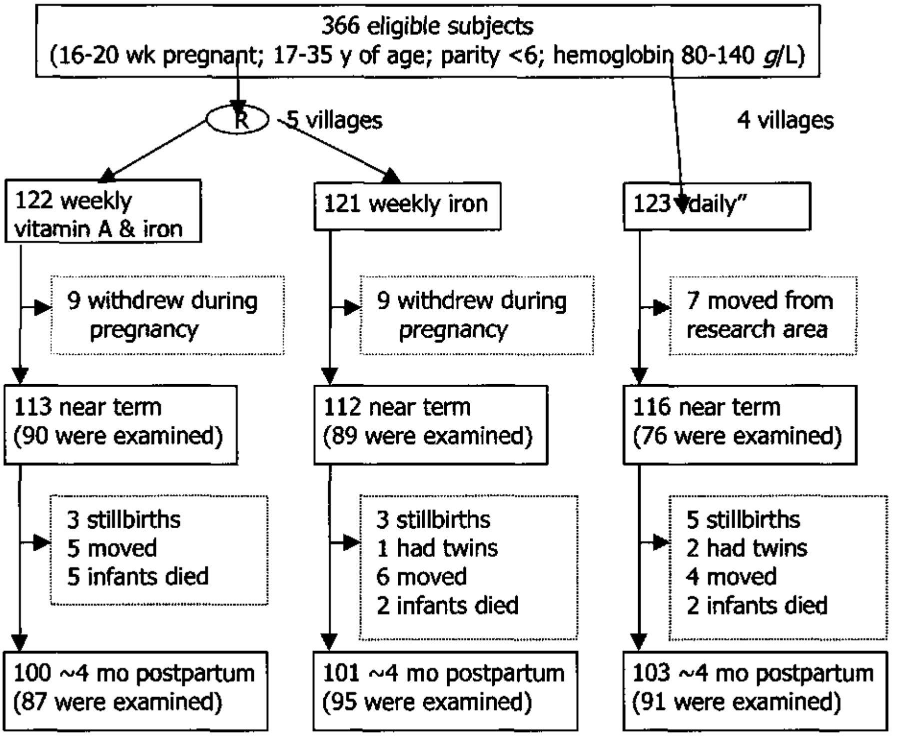


FIGURE 1 Subjects selection, retention and drop out.

Indonesian government policy stipulates that each pregnant woman should receive at least 90 – 120 iron tablets, taken daily throughout pregnancy, from health services. Hence, inclusion of a placebo group is unethical in these circumstances. Pregnant women from 4 neighboring villages with similar characteristics were recruited at the same time to serve as a control ("daily") group. These women had free access to iron tablets from government health services, such as health centers, midwives, or integrated health posts (*Posyandu*). Intake of iron tablets in this group was assessed through interview at a postnatal home visit. Median iron tablets intake was 50. The iron tablets used for the national program had the same content of iron and folic acid and physical appearance as those for weekly supplementation (PT Kimia Farma, Indonesia).

Three villages were in the catchment area of the main health center, while the other 6 villages were in the catchment area of two smaller health centers. These two small health centers were under supervision of the main health center. Each health center had villages allocated for either weekly or "daily" group.

Household characteristics, hygiene facilities, and pregnancy history assessment. At enrollment, interviews using structured questionnaires were carried out to assess household characteristics and pregnancy history. Variables included in the household characteristics were education, occupation, possessions (such as land, cattle, chairs, bicycle, television sets, radio/cassette recorder), type of house and hygiene facilities (place of defecation and source of drinking water). Pregnancy history assessed the number of pregnancies and miscarriages experienced by the subjects.

Anthropometry and pregnancy assessments. Anthropometric assessments were carried out twice during pregnancy (enrollment and near term) and once postpartum. Body weight was measured using a UNICEF weighing scale (SECA 890; Hamburg, Germany) to the nearest 0.1 kg; height was measured using a standing height measurement 'microtoise' to the nearest 0.1 cm; and mid-upper arm circumference was measured using a plastic measuring tape to the nearest 0.1 cm. Midwives estimated the age of gestation by palpation method and the last date of menstruation, which was verified later against the actual date of childbirth.

Dietary assessment. Subjects were visited at home for dietary assessment when they were 30 wk pregnant. A single 24-h recall was used to assess the dietary intake in 50% of the subjects selected randomly.

Blood drawing and biochemical analysis. On the same day as anthropometric assessments were carried out, venous blood samples (~5 mL) were collected in a tube without anticoagulant between 0900 and 1200 h. Hemoglobin concentration was determined using the cyanmethemoglobin method (Merck test 3317; Merck, Darmstadt, Germany) at the Nutrition Research and Development Center laboratory, Bogor. Serum ferritin concentration was assessed by enzyme immunoassay using a commercial kit (IMX System, Abbott, Abbott Park, IL) at the SEAMEO TROPMED laboratory, Jakarta. Serum retinol concentration was analyzed using high-pressure liquid chromatography at the Division of Human Nutrition and Epidemiology, Wageningen University. All analyses had been described in more detail earlier [11].

Statistical analysis. Since only the two weekly groups were randomized while the "daily" group was not, this was taken into account during analysis. To test the household characteristics in relation with allocation groups, the two weekly groups were compared and then the combined weekly groups were compared with the "daily" group. All data were pooled to investigate the association of household characteristic variables with body mass index and dietary intake. To determine the effect of vitamin A supplementation on the vitamin A and iron status, only the two weekly groups were compared. The "daily" group was compared to the weekly iron group to test the effect of iron supplementation regiment (weekly or daily) on the iron status. The associations between prevalence of micronutrient status and household characteristics were carried out independently in each group. The normality of data distribution was checked using the Kolmogorov-Smirnov test. Normally distributed data are reported as mean and SD. ANOVA and t test were used to examine the relationship between maternal nutritional status and household and other individual characteristics. Pearson or Spearman correlation coefficients were calculated for the relationship between continuous variables as appropriate. Energy and nutrient intake data are presented as median and 25^{th} - 75^{th} percentiles, and Mann-Whitney U or Kruskal-Wallis test was employed to test the difference between or among groups. Energy and nutrient intake was calculated based on Indonesian food composition tables. For dichotomous variables, McNemar test was employed to test the two related dichotomous variables, while χ^2 test was used to test the difference between groups. 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.

RESULTS

All of the households were Moslems, 95% Sundanese and 95% of the women were not involved in money earning activities. General households' characteristics are described in **Table 1**. Most of the households had 3 – 5 members. The majority of pregnant women had elementary school education and their husbands were daily paid workers including farmers. Farmers were included in the daily paid workers group because they took another job on a daily paid basis when they did not work on their own land. Only few households owned land. The most popular household belongings were radio/radio cassette player, set of chairs, and television. The household characteristics did not differ between the weekly groups or between the weekly groups and the "daily" group. However, more households in the "daily" group used river/pond as place of defecation (58%) than in the weekly groups (40%, $P < 0.01$). Of all women at enrollment, 30% were primiparous, 11% ever experienced a miscarriage, and 51% had one or more children below five years old.

The age of the pregnant women at enrollment was 24.2 ± 4.6 y (mean ± SD), the time of gestation 17.7 ± 2.1 wk, mid-upper arm circumference 24.6 ± 2.4 cm, body weight 48.9 ± 6.4 kg, height 149.2 ± 4.8 cm, and body mass index 21.9 ± 2.5 kg/m^2. Body weight was significantly correlated with mid-upper arm circumference (r = 0.812, $P < 0.001$) and height (r = 0.463, $P < 0.001$). At ~4 mo postpartum mid-upper arm circumference was similar to that at enrollment while body mass index was slightly decreased by 0.8 ± 1.2 kg/m^2 ($P < 0.001$). The proportion of women with body mass index ≤18.5 kg/m^2 at enrollment and ~4 mo postpartum was 4.4% and 10.3% respectively, while the proportion of women with body mass index ≤21.0 kg/m^2 was 36.9% and 52.4% respectively. Body mass index at enrollment and at ~4 mo postpartum were highly correlated (r = 0.885, $P < 0.01$). None of the anthropometric parameters differed among the groups during pregnancy nor at postpartum.

Median (25th - 75th percentiles) daily intake of energy and nutrient were as follows: energy, 4.88 (3.40 – 6.42) MJ; protein, 38.9 (29.2 – 58.4) g; fat, 32.5 (18.3 – 53.8) g; iron, 5.80 (3.67 – 8.62) mg; and vitamin A, 270 (78.5 – 551.5) RE. Heme iron intake was only 0.42 (0.19 – 0.69) mg/d and vitamin A intake from animal sources was 0 (0 – 21.5) RE/d. The proportion of subjects who met 100% of the Indonesian RDA were 10% for energy, 22% for protein, 19% for vitamin A, and none for iron.

TABLE 1

Household characteristics of the studied population at enrollment

	n	(%)
Number of person in the household:		
2 person	36	9.8
3 – 5 person	227	59.3
>5 person	97	30.9
Educational level:		
Elementary school	269	73.5
Secondary/high school	97	26.5
Husband's occupation[1]:		
Daily paid worker	165	45.1
Monthly paid worker	128	35.0
Trader	73	19.9
Possession of:		
Land	99	27.0
Big cattle (goat, cow, buffalo)	31	8.5
Poultry (chicken, duck)	196	53.6
Fish pond	37	10.1
Household belongings:		
Television	210	57.4
Radio/radio cassette player	258	70.5
Bicycle	112	30.6
Motorcycle	42	11.5
Set of chairs	235	64.2
Place of defecation:		
Latrine	197	53.8
River/pond	169	46.2
Source of drinking water:		
Well	290	79.2
Spring water	76	20.8
Type of house:		
Cement floor	335	91.5
Brick wall	259	70.8
Tile roof	332	90.7
Cement floor, brick wall, and tile roof	244	66.7

[1] Daily paid workers include farmers, laborers, drivers; Monthly paid workers include teachers, government and private employees.

The intake of energy, protein, fat, iron, and vitamin A intake were similar between the weekly groups or between the weekly groups and the "daily" group ($P > 0.05$). Energy intake was highly correlated with protein, fat and iron intake ($0.778 < r < 0.807$, $P < 0.01$) and to a lesser extent with vitamin A intake ($r = 0.200$, $P < 0.05$).

Energy intake was not correlated with nutritional status of women either during pregnancy or postpartum. Energy intake and body mass index at enrollment of women with secondary/high school education was significantly higher compared to women with elementary education (**Table 2**). Husband's occupation, possession of television, motorcycle, and set of chairs were significantly associated with energy intake. Having a latrine as place of defecation, well as a source of drinking water or a house with better quality materials was associated with a higher energy intake. In addition possession of a bicycle, motorcycle, or a set of chairs was associated with higher body mass index at ~18 wk pregnant (Table 2) and at ~4 mo postpartum (data not shown). Possession of land, cattle, or poultry was not associated with energy intake or nutritional status of women during pregnancy or postpartum (data not shown).

At enrollment, proportion of women with anemia (hemoglobin concentrations <110 g/L) was 46%, iron deficiency (serum ferritin concentrations <12 μg/L) 45%, and marginal vitamin A deficiency (serum retinol concentrations <0.70 μmol/L) 16.5%. Vitamin A intake above the median (>270 RE/d) was associated with lower prevalence of marginal vitamin A deficiency (10% vs. 24%, $P < 0.05$). During pregnancy, the prevalence of marginal vitamin A deficiency remained constant in the weekly vitamin A and iron group, but increased in the groups which did not receive vitamin A supplementation (**Figure 2**). At ~4 mo postpartum, the weekly vitamin A and iron group still had lower proportion of marginal vitamin A deficiency compared to the weekly iron group. The prevalence of anemia during pregnancy in the weekly vitamin A and iron group slightly but not significantly ($P = 0.061$) decreased, but the prevalence of iron deficiency increased significantly. In the "daily" iron group, the prevalence of iron deficiency also increased significantly during pregnancy. In all groups the prevalence of anemia and iron deficiency were persistently above 40% during pregnancy and at ~4 mo postpartum (Figure 2).

TABLE 2

Energy intake[1] and body mass index[2] of pregnant women according to household characteristics

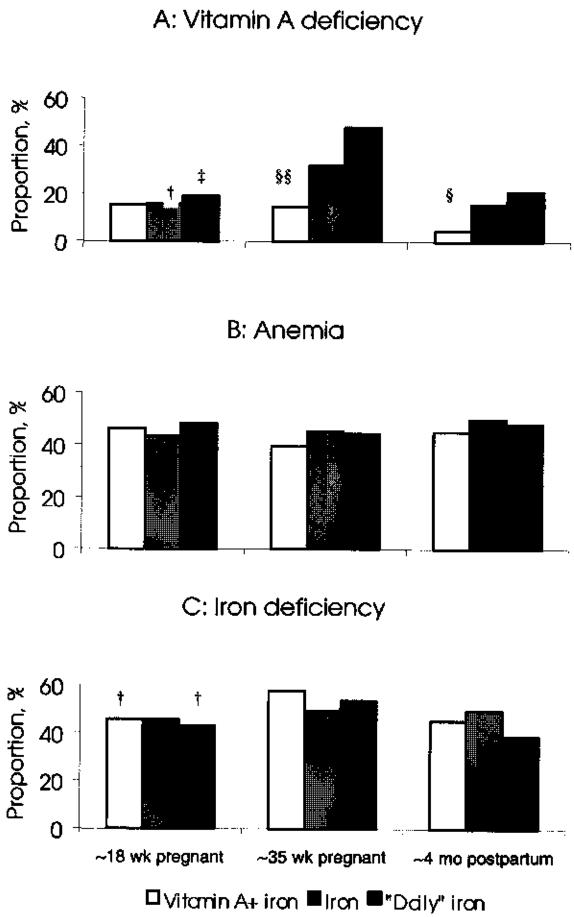
	n	Energy intake, MJ	n	~18 wk pregnant body mass index, kg/m²
Educational level				
Elementary school	123	4.58 (3.13 – 5.85)	269	21.7 ± 2.5
Secondary/high school	42	6.39 (4.10 – 9.58)**	97	22.5 ± 2.6§
Husband's occupation[3]				
Daily paid worker	81	4.12 (3.09 – 5.55)	165	21.7 ± 2.5
Monthly paid worker	57	5.88 (4.59 – 8.27)	128	22.2 ± 2.5
Trader	27	4.41 (2.90 – 6.32)‡	73	21.9 ± 2.6
Television				
Yes	93	5.40 (3.97 – 7.57)	210	22.1 ± 2.8
No	72	4.15 (2.97 - 5.64) **	156	21.7 ± 2.0
Bicycle				
Yes	53	5.26 (3.98 – 7.43)	112	22.4 ± 2.8
No	112	4.69 (3.31 – 6.36)	254	21.7 ± 2.3§
Motorcycle				
Yes	19	6.43 (4.12 – 8.89)	42	23.5 ± 3.8
No	146	4.69 (3.28 - 6.23) **	324	21.7 ± 2.2§§
Set of chairs				
Yes	105	5.47 (3.91 – 7.57)	235	22.1 ± 2.6
No	60	4.30 (3.01 – 5.49) **	131	21.6 ± 2.3§
Place of defecation				
Latrine	87	5.40 (3.80 – 7.76)	197	22.1 ± 2.7
River/pond	78	4.52 (3.15 – 5.82) *	169	21.7 ± 2.4
Source of drinking water				
Well	133	5.21 (3.53 - 6.78)	290	22.0 ± 2.3
Spring water	32	3.98 (2.61 – 5.33) *	76	21.7 ± 3.1
House				
Had cement floor, brick wall and tile roof	109	5.21 (3.93 – 6.78)	244	22.1 ± 2.7
Other	56	4.12 (2.83 – 5.81) *	122	21.7 ± 2.2

[1] Presented as median (25^{th} - 75^{th} percentiles); [2]Presented as mean ± SD; [3]Daily paid workers include farmers, laborers, drivers; Monthly paid workers include teachers, government and private employees.

* Significantly different between groups, $P < 0.05$; ** $P < 0.01$ (Mann-Whitney U test).

‡ Significantly different among groups, $P < 0.01$ (Kruskal-Wallis test).

§ Significantly different between groups, $P < 0.05$, §§ $P < 0.01$ (ANOVA controlling for gestational age).



[†] Significantly different between ~18 pregnant and ~35 wk pregnant, $P < 0.05$; [‡] $P < 0.01$ (McNemar test)

[§] Significantly different between the weekly vitamin A and iron group and the weekly iron group, $P < 0.05$; [§§] $P < 0.01$(χ^2-test).

FIGURE 2 Prevalence of vitamin A deficiency (serum retinol concentration <0.70 µmol/L; panel A), anemia (hemoglobin concentration <110 g/L for during pregnancy and <120 g/L during postpartum period; panel B), and iron deficiency (serum ferritin concentration <12 µg/L; panel C) of women during pregnancy and lactation.

At enrollment, anemic women were more frequently ($P < 0.05$) found in houses with better quality materials (50%) than in houses of poorer quality (38%). On the other hand, having a television set or a set of chairs were both associated with lower ($P < 0.01$) proportions of iron deficiency (38% vs. 54% and 38% vs. 57%, respectively). At ~4 mo postpartum, having a television set was still associated with lower ($P < 0.01$) proportion of iron deficiency (37% vs. 63%) in the two weekly groups. Across groups and time of assessments (pregnancy or postpartum), other household characteristics were not consistently associated with prevalence of anemia, iron deficiency or marginal vitamin A deficiency.

DISCUSSION

In this study, we had shown that a large proportion of the women in our study had a marginal nutritional status during pregnancy and ~4 mo postpartum. In addition energy and nutrient intake in this population was low. Low nutritional status and energy intake were associated with household characteristics reflecting a lower socioeconomic status. Although nutritional status of women in our study was better than those of women from Madura [3] it was comparable to that of Indonesian lactating women in a more recent study [16]. However the energy intake of our population was lower than that in other studies from Central Java [17], Madura [18] and West Java [19]. We are well aware of the limitations of the 24-h recall in determining nutrient intake [20; 21] and underreporting could have occurred. There are a number of factors which could contribute to a low energy intake of the women in our study: activity of pregnant women in Indonesia has been reported earlier to be low [18], most women in our study were not involved in money earning activities, and weight gain from enrollment (~18 wk pregnant) until delivery was only 5 kg [11]. In addition, the economic crisis in Indonesia, which started since July 1997, might have contributed to a lower food intake, but this was not assessed in our study.

Women from households with a monthly income had higher energy intake reflecting the better food security of the household. In Bangladeshi women, increases in household income were associated with higher intake of iron from animal sources [22]. In our study energy intake were associated with vitamin A and iron intake; thus improving the food intake of women may also lead to a better micronutrient status. Unfortunately we did not collect data on income and expenditure and therefore we cannot predict the effect of income generating activities on food intake. However considering the relatively low content and bioavailability of vitamin A and iron in food [16; 19] increasing food intake alone may not be enough to prevent the more serious consequences of vitamin A and iron deficiency [7; 13].

Vitamin A supplementation during pregnancy prevented the increase of the prevalence of marginal vitamin A deficiency even until ~4 mo postpartum. An Indian study also reported that daily supplementation of vitamin A prevented a decline of retinol concentration in plasma during pregnancy [23]. The positive association between vitamin A intake from food and lower prevalence of vitamin A deficiency was in accordance with another study among mothers of children aged ≤24 mo which revealed that the distribution curves of serum retinol concentrations shifted toward a higher value when vitamin A intake was above median, either from plant (≥280 RE/d) or animal (≥50 RE/d) foods [24]. Vitamin A deficiency has been associated with lower household socioeconomic condition and parasite infestation [24-26]. We did not find such an association, except that use of latrine was significantly associated with lower prevalence of *Ascaris* infestation (data not shown).

We did not observe a significant reduction of prevalence of anemia and iron deficiency as a result of vitamin A supplementation [11]. Our findings are not in line with those reported earlier [12]. This may be because our subjects received the supplement weekly and we did not select anemic subject only. Also, the daily and weekly iron supplementation regimes did not affect the prevalence of anemia and iron deficiency.

Prevalence of anemia and iron deficiency was high during pregnancy and postpartum while iron intake from food was low as had been reported earlier in Indonesian pregnant women [5]. It is disturbing that the prevalence of anemia and iron deficiency remained high despite iron supplementation. In our population, 46% (n = 71) of anemic women had serum ferritin concentrations <12 µg/L, which signifies iron deficiency anemia. It might be that other factors contributed to the high prevalence of anemia, such as deficiencies of folate and vitamin B12, and heavy worm load. Malaria was not signified as public health problem in the study area.

As had been mentioned previously, iron consumption from animal products is income elastic [22]; hence iron consumption is higher in the richer households than in the poorer ones. We showed that possession of a television set and of a set of chairs were associated with lower prevalence of iron deficiency suggesting that the effect of household possessions on the iron status was mediated through their food intake. The finding that having a better house type was associated with a higher prevalence of anemia was unexpected and may be to a chance. Considering the negative effect of iron deficiency anemia on pregnancy outcome and on time spent on household work and social activities [27; 28], additional interventions aimed at improving iron status of these women during pregnancy and postpartum would be beneficial.

In conclusion, the low nutritional status and energy intake in our population was associated with household characteristics reflecting a lower socioeconomic status. Supplementation with vitamin A together with iron during pregnancy prevented an increase in the prevalence of marginal vitamin A deficiency until postpartum period, whereas no positive effects were observed on the prevalence of anemia and iron deficiency. In order to have a greater impact of intervention programs aimed at improving nutritional status of women, the role of household characteristics reflecting the socioeconomic condition should be taken into account. Considering the inadequate nutritional status as assessed by body mass index and micronutrient status of the women in our study, intervention programs aimed at improving income or increasing food intake together with integration of vitamin A and iron supplementation, would be beneficial.

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6

Discussion

The overall aim of the study was to examine whether supplementation with iron and vitamin A during gestation, which is one of the most critical stages in the human life cycle, could prevent growth retardation. The studies described in this thesis concern outcomes in the mothers and the neonates. In this study, iron supplementation refers to iron and folic acid supplementation. Compared with weekly supplementation with iron alone, supplementation with vitamin A and iron, given at the time when women entered their second trimester of pregnancy until delivery, prevented the deterioration of vitamin A status near term and ~4 month postpartum, and increased retinol concentration in breast milk. Weekly supplementation with iron and vitamin A improved the hemoglobin concentration of mothers but did not decrease the prevalence of anemia near term. Weekly iron supplementation did not differ from the ongoing daily iron supplementation program in its effect on the iron status during pregnancy and lactation. The prevalence of anemia during pregnancy and lactation remained >40% despite supplementation with iron or with iron and vitamin A. The intervention did not influence the neonatal weight and length. The gestational age, maternal weight at the beginning of the second trimester and infant gender were the main predictors of neonatal weight and length. Women from households reflecting higher socioeconomic characteristics had a better nutritional status.

RELATIVE BENEFITS OF WEEKLY VERSUS DAILY IRON SUPPLEMENTATION DURING PREGNANCY ON IRON STATUS

The ongoing debate on daily versus weekly or intermittent iron supplementation started in 1990. With respect to this controversy, particular attention has been given to pregnant women as a target group considering their vulnerability due to the highest need for iron [1] and the adverse effects on pregnancy outcomes associated with iron deficiency and anemia [2]. The report by Beaton and McCabe [3] concludes that weekly iron supplementation is associated with 1.34 (95% CI, 1.20 – 1.49) higher risk of anemia, therefore weekly iron supplementation should not be recommended during pregnancy, regardless of the degree of supervision. A recent study reported the superiority of daily compared with twice weekly iron supplementation among anemic pregnant women in Pakistan in improving hemoglobin concentrations and iron stores [4].

As reported from a previous study in Indonesia [5] and a recent study in Malawi [6], weekly iron supplementation with ensured compliance performed similarly to the ongoing daily iron supplementation program, and even better in those women whose compliance was low (intake of iron tablets <50, ***Chapter 2***). However, such iron supplementation did not result in the improvement of hemoglobin or serum ferritin concentrations and anemia prevalence remained high (>40%), even until ~4 months postpartum (***Chapter 3***).

Women need to have adequate iron stores before entering pregnancy because such iron stores determine the iron status during pregnancy [7]. Therefore, weekly iron supplementation is more appropriate to improve and build iron stores among adolescent girls before entering pregnancy [8-10] rather than to correct anemia during pregnancy. Weekly iron supplementation has been shown to reduce the adverse side effects among pregnant women, hence improving compliance [5; 6]. The relative benefits of weekly versus daily iron supplementation on aspects of adverse side effect, compliance, cost, feasibility in the field under normal condition and probability of iron load were not assessed in this study.

BENEFITS OF VITAMIN A AND IRON SUPPLEMENTATION DURING PREGNANCY ON IRON AND VITAMIN A STATUS

The modest improvement of hemoglobin concentrations but a decline in serum ferritin concentrations with vitamin A supplementation confirmed the involvement of vitamin A in iron metabolism (***Chapter 2***). In addition, improvement of hemoglobin concentrations with vitamin A supplementation was only observed in anemic subjects. However the benefit on hemoglobin concentrations was not sustained postpartum (***Chapter 3***). The mechanism by which vitamin A deficiency affects iron metabolisms was not addressed in this study. Our findings are in line with results from a study in India [11], but the hemoglobin response is less pronounced than that found in a study in Indonesia reported earlier [12]. The moderate effects of iron and vitamin A supplementation on hemoglobin concentrations might be due to the fact that the subjects had low iron stores whereas the iron provided as supplement might not be sufficient to ensure adequate erythropoiesis or to build up iron stores (as shown by a decrease of serum ferritin concentration). A recent study among anemic adolescent girls in Bangladesh [13] found similar findings to the earlier Indonesian study among anemic pregnant women [12], despite that the studies were carried out in two physiologically different population groups and the supplement was administered weekly in the former but daily in the latter study.

The main benefit of vitamin A supplementation was the prevention of declining serum retinol concentrations during pregnancy (***Chapter 2***) and their maintenance throughout the lactation period. This was demonstrated by the lower proportion of subjects with marginal vitamin A deficiency and higher concentrations of retinol in breast milk (***Chapter 3***). However, the overall mean value of serum retinol concentration of 0.98 μmol/L at the beginning of the second trimester of pregnancy and 1.10 μmol/L at ~4 months postpartum indicating that the vitamin A status of the population studied is generally marginal. We did not observe any clinical manifestations of vitamin A deficiency among our study population. The improvement of retinol concentrations in breast milk following vitamin A supplementation during pregnancy is encouraging. However, the retinol concentration in breast milk (mean value 1.16 μmol/L and 0.05 μmol/g fat) is still inadequate to build up vitamin A stores in infants. The low concentration of retinol in breast milk was in line with the low concentration of retinol in serum in postpartum women. Our findings suggest that there is a need to improve the vitamin A status of women both during pregnancy and lactation for the benefit of themselves and of their infants. Weekly supplementation with vitamin A during pregnancy is considered as a safe procedure as has been carried out in this study and in Nepal [14] as long as the dose does not exceed 25,000 IU (25.25 μmol or 7,500 RE).

The concentration of iron in breast milk was within reported values in earlier studies [15] and was not influenced by the iron status during pregnancy and lactation.

BENEFITS OF VITAMIN A AND IRON SUPPLEMENTATION ON PREGNANCY OUTCOME

Compared with iron supplementation, vitamin A and iron supplementation did not show a direct benefit on pregnancy outcome as shown by the lack of differences in neonatal weight and length (***Chapter 4***). In addition, iron status during pregnancy did not have any influence on neonatal weight and length. Indeed, many intervention studies with micronutrients supplementation during pregnancy have not shown any improvement in infant birth weight [16-20]. In a study in Indonesia, iron tablet intake of >1 per week (60 mg iron and 250 μg folic acid) during pregnancy was associated with an increase in weight and length in the neonates [21]. However, it should be noted that maternal variables including iron tablet intake explained <10% of the association in that study.

A review of the role of iron in pregnancy outcomes identified only one study showing that iron supplementation had a positive effects on birth weight [22], whereas observational studies consistently show a U-shaped relationship between hemoglobin concentration and birth weight, duration of gestation and perinatal mortality [2; 22-25]. Our study design did not allow us to test whether iron supplementation influenced birth weight because of the lack of a placebo group. Neither iron status nor hemoglobin concentration were associated with neonatal weight or length. This may be because of the small sample size or the presence of confounding factors, such as age at neonatal measurement.

We could not assess the prevalence of low birth weight due to the relative delay in measurement of weight and length after birth. However, among 202 infants measured within 7 days, 13 (6.4%) had birth weights below 2500 g. In addition, 15 (4.4%) infants, including 2 pairs of twins and one twin, died shortly after birth.

NUTRITIONAL STATUS, FOOD INTAKE AND HOUSEHOLD CHARACTERISTICS OF PREGNANT AND LACTATING WOMEN

Women in our population had a marginal nutritional status as assessed by the proportion of body mass index ≤21.0 kg/m² of 37% at the beginning of the second trimester of pregnancy and of 52% at ~4 months postpartum. In addition, the prevalence of anemia, iron deficiency, iron deficiency anemia, and marginal vitamin A deficiency were 46%, 45%, 21% and 16.5% at second trimester of pregnancy, respectively, and 48%, 45%, 28% and 14% at ~4 months postpartum, respectively (***Chapter 5***). The nutritional status of pregnant women in our population was comparable to that reported earlier from West Java [26], but better than that reported from East Java [21; 27]. The marginal nutritional status was related to a relatively low intake of energy and nutrient (***Chapter 2 and 5***) and household characteristics reflecting poor socioeconomic status (***Chapter 5***). The socioeconomic variables, such as educational level and household possessions were associated with the nutritional status of the pregnant and lactating women (***Chapter 4 and 5***) but not with neonatal weight and length (***Chapter 4***).

Maternal status both before and during pregnancy, such as pre-pregnancy weight, pregnancy weight gain, and stature [28-30] are the main determinants of birth weight. However, the association between socioeconomic factors and pregnancy outcomes, i.e. low birth weight, is mediated through its effect on the mother [31; 32]. The recommendation to gain 6 kg weight (1 kg per month) during the second and third trimesters of pregnancy [29] was met by our population, which resulted in the satisfactory neonatal weight and length.

The actual energy and nutrient intake of women in developing countries are only two thirds of the recommended daily intake, whereas women during pregnancy tend to increase their food consumption only slightly despite the increased requirements [33]. In our study, only very few women met the recommended dietary intake. Although the method we used to assess food intake might underestimate true intake, energy intake reported in our study was comparable to that in other Indonesian studies [34-36]. The associations between energy intake and vitamin A and iron intake and between vitamin A intake with vitamin A status imply the importance of dietary approaches to improve vitamin A status. Current evidence suggested that animal foods and yellow/orange-colored vegetables and fruit are better sources of vitamin A than dark green leafy vegetables [37; 38].

CURRENT ISSUES ON ANEMIA DURING PREGNANCY

It is generally acceptable that little progress has been made in reducing the prevalence of anemia in general and iron deficiency anemia in particular despite measures being available and continuous efforts being made for more than three decades. The success of programs for the prevention of anemia during pregnancy depends not only on biological but also programmatic factors [7; 39-42]. Biologically, anemia is not only due to iron deficiency [43; 44], and iron supplementation can only reduce the prevalence of iron deficiency anemia. Programmatically, all necessary components for the functioning of the program, such as availability of the supplements, accessibility of the pregnant women to the supplements, behavior of the providers (knowledge, motivation, and supervision) and of pregnant women (knowledge and compliance) should be in place and functioning [45].

It is suggested that the cut off point for the hemoglobin concentration to define anemia should be based on functional outcomes, such as health of women or infants. The current cut off point based on the maximal hemoglobin concentration which can be achieved with iron supplementation in healthy populations might over estimate the need for iron during pregnancy leading to unnecessary administration of high dose iron supplements [40; 44]. Studies up until now have suggested that only severe anemia (hemoglobin <80 g/L) is associated with adverse outcomes, such as low birth weight [22] and maternal mortality [41]. In addition, although the prevalence of anemia is relatively constant among countries, the prevalence of severe anemia differs indicating that causes of anemia are area-specific and that there are differences in the extent of implementation of anemia control program including those based on iron and folic acid supplementation [39]. Therefore a lower cut off for hemoglobin concentration of 90 g/L for use in anemia surveillance has been proposed [39; 40]. In addition, the etiology of anemia in the population of interest needs to be re-examined [7].

In our study, half of the anemic women (both during pregnancy and lactation) were iron deficient whereas the prevalence of iron deficiency without anemia was 23%. In addition, 45% and 62% of lactating women had serum ferritin concentrations <12 µg/L and <20 µg/L, respectively, indicating that women had minimum iron stores. These findings have two important implications. Firstly, iron supplementation can only correct half of the cases of anemia. Therefore anemia prevention programs cannot depend solely on iron supplementation. Supplementation not only with iron but also with vitamin A improved hemoglobin concentrations better than did iron supplementation alone. From previous studies it is known that vitamin A contributed one-third of the improvement in hemoglobin concentrations [12]. Other causes of anemia such as infection with intestinal parasites, particularly hookworm; malaria in malaria-endemic areas; chronic infections; deficiencies of other micronutrients such as riboflavin, vitamin B12, and copper; and hemoglobinopathies such as thalassemia and sickle cell need to be sought for and, if found, quantified. Secondly, the iron stores of women need to be improved, not only in preparation for the next pregnancy but also for the well being of themselves and their children. This is achieved through improvement in work capacity and in child care. Until an adequate supply of iron from dietary sources can be achieved, weekly iron supplementation is a suitable approach to build up iron stores in women of reproductive age.

CONCLUSIONS

From the studies described in this thesis, the following conclusions can be drawn:

1. Weekly supplementation with iron and vitamin A from the second trimester of pregnancy until near term improved hemoglobin concentrations, but the improvement was not significantly different from iron supplementation alone, either weekly or daily.
2. Weekly iron supplementation during pregnancy was as effective as daily iron supplementation in improving hemoglobin concentration when compliance was good. However both approaches did not reduce the prevalence of anemia.

3. Weekly supplementation with vitamin A and iron during pregnancy prevented the decline near term of serum retinol concentrations and improved retinol concentrations in breast milk.
4. Neither weekly supplementation with vitamin A and iron or with iron alone during gestation nor maternal iron and vitamin A status during gestation influenced the neonatal weight and length.
5. Gestational age, maternal weight at the beginning of the second trimester and infant gender were the main predictors of neonatal weight and length.
6. Low maternal nutritional status (in term of body mass index) during pregnancy and lactation was associated with household characteristics reflecting low socioeconomic status. However, the household characteristics were not associated with neonatal weight and length.

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 [46; 47].

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 [44; 48]. An earlier study on iron and vitamin A supplementation carried out among anemic women, in which the supplements were administered daily but for a shorter duration, found a higher response on hemoglobin concentrations [12]. We conducted the study as close as possible to the 'real life' setting in the community, therefore women were admitted into the study irrespective of whether they were suffering from anemia 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 Marjanka K. Schmidt and also appear in her thesis [47].

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 [49]. As has been reported from other studies [45; 49; 50], indeed, the compliance was poor and the variation of 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 the "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 tablets 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.

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 vs. 5.2 ± 3.3 days, $P < 0.01$; 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.

Indonesian crises

The economic crisis in Indonesia and other Southeast Asian countries started three 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 3,845 to 17,000. In one year the per capita growth in Gross Domestic Product declined from +3.3% to –14.8% [51]. 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 [52]. 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.

GENERAL CONCLUSIONS

The present study [46; 47] 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 anemia 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 two treatment groups with respect to iron status, nutritional status, or development of infants.

Almost half of the women were anemic 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 aged 6 to 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.

IMPLICATIONS

Although the programmatic issues, such as availability of supplements, accessibility, and compliance of prenatal iron supplementation for the prevention of anemia need to be resolved further, the inclusion of vitamin A in iron supplementation should be considered. In this study, pregnant women were supplemented with 4,800 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 7,500 RE [53]. No congenital malformations were observed in any of the infants born during the study. In a study in Nepal [14] using a dose of 7,000 RE, no malformations were reported neither. The delivery of vitamin A and iron supplements should be incorporated in a safe motherhood program, which among other measures to improve the program 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 [46]). 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 anemia among pregnant women is not an alternative solution to overcoming the low effectiveness of the current iron supplementation program. In order to prevent anemia 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 anemia should be elucidated and quantified.

Immunization 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 immunization 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.

FUTURE RESEARCH

In order to elucidate the role of iron and vitamin A in fetal 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 respective nutrient, the content of 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 assessable foods rich in iron and vitamin A, or food enrichment by plant breeding or fortification, need to be addressed.

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Annex

Bioavailability of Iron from Iron plus Vitamin A Tablets Relative to that from an Aqueous Iron Solution

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ABSTRACT

Background. Iron supplementation is the most common method of preventing iron deficiency anemia. It is much more effective in vitamin A deficient subjects when vitamin A supplements are also provided. Thus we have measured the bioavailability of iron from tablets containing iron, folic acid and vitamin A relative to an aqueous solution containing iron.

Methods. Five apparently healthy female subjects each received 2 tablets each containing ferrous sulfate (60 mg Fe)/folic acid and vitamin A or an aqueous preparation of ferrous sulfate (120 mg Fe) in a randomized study with crossover design with a one-week washout period between the two study periods. Serum iron concentrations were measured immediately before and 2, 4, 6, and 8 hours after dosing.

Results. The maximum serum iron concentration and time to reach the peak serum iron concentration after ingestion of the tablets did not differ from when the aqueous preparation was ingested. The mean bioavailability of iron from the tablets was 116% relative to that from the aqueous solution.

Conclusion. The bioavailability of iron from the tablets containing iron, folic acid and vitamin A was similar to that from the aqueous solution.

The importance of vitamin A in iron metabolism was recognized by Hodges et al. [1] by showing that vitamin A-depleted subjects developed anemia did not respond to iron treatment. Further studies revealed that vitamin A deficiency led to the failure of iron mobilization from the body stores into the circulation and thus into hematopoietic tissues [2; 3].

In Indonesia, studies showed that almost half of pregnant women suffer from iron deficiency anemia [4] and that one fifth of them suffer from marginal vitamin A deficiency [5]. In pregnant women both iron and vitamin A status had been shown to improve after daily supplementation of iron and vitamin A [6; 7], whereas hemoglobin concentration increases with weekly supplementation of iron and vitamin A [8].

Anemia is prevalent both in the developing and developed countries. It affects more than half of women and children with South East Asia having the highest prevalence [9]. The most common cause of anemia is iron deficiency [10]. Programs in which pregnant women are advised to take iron supplement daily are common in most countries, including Indonesia. Among the important factors that influence the success of iron supplementation programs is the bioavailability of iron in the preparations used. The bioavailability of iron from iron tablets used in supplementation programs is high [11].

We conducted a community-based study to investigate the effect of weekly vitamin A and iron supplementation during pregnancy on infant growth in the first year of life [8]. As a prerequisite of the study, we evaluated the bioavailability of iron from the tablets containing iron, vitamin A, and folic acid relative to that from an aqueous solution containing ferrous sulfate. The tablets had the same physical appearance and iron/folic acid content with the iron tablets used for the ongoing national iron supplementation program in Indonesia and were produced by the same manufacturer.

SUBJECTS AND METHODS

Subjects. The bioavailability study was performed in 5 apparently healthy non-pregnant, female volunteers in October 1998. Prior to the study, all subjects received detailed information on the objectives and nature of the study and each participant provided a written informed consent. The study protocol was approved by the Ethical Committee of the South East Asian Ministers of Education Organization/Tropical Medicine, Jakarta, Indonesia.

Study design. A crossover study design with a one-week washout period in between was used. On the first day, 3 volunteers were allocated randomly to receive 2 iron plus vitamin A tablets each containing 60 mg elemental iron in the form of $Fe_2SO_4.7H_2O$, 250 µg folic acid, and 10,000 IU (3,000 RE) vitamin A supplied by PT Kimia Farma, Indonesia. The other 2 volunteers received an aqueous solution containing 120 mg elemental iron (597 mg $Fe_2SO_4.7H_2O$). After receiving the iron plus vitamin A tablets or the aqueous iron solution, the volunteers were asked to drink 200 mL tap water. On the second test day, subjects were crossed over to receive the other preparation vice versa. The subjects received the tablets or iron solution at 08.00. Twelve hours prior to the beginning of the study, the volunteers were requested to fast and drink only tap water to minimize the variation of serum iron concentration. On the day of the experiment, the volunteers received a standardized meal comprising white bread, butter, fruit jam (pineapple, strawberry, orange, mixed fruit) and water, started 2 hours after dosing.

Samples of venous blood (5 mL) were drawn by a trained technician to assess hemoglobin concentration before and serum iron concentrations before and 2, 4, 6, and 8 hours after dosing. Hemoglobin concentration was measured in duplicate by the cyanmethemoglobin method (Merck test 3317; Merck, Darmstadt, Germany), while serum iron concentration was measured by the Ferrozin method (Merck test 12978, Merck, Darmstadt, Germany) directly after blood was collected. All examinations were carried out at the laboratory of the South East Asian Ministers of Education Organization/Tropical Medicine, Jakarta, Indonesia.

The area under the curve (AUC) was calculated from the serum iron concentrations at the various times after dosing. The serum iron concentration before dosing (0 hour) was considered as the baseline level. The relative bioavailability of iron in the tablets was determined by comparing the area under the time-concentration curve of serum iron 8 hours after administration of the tablets with that of after administration of the aqueous iron solution.

Statistical analysis. The area under the curve (AUC) was calculated using GraphPad Prism (GraphPad Software Inc. San Diego, CA). Data processing and analysis were carried out using SPSS software package (Windows version 7.5.2. SPSS, Chicago, IL). Paired t-test was used to determine within subject differences of serum iron concentration after receiving the tablets and aqueous iron solution. It was also used to test the significance of the difference of the time-concentration curves (AUC), maximum serum iron concentration (C-max), and times to reach C-max between the two preparations.

TABLE 1

Characteristics of volunteers participating in the study

Subject	Age (y)	Weight (kg)	Height (cm)	Hemoglobin (g/L)	
				1st test day	2nd test day
1	26	56.0	154.6	121.4	123.4
2	29	64.8	163.2	135.4	134.7
3	36	58.8	160.0	115.5	121.0
4	28	55.6	156.7	127.3	125.1
5	24	43.0	157.0	122.5	124.5
Mean	28.6	55.6	158.3	124.4	125.7
SD	4.56	8.0	3.3	7.4	5.2

RESULTS

Characteristics of the volunteers are presented in **Table 1**. One subject had a hemoglobin concentration <120 g/L, the cut off point for anemia, on the first day, but not the second day. The hemoglobin concentrations did not differ between the first and second test day. The changes in serum iron concentrations after the ingestion of the tablets did not differ from those after the ingestion of the aqueous iron solution. The serum iron concentrations started to decrease 4 hours after the ingestion of tablets or aqueous solution. Between the tablets and aqueous solution, there were no differences in the maximum concentration of serum iron (3.86 ± 0.79 vs. 4.12 ± 1.45 g/L, respectively) and the time to reach the peak of serum iron concentrations

(3.2 ± 1.7 vs. 3.2 ± 1.9 hours, respectively). On the basis of combining the individual areas under the curve, the relative bioavailability ranged between 54% and 176%, with a mean of 116% (**Table 2**).

TABLE 2

Relative bioavailability of iron from the tablets compared to that from an aqueous solution.

Subject	Area under the curve (g/L x h) Iron plus Vitamin A tablets	Area under the curve (g/L x h) Aqueous iron solution	Relative bioavailability (%)
1	9.6	5.6	176
2	19.4	14.8	131
3	14.0	26.2	54
4	23.8	16.1	148
5	21.8	31.2	70
Mean	17.8	18.8	116
SD	5.8	10.1	52
Median	19.4	16.1	131
Minimum	9.8	5.6	54
Maximum	23.8	31.2	176

DISCUSSION

Based on the area under the time concentration curve, our study showed that the bioavailability of iron from the iron/folic acid/vitamin A tablets was comparable to that from the aqueous ferrous sulfate preparation. In addition, the maximum concentration of serum iron and the time to reach the peak of serum iron concentration did not differ between the two preparations. It should be noted that our study had only a small number of volunteers which could not circumvent the large between-subject variation after iron ingestion. Such variation is commonly observed and can be attributed, at least in part, to differences in iron status [12].

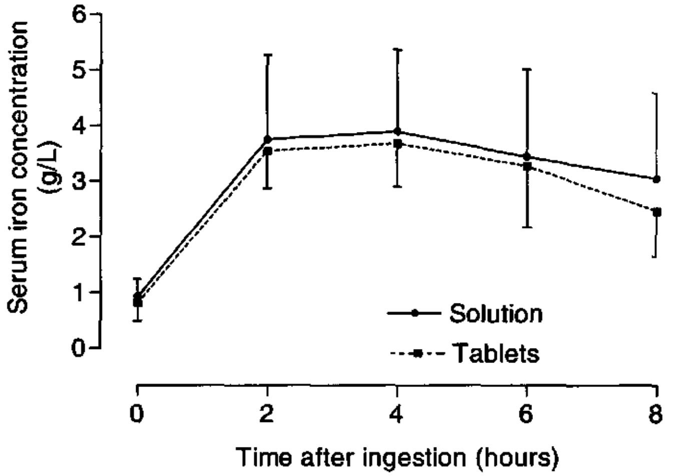

Figure 1. Serum iron concentration after the ingestion of tablets containing ferrous sulfate (120 mg Fe), folic acid and vitamin A (6,000 RE) (■) and an aqueous solution containing ferrous sulfate (120 mg Fe) (•). The vertical bars represent SD.

Schümann et al. [12] has argued that measurements of plasma iron concentrations to assess iron bioavailability will result in erroneous conclusions because plasma iron concentrations are influenced greatly by shifts of iron between the erythron and the iron storage compartment as well as by the rate of intestinal absorption which is regulated closely. Thus, it has been recommended that the rate of increase in hemoglobin concentration should be measured as this represents the metabolic utilization of iron [13]. We did not measure hemoglobin concentration as the endpoint measurement because of the time required for ingestion of iron to have an effect on hemoglobin concentrations.

The reasons for using an aqueous iron sulfate solution as reference were the high bioavailability and their frequent use by others making it possible to compare the results with other studies [11; 14]. Thorand et al. [11] has reported that the tablets used in governmental iron supplementation program in Indonesia are efficacious as shown by 116% relative bioavailability of iron. The tablets containing vitamin A used were produced by the same company and had similar physical appearance and iron/folic acid content as the tablets used in the government program. The vitamin A appeared not to impaired iron bioavailability and may have even improved it.

Iron absorption from rice, corn, and wheat meals doubled when vitamin A was provided in a study in Venezuela in which isotopically-labelled iron was administered and measured in the blood 15 days after ingestion [15]. The authors hypothesized that vitamin A binds iron liberated during the digestive process and forms a complex preventing the inhibitory effect of phytates and polyphenols on non-heme iron absorption.

Besides its role in immunity, growth and vision [16], vitamin A has been shown to have a relationship with iron nutrition [1; 3]. In areas with vitamin A deficiency, iron supplementation alone might be less effective than combined iron and vitamin A supplementation in preventing iron deficiency anemia. Indonesian anemic pregnant women have been shown to become non-anemic after daily vitamin A and iron supplementation [6]. We demonstrated increased hemoglobin concentrations among pregnant women after weekly supplementation with vitamin A and iron [8].

For further study examining the iron bioavailability in a combined supplement with vitamin A, we recommend to take into account the number of subjects and the homogeneity of the iron status and physiological condition of the subjects [12] and with a better method, such as a method based on the use of stable isotopes. While adding vitamin A did not impair iron bioavailability, further study is needed to examine the bioavailability of vitamin A in the presence of iron. We observed that vitamin A content in the iron plus vitamin A tablet decreased after 1.5 years of storage [8]. We have shown that the iron bioavailability was not impaired when vitamin A was added in the iron supplement. Aside from being more practical in term of logistic, administration, and monitoring, supplements containing iron and vitamin A will be more effective in preventing anemia.

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Summary

Many preschoolers are stunted and the process starts as early as 3 months after birth, or even in utero. Stunting is associated with maternal nutrition as well as postnatal factors such as the quality of care and living conditions rather than with genetic background. Nutrition during pregnancy is important for women's health, outcome of pregnancy and child survival. Therefore, improving nutritional status of women during pregnancy will not only benefit them, but also optimize the growth in utero and postnatally of their infants.

In most cases, micronutrient deficiencies share the same etiology; deficiency of one micronutrient is often associated with deficiencies of others; and the deficiency of one micronutrient can exacerbate the deficiency of another. Thus micronutrient deficiencies often occur concomitantly. In Indonesia, more than half of pregnant women suffer from iron deficiency anemia and one third of pregnant and lactating women are marginally vitamin A deficient. Both iron and vitamin A deficiencies may have a negative influence on pregnancy outcomes and child growth.

National iron supplementation programs are common practice where the prevalence of iron deficiency anemia is high, particularly in developing countries such as Indonesia. However, it is generally accepted that little progress has been made on the reduction of anemia and iron deficiency anemia, particularly among pregnant women. Weekly iron supplementation has been proposed as the method of choice for providing iron supplements. With the assumption that it is as effective as daily supplementation, weekly supplementation with iron offers potential advantages on the reduction of side effects due to lower frequency of iron ingestion and hence improvement of compliance.

From November 1997 until November 1999, we conducted a community-based study with the aim of investigating the effect of weekly vitamin A and iron supplementation during pregnancy on infant growth in the first year of life. An additional aim of the study was to investigate whether weekly iron supplementation was as effective as the ongoing national iron supplementation program in improving iron status. The study was conducted in 9 villages in Leuwiliang subdistrict, Bogor district, West Java, Indonesia among women who were 16 – 20 weeks pregnant, aged 17 – 35 years, parity <6, and with hemoglobin concentrations 80 – 140 g/L. Initially, 366 pregnant women were recruited between November 1997 and May 1998. Women from 5 villages were randomly allocated on an individual basis to receive a weekly

supplement either with 120 mg iron as Fe_2SO_4 and 500 μg folic acid (n = 121) or the same amount of iron and folic acid plus 4,800 RE vitamin A (n = 122). The supplement intake was supervised by volunteer health workers. A third group participating in the ongoing national iron supplementation program in which women are advised to take iron tablets daily during pregnancy ("daily" group) was recruited at the same time from 4 neighboring villages (n = 123). The iron-folic acid tablet intake in the "daily" group was not supervised and the adherence of iron tablet intake was assessed through interview at the postnatal home visit. Subjects were considered to have dropped out when they withdrew from the study, moved from the research area, had twins, or when their infant died but not when they did not attend. In this thesis, outcomes from the study on mothers and neonates are described, while the thesis entitled "***The role of maternal nutrition in growth and health of Indonesians infants: a focus on vitamin A and iron***" by Marjanka K. Schmidt presents outcomes on infants' growth, morbidity and development.

Of the 366 subjects initially enrolled, 190 subjects had complete biochemical data both at baseline (~18 week of pregnancy) and near term (35 week of pregnancy). The median iron tablets intake in the "daily" group was 50. Only 17% of the subjects took ≥90 and 43% took <30 tablets. The iron status of pregnant women near term in the group supplemented weekly with iron was not different from the "daily" group (***Chapter 2***). However, iron status decreased with daily iron supplementation if <50 iron tablets were ingested. The hemoglobin concentrations in the group supplemented weekly with iron and vitamin A increased from 110.1 ± 1.4 g/L to 114.0 ± 1.4 g/L (mean ± SEM), but the increase was not significantly different from that in group supplemented weekly with iron alone or from the "daily" group. Among anemic subjects, the increase of hemoglobin concentration in the weekly iron and vitamin A group was significantly higher than in the weekly iron group (10.7 ± 2.3 g/L, n = 35 vs. 6.6 ± 2.3 g/L, n = 29; $P < 0.05$). Serum ferritin concentrations decreased significantly in the weekly iron and vitamin A group, suggesting that vitamin A improved utilization of iron for hematopoiesis. The concentrations of serum transferrin receptor increased significantly in all groups, which might reflect both the increased hematopoiesis and tissue iron depletion as gestation proceeded. At baseline, the serum retinol concentrations did not differ among the three groups. However, it remained constant in the weekly iron and vitamin A group (0.97 ± 0.03 μmol/L) but decreased significantly in the other two groups (0.88 ± 0.04 μmol/L and 0.79 ± 0.07 μmol/L in the weekly iron group and "daily" group, respectively).

Complete biochemical data of women ~4 months postpartum from the two weekly groups was available from 170 subjects (***Chapter 3***). Compared with the weekly iron group (n = 88), the weekly iron and vitamin A group (n = 82) had significantly fewer ($P < 0.01$) subjects with serum retinol concentration ≤0.70 µmol/L (18 vs. 5%) and tended to have fewer ($P = 0.066$) subjects with serum retinol concentration ≤1.05 µmol/L (52% vs. 38%). The iron status of women in the weekly iron and vitamin A group did not differ from that of women in the weekly iron group. The concentrations of iron and retinol in transitional milk (4 – 7 days postpartum) was almost double than that in mature milk (3 months postpartum), whereas fat concentration did not differ. The proportions of all subjects having concentrations of retinol in mature milk ≤1.05 µmol/L and ≤0.028 µmol/g fat were 51 and 21%, respectively. Compared with the weekly iron group, the weekly iron and vitamin A group had significantly higher ($P < 0.05$) concentrations of retinol in transitional milk [2.29 (1.80 – 1.22) vs. 3.37 (2.47 – 4.26) µmol/L; mean (95% CI)] and in mature milk [0.044 (0.037 – 0.052) vs. 0.053 (0.044 – 0.063) µmol/g fat].

As many as 326 singleton infants were born, of which weight and length measured within 15 days after birth were available from 296 infants (91%) (***Chapter 4***). Neonatal weight (3094 ± 440 g) and length (49.1 ± 2.0 cm) did not differ among the three groups and boys were 100 g heavier and 0.53 cm longer than girls. Iron and vitamin A status during pregnancy did not influence the neonatal weigh and length. Gestational age, maternal weight at the beginning of the second trimester and infant gender were the main predictors of neonatal weight and length. Pregnancy weight gain (0.29 ± 0.14 kg/week) was positively associated with the neonatal weight and length.

The proportion of women with a body mass index ≤21.0 kg/m^2 was 37% at the beginning of the second trimester of pregnancy and 52% at ~4 months postpartum. In addition, the prevalence of anemia (hemoglobin concentration <110 g/L during pregnancy and <120 g/L postpartum), iron deficiency (serum ferrition concentration <12 µg/L), iron deficiency anemia, and marginal vitamin A deficiency (serum retinol concentration <0.70 µmol/L) were 46%, 45%, 21% and 16.5% at the beginning of the second trimester of pregnancy, respectively, and 48%, 45%, 28% and 14% at ~4 months postpartum, respectively (***Chapter 5***). Low nutritional status of the women was associated with household characteristics reflecting a lower socioeconomic status, such as river/pond as place of defecation and did not possess bicycle, motorcycle or a set of chairs. Energy intake was associated

with some household characteristics as well, such as husband's occupation and possession of television, motorcycle, or a set of chairs, but energy intake was not associated with nutritional status. However, vitamin A intake above the median (>270 RE/day) was associated with lower prevalence of marginal vitamin A deficiency (10% vs. 24%, $P < 0.05$). Possession of television was associated with lower proportion of iron deficiency during pregnancy and postpartum. Across groups and time of assessments, other household characteristics were not consistently associated with prevalence of anemia, iron deficiency, or marginal vitamin A deficiency.

In conclusion, compared with weekly supplementation with iron alone, supplementation with vitamin A and iron given at the time when women entered their second trimester of pregnancy prevented the deterioration of vitamin A status near term and ~4 month postpartum, and increased retinol concentration in breast milk. Weekly supplementation with iron and vitamin A improved the hemoglobin concentration but did not decrease the prevalence of anemia near term. The performance of weekly iron supplementation did not differ from the ongoing daily iron supplementation program in improving the iron status during pregnancy and lactation. The prevalence of anemia during pregnancy and lactation remained >40% despite iron supplementation either with or without vitamin A. The intervention did not influence the neonatal weight and length. The gestational age, maternal weight at the beginning of the second trimester and infant gender were the main predictors of neonatal weight and length. Women from households reflecting higher socioeconomic characteristics had better nutritional status. The delivery of iron-folic acid supplement on a weekly basis for the prevention of anemia among pregnant women is not an alternative solution to overcome the low effectiveness of currently ongoing iron supplementation. For anemia and iron deficiency prevention program during pregnancy, daily iron supplementation program need to be continued provided that the compliance is improved and side effects due to iron tablet ingestion are reduced. In addition to that, other causes of anemia need to be elucidated and quantified. It is recommended to include vitamin A in the iron supplementation program.

Samenvatting

Veel kinderen die nog niet naar school gaan zijn te klein voor hun leeftijd (*'stunted'*). Dit proces begint al 3 maanden na de geboorte, of zelfs al in de baarmoeder. *Stunting* is geassocieerd met zowel de voeding van de moeder als met postnatale factoren, zoals de kwaliteit van zorg en leefomstandigheden, en niet zozeer met de genetische achtergrond. De voeding tijdens de zwangerschap is belangrijk voor de gezondheid van de moeder, voor het ter wereld brengen van een gezond kind (zwangerschapsresultaat) en voor de overleving van het kind. Daarom zal een verbetering in de voedingstoestand van vrouwen tijdens de zwangerschap niet alleen ten goede komen aan de vrouwen zelf, maar ook de groei van hun kind, bevorderen, zowel voor als na de geboorte.

Tekorten aan micronutriënten in de voeding (deficiënties) hebben in de meeste gevallen eenzelfde etiologie: deficiëntie aan het ene micronutriënt gaat vaak samen met een deficiëntie aan een andere. Ook kan een deficiëntie aan het ene micronutriënt de deficiëntie aan een andere verergeren. Deficiënties aan verschillende micronutriënten gaan dus vaak samen. In Indonesië lijdt meer dan de helft van de zwangere vrouwen aan bloedarmoede door ijzerdeficiëntie en meer dan een derde van de zwangere en lacterende vrouwen heeft een marginale vitamine A-deficiëntie. Zowel ijzer- als vitamine A-deficiëntie zouden een negatieve invloed kunnen hebben op het zwangerschapsresultaat en op de groei van het kind.

Op plaatsen waar de prevalentie van bloedarmoede door ijzerdeficiëntie hoog is, zoals in ontwikkelingslanden als Indonesië, zijn vaak nationale suppletieprogramma's voor ijzer opgezet. In het algemeen is men het er echter over eens dat er nog maar weinig vorderingen zijn gemaakt in het terugdringen van bloedarmoede door ijzerdeficiëntie, in het bijzonder onder zwangere vrouwen. Wekelijkse toediening van ijzersupplementen wordt naar voren gebracht als de beste methode om in de behoefte aan ijzer te voorzien. Aangenomen dat wekelijkse suppletie even effectief is als dagelijkse suppletie, zou wekelijkse suppletie als voordeel hebben dat er zich minder bijwerkingen voordoen vanwege de lagere frequentie van ijzerinname. Dit zou de innametrouw van de ijzersupplementen kunnen verbeteren.

Van november 1997 tot november 1999 hebben wij in een veldstudie het effect onderzocht van wekelijkse ijzer- en vitamine A-suppletie tijdens de zwangerschap op de groei van het kind in het eerste levensjaar. Daarnaast hebben wij bestudeerd of wekelijkse ijzersuppletie net zo effectief was in het verbeteren van de ijzerstatus als het nationale ijzersuppletieprogramma. Het onderzoek werd uitgevoerd onder zwangere vrouwen in 9 dorpen in het subdistrict Leuwiliang, Bogor, West Java, Indonesië. De vrouwen waren 16 – 20 weken zwanger, hun leeftijd was 17 – 35 jaar, ze hadden minder dan 6 kinderen gebaard en hun hemoglobine concentraties waren 80 – 140 g/L. Aanvankelijk werden 366 zwangere vrouwen voor het onderzoek gerekruteerd tussen november 1997 en mei 1998. De vrouwen uit 5 dorpen werden aselect toegewezen aan wekelijkse suppletie met 120 mg ijzer als Fe_2SO_4 en 500 µg foliumzuur (n=121), of aan wekelijkse suppletie met dezelfde hoeveelheden ijzer en foliumzuur plus 4.800 RE vitamine A (n=122). Vrijwillige gezondheidswerkers hielden toezicht op de inname van de supplementen. Een derde onderzoeksgroep werd gevormd door zwangere vrouwen uit 4 dorpen in de buurt (n=123) die deelnamen aan het lopende nationale ijzersuppletieprogramma, waarbij de vrouwen worden geadviseerd om tijdens de zwangerschap dagelijks ijzertabletten in te nemen ("dagelijkse" groep). In deze groep werd geen toezicht gehouden op het innemen van de ijzer-foliumzuurtabletten, maar de inname werd naderhand nagevraagd door middel van een interview tijdens het huisbezoek na de bevalling. Personen die tijdens het onderzoek uitvielen waren vrouwen die zich uit het onderzoek terugtrokken, uit het onderzoeksgebied verhuisden, een tweeling kregen of wanneer hun kind was overleden. Vrouwen die een keer niet aanwezig waren bij de metingen werden niet als uitvallers beschouwd. In dit proefschrift wordt het onderzoek naar de moeders en pasgeborenen beschreven, terwijl in het proefschrift met als titel **"The role of maternal nutrition in growth and health of Indonesian infants: a focus on vitamin A and iron"** door Marjanka K. Schmidt het onderzoek naar de groei, ziekte en ontwikkeling van de kinderen wordt beschreven.

Van de 366 deelnemers die aanvankelijk aan het onderzoek begonnen zijn waren uiteindelijk van 190 deelnemers alle biochemische data verzameld, zowel aan het begin van het onderzoek (~18e zwangerschapsweek) als vlak voor de bevalling (35e zwangerschapsweek). De mediane inname van ijzertabletten in de "dagelijkse" groep was 50; slechts 17% van de deelnemers namen ≥90 en 43% nam <30 tabletten in. Vlak voor de bevalling was er geen verschil in ijzerstatus tussen de groep die wekelijks supplementen innam en de "dagelijkse" groep (***Hoofdstuk 2***). Echter, de ijzerstatus in de "dagelijkse" groep nam af wanneer <50 tabletten waren ingenomen. De haemoglobineconcentratie in de groep die wekelijks ijzer en vitamine A kreeg, steeg van 110,1 ± 1,4 g/L tot 114,0 ± 1,4 g/L (gemiddelde

± SEM), maar deze stijging was niet significant verschillend van de stijging in de groep die wekelijks alleen ijzer kreeg, of van die in de "dagelijkse" groep. Onder de vrouwen die al bloedarmoede hadden, was de stijging in de haemoglobineconcentratie in de groep die wekelijks zowel ijzer als vitamine A kreeg groter dan in de groep die wekelijks alleen ijzer kreeg (10,7 ± 2,3 g/L, n=35 vs. 6,6 ± 2,3, n=29; P<0,05). De ferritineconcentratie in serum daalde significant in de groep die wekelijks zowel ijzer als vitamine A kreeg, hetgeen suggereert dat vitamine A het gebruik van ijzer in de aanmaak van bloedcellen verbeterde. De concentraties van transferrinereceptor in serum namen significant toe in alle groepen, wat te maken zou kunnen hebben met de toenemende aanmaak van bloedcellen en een toenemende uitputting van ijzer in weefsels tijdens de zwangerschap. Aan het begin van het onderzoek was er geen verschil in de concentratie van retinol in het serum tussen de groepen. De groep die wekelijks zowel ijzer als vitamine A kreeg, was de enige waarin de retinolconcentratie in het serum constant bleef (0,97 ± 0,03 µmol/L); in de andere twee groepen daalde deze significant (respectievelijk 0,88 ± 0,04 µmol/L in de groep die wekelijks ijzer kreeg en 0,79 ± 0,07 µmol/L in de "dagelijkse" groep).

Van 170 vrouwen in de beide wekelijkse suppletiegroepen werden ongeveer 4 maanden na de geboorte van hun kind opnieuw biochemische gegevens verzameld (***Hoofdstuk 3***). Vergeleken met de groep die wekelijks alleen ijzer kreeg (n=88), waren er in de groep die wekelijks zowel ijzer als vitamine A kreeg (n=82) minder vrouwen met serum retinolconcentraties ≤0,70 µmol/L (18% vs. 5%, P< 0,01) en was er in die groep een tendentie naar minder vrouwen met serum retinolconcentraties ≤1,05 µmol/L (52% vs. 38%, P=0.066). De ijzerstatus van de vrouwen in de groep die zowel ijzer als vitamine A kreeg, verschilde niet van die in de groep die alleen ijzer kreeg. De ijzer- en retinolconcentraties in de overgangsmelk (4 – 7 dagen na de bevalling) was bijna het dubbele van dat in rijpe moedermelk (3 maanden na de bevalling) zonder verschil in vetconcentraties. Van alle vrouwen had 51% een retinolconcentratie ≤1,05 µmol/L in de rijpe moedermelk, en bij 21% was deze concentratie ≤0,028 µmol/g vet. Vergeleken met de groep die alleen ijzer kreeg, had de groep die zowel ijzer als vitamine A kreeg hogere retinolconcentraties in de overgangsmelk [2,29 (1,80 – 1,22) vs. 3,37 (2,47 – 4,26) µmol/L; gemiddelde (95% CI)] en ook in de rijpe melk [0,044 (0,037 – 0,052) vs. 0,053 (0,044 – 0,063) µmol/g vet].

In totaal werden 326 eenlingen geboren en bij 296 van deze kinderen (91%) werd binnen 15 dagen na de geboorte gewicht en lengte bepaald

(***Hoofdstuk 4***). Gewicht (3094 ± 440 g) en lengte (49,1 ± 2,0 cm) van de pasgeborenen verschilde niet tussen de drie groepen. Jongetjes waren gemiddeld 100 g zwaarder en 0,53 cm langer dan meisjes. De ijzer- en vitamine A-status van de vrouwen tijdens de zwangerschap was niet van invloed op gewicht en lengte van de pasgeborenen. De belangrijkste voorspellers van geboortegewicht en -lengte waren de hoeveelheid doorgemaakte zwangerschappen, gewicht van de moeder aan het begin van het tweede trimester en het geslacht van het kind. De gewichtstoename tijdens de zwangerschap (0,29 ± 0,14 kg/ week) was positief geassocieerd met geboortegewicht en -lengte.

Het percentage vrouwen met een Quetelet Index ≤21,0 kg/m^2 was aan het begin van het tweede trimester van de zwangerschap 37% en ongeveer 4 maanden na de bevalling 52%. De prevalentie van bloedarmoede (haemoglobineconcentraties <110 g/L tijdens zwangerschap en <120 g/L na de bevalling), ijzerdeficiëntie (serum ferritineconcentraties <12 µg/L), bloedarmoede door ijzergebrek en marginale vitamine A deficiëntie (serum retinolconcentratie <0,70 µmol/L) waren respectievelijk 46%, 45%, 21% en 16,5% aan het begin van het tweede trimester van de zwangerschap, en respectievelijk 48%, 45%, 28% en 14% ongeveer 4 maanden na de bevalling (***Hoofdstuk 5***). Een slechte voedingsstatus van de vrouwen was geassocieerd met huishoudkarakteristieken die een lage sociaal-economische reflecteren, zoals gebruik van rivier of vijver als toilet en het niet bezitten van een fiets, brommer of zitmeubels. De inname van energie was ook geassocieerd met bepaalde huishoudkarakteristieken, zoals de baan van de echtgenoot en het bezit van een televisie, brommer of zitmeubels, maar de inname van energie was niet geassocieerd met voedingsstatus. Inname van vitamine A boven de mediaan (>270 RE/dag) was geassocieerd met een lagere prevalentie van vitamine A deficiëntie (10% vs. 24%, $P<0,05$). Het bezit van een televisie was geassocieerd met een lager percentage ijzerdeficiëntie tijdens en na de zwangerschap. Door de groepen en tijdstippen van bepalingen heen, werden verder geen andere consistente verbanden gevonden tussen huishoudkarakteristieken en de prevalentie van bloedarmoede, ijzer- en vitamine A-deficiëntie.

Concluderend, vergeleken met wekelijkse suppletie met alleen ijzer kan suppletie met zowel ijzer als vitamine A vanaf het tweede trimester van de zwangerschap een verslechtering van de vitamine A status tot kort voor de bevalling en tot ongeveer 4 maanden na de bevalling voorkomen en de retinolconcentratie in moedermelk verhogen. Wekelijkse suppletie met zowel ijzer als vitamine A verbeterde de haemoglobineconcentratie, maar verlaagde niet de prevalentie van bloedarmoede vlak voor de bevalling. Het resultaat van wekelijkse ijzersuppletie was niet anders dan van het lopende, dagelijkse, ijzersuppletieprogramma voor wat betreft het verbeteren van de ijzerstatus tijdens zwangerschap en lactatie. De prevalentie van bloedarmoede tijdens zwangerschap en lactatie bleef >40% ondanks ijzersuppletie met of zonder vitamine A. De interventie had geen invloed op geboortegewicht en -lengte van de pasgeborenen. De hoeveelheid doorgemaakte zwangerschappen, gewicht van de moeder aan het begin van het tweede trimester en het geslacht van het kind waren de belangrijkste voorspellers van geboortegewicht en -lengte. Vrouwen uit huishoudens met karakteristieken die een hogere sociaal-economische status aangeven, hadden een betere voedingsstatus. Wekelijkse suppletie met een ijzer-foliumzuur supplement is geen alternatieve oplossing voor de lage effectiviteit van het huidige ijzersuppletieprogramma voor de preventie van bloedarmoede onder zwangere vrouwen. Het dagelijkse ijzersuppletieprogramma moet gecontinueerd worden op voorwaarde dat de innametrouw van de ijzertabletten wordt verbeterd en de bijwerkingen worden verminderd. In aanvulling daarop moeten andere oorzaken van bloedarmoede worden opgehelderd en gekwantificeerd. Opname van vitamine A in het ijzersuppletieprogramma is aan te bevelen.

Ringkasan

Banyak anak prasekolah yang *stunted* (kurang tinggi menurut umur) dan proses tersebut telah dimulai sejak usia 3 bulan, bahkan mungkin sejak di dalam kandungan. *Stunting* lebih berhubungan dengan keadaan gizi ibu dan faktor di luar kandungan seperti kualitas pengasuhan dan keadaan lingkungan hidup daripada hanya faktor keturunan. Gizi selama kehamilan penting untuk kesehatan ibu, kualitas kehamilan, dan keselamatan hidup bayi. Oleh karena itu, perbaikan keadaan gizi ibu selama kehamilan tidak hanya akan bermanfaat untuk ibu tersebut, tetapi juga untuk mengoptimalkan pertumbuhan bayi mereka di dalam dan di luar kandungan.

Pada beberapa kasus, defisiensi gizi mikro mempunyai penyebab yang sama; defisiensi gizi mikro yang satu sering berhubungan dengan defisiensi gizi mikro lainnya; dan defisiensi gizi mikro yang satu dapat memperburuk defisiensi gizi mikro yang lainnya. Oleh karena itu, defisiensi gizi mikro sering terjadi bersamaan waktunya. Di Indonesia, lebih dari separuh ibu-ibu hamil menderita anemia defisiensi besi dan sepertiga dari ibu-ibu hamil dan menyusui mempunyai status vitamin A yang tidak adekuat. Defisiensi besi dan vitamin A mungkin mengakibatkan pengaruh negatif terhadap kualitas kehamilan dan pertumbuhan bayi.

Program nasional suplementasi zat besi umum dilaksanakan di daerah-daerah dengan prevalensi anemia defisiensi besi-nya tinggi, terutama di negara-negara berkembang seperti Indonesia. Akan tetapi, umumnya disepakati bahwa tidak banyak kemajuan yang telah dicapai dalam penurunan prevalensi anemia dan defisiensi besi, terutama pada ibu hamil. Suplementasi besi secara mingguan telah diusulkan sebagai salah satu cara penyediaan suplemen besi. Dengan asumsi mempunyai efektifitas yang sama dengan suplementasi harian, suplementasi mingguan besi menawarkan keuntungan dalam hal penurunan efek samping karena bekurangnya frekuensi minum tablet besi yang pada akhirnya akan memperbaiki kepatuhan dalam minum tablet besi.

Dari bulan November 1997 hingga November 1999, sebuah penelitian di masyarakat telah dilaksanakan yang bertujuan untuk meneliti pengaruh suplementasi mingguan vitamin A dan besi selama kehamilan terhadap pertumbuhan anak selama satu tahun pertama. Tujuan lain dari penelitian

ini adalah untuk mengetahui apakah suplementasi besi secara mingguan sama efektifnya dengan program nasional suplementasi besi dalam perbaikan status besi. Penelitian dilaksanakan di 9 desa di kecamatan Leuwiliang, kabupaten Bogor, Jawa Barat, Indonesia terhadap ibu-ibu yang hamil 16 - 20 minggu, berumur 17 – 35 tahun, melahirkan <6 kali, dan mempunyai konsentrasi hemoglobin 80 – 140 g/L. Pada awalnya, 366 ibu hamil terdaftar sebagai subyek dalam penelitian yang pendaftarannya dilakukan antara bulan November 1997 sampai dengan bulan Mei 1998. Ibu-ibu dari 5 desa dikelompokkan secara acak per orang dalam dua grup untuk menerima suplementasi mingguan yang mengandung 120 mg besi sebagai Fe_2SO_4 (besi sulfat) dan 500 µg asam folat (n = 121) atau besi dan asam folat dengan kandungan yang sama ditambah dengan 4,800 RE vitamin A (n = 122). Konsumsi suplemen diawasi oleh kader. Kelompok ketiga yang berpartisipasi dalam program nasional suplementasi besi, yaitu setiap ibu hamil disarankan untuk minum tablet besi setiap hari selama kehamilan (kelompok "harian") diikutkan dalam penelitian pada saat yang bersamaan dari 4 desa lainnya (n = 123). Konsumsi tablet besi-asam folat di kelompok "harian" tidak diawasi, dan kepatuhan minum tablet besi diperiksa melalui wawancara pada saat kunjungan pasca melahirkan. Subyek dianggap drop out ketika mereka mengundurkan diri dari penelitian, pindah dari lokasi penelitian, mempunyai bayi kembar, atau ketika bayinya meninggal, akan tetapi tidak dianggap drop out apabila mereka tidak datang pada saat pemeriksaan. Tesis ini menampilkan hasil-hasil penelitian pada ibu dan neonatus, sedangkan tesis yang berjudul **"The role of maternal nutrition in growth and health of Indonesian infants: a focus on vitamin A and iron"** oleh Marjanka K. Schmidt menampilkan hasil-hasil penelitian pada pertumbuhan, kesakitan dan perkembangan bayi.

Dari 366 subyek yang pada awalnya direkrut, 190 subyek mempunyai data biokimia darah yang lengkap pada saat awal (kehamilan ~18 minggu) dan menjelang melahirkan (kehamilan 35 minggu). Median konsumsi tablet besi di kelompok "harian" adalah 50. Hanya 17% subyek yang minum ≥90 tablet besi dan 43% subyek minum <30 tablet besi. Status besi ibu-ibu hamil menjelang melahirkan di kelompok mingguan besi tidak berbeda nyata dari kelompok "harian" (***Bab 2***). Akan tetapi, status besi menurun dengan suplementasi besi harian jika minum <50 tablet besi. Konsentrasi hemoglobin di kelompok mingguan besi dan vitamin A meningkat dari 110.1 ± 1.4 g/L menjadi 114.0 ± 1.4 g/L (rerata ± galat baku), akan tetapi peningkatan tersebut tidak berbeda nyata dari kelompok mingguan besi atau kelompok mingguan "harian". Diantara subyek yang anemi, peningkatan konsentrasi hemoglobin dalam kelompok mingguan besi dan vitamin A lebih tinggi dan berbeda nyata daripada dalam kelompok mingguan besi (10.7 ± 2.3 g/L, n = 35 vs. 6.6 ± 2.3 g/L, n = 29; $P < 0.05$). Konsentrasi serum ferritin menurun secara bermakna dalam kelompok mingguan besi dan vitamin A, yang menunjukkan bahwa vitamin A mungkin memperbaiki penggunaan besi dalam proses pembentukan sel darah. Konsentrasi serum transferin reseptor meningkat secara bermakna pada semua kelompok, yang mungkin menunjukkan proses peningkatan pembentukan sel darah dan juga penurunan besi di jaringan sehubungan dengan proses kehamilan. Pada saat awal, konsentrasi serum retinol tidak berbeda di ketiga kelompok. Akan tetapi, pada saat menjelang melahirkan, konsentrasi serum retinol di kelompok mingguan besi dan vitamin A tetap konstan (0.97 ± 0.03 µmol/L), sedangkan di dua kelompok lainnya menurun secara bermakna (0.88 ± 0.04 µmol/L di kelompok mingguan besi dan 0.79 ± 0.07 µmol/L di kelompok "harian").

Data lengkap biokimia darah diperoleh dari 170 ibu-ibu ~4 bulan pasca melahirkan dari dua kelompok mingguan (***Bab 3***). Dibandingkan dengan kelompok mingguan besi (n = 88), kelompok mingguan besi dan vitamin A (n = 82) mempunyai lebih sedikit ($P < 0.01$) subyek dengan konsentrasi serum retinol ≤0.70 µmol/L (18% vs. 5%) dan cenderung mempunyai lebih sedikit ($P = 0.066$) subyek dengan konsentrasi serum retinol ≤1.05 µmol/L (52% vs. 38%). Status besi dari subyek di kelompok mingguan besi dan vitamin A tidak berbeda nyata dari subyek di kelompok mingguan besi. Konsentrasi besi dan retinol di air susu ibu pada awal melahirkan (4 – 7 hari pasca melahirkan) hampir dua kali lebih banyak dari pada di air susu ibu biasa (3 bulan pasca melahirkan), sedangkan konsentrasi lemak tidak berbeda nyata. Proporsi semua subyek yang mempunyai konsentrasi retinol di air susu ibu ≤1.05 µmol/L dan ≤0.028 µmol/g lemak masing-masing adalah 51% dan 21%. Dibandingkan dengan kelompok mingguan besi, kelompok mingguan besi dan vitamin A mempunyai lebih tinggi ($P < 0.05$) konsentrasi retinol di air susu ibu pada awal melahirkan [2.29 (1.80 – 1.22) vs. 3.37 (2.47 – 4.26) µmol/L;

rerata (95% CI)] dan di air susu ibu biasa [0.044 (0.037 – 0.052) vs. 0.053 (0.044 – 0.063) µmol/g lemak].

Sebanyak 326 bayi tunggal dilahirkan, sedangkan 296 (91%) bayi mempunyai berat dan panjang yang diukur dalam waktu 15 hari sesudah kelahiran (***Bab 4***). Berat (3094 ± 440 g) dan panjang (49.1 ± 2.0 cm) neonatus tidak berbeda nyata diantara ketiga kelompok dan bayi-bayi laki-laki 100 g lebih berat dan 0.53 cm lebih panjang daripada bayi-bayi perempuan. Umur kehamilan, berat ibu pada waktu awal trimester kedua dan jenis kelamin adalah faktor-faktor penting yang mempengaruhi berat dan panjang neonatus. Kenaikan berat badan ibu selama kehamilan (0.29 ± 0.14 kg/minggu) berhubungan positif dengan berat dan panjang neonatus.

Proporsi ibu-ibu dengan indeks masa tubuh ≤21.0 kg/m^2 pada awal trimester kedua adalah 37%, sedangkan pada ~4 bulan pasca melahirkan adalah 52%. Selain itu, prevalensi anemia (konsentrasi hemoglobin <110 g/L selama kehamilan dan <120 g/L pasca melahirkan), defisiensi besi (konsentrasi serum ferritin <12 µg/L), anemia defisiensi besi, dan defisiensi vitamin A (konsentrasi serum retinol <0.70 µmol/L) pada awal trimester kedua masing-masing adalah 46%, 45%, 21% dan 16.5%, sedangkan pada ~4 bulan pasca melahirkan masing-masing adalah 48%, 45%, 28% dan 14% (***Bab 5***). Kekurangan gizi dalam hal indeks masa tubuh berhubungan dengan karakteristik rumah tangga yang menggambarkan keadaan sosial ekonomi yang rendah, seperti tempat buang air besar di sungai/kolam dan tidak mempunyai sepeda, sepeda motor atau kursi tamu. Asupan energi juga berhubungan dengan beberapa karakteristik rumah tangga, seperti pekerjaan suami dan kepemilikan televisi, sepeda motor, atau kursi tamu, tetapi asupan energi tidak berhubungan dengan keadaan status gizi. Akan tetapi, asupan vitamin A diatas median (>270 RE/hari) berhubungan dengan prevalensi defisiensi vitamin A yang lebih rendah (10% vs. 24%, $P < 0.05$). Kepemilikan televisi berhubungan dengan sedikitnya proporsi defisiensi besi selama kehamilan dan pasca melahirkan. Antar kelompok dan waktu pemeriksaan, karakteristik rumah tangga yang lain tidak selalu konsisten hubungannya dengan prevalensi anemia, defisiensi besi, atau defisiensi vitamin A.

Disimpulkan bahwa, dibandingkan dengan suplementasi mingguan dengan besi saja, suplementasi dengan vitamin A dan besi yang diberikan pada waktu ibu-ibu memasuki trimester kedua kehamilan mencegah penurunan status vitamin A pada saat menjelang melahirkan dan ~4 pasca melahirkan, serta meningkatkan konsentrasi retinol di air susu ibu. Suplementasi mingguan dengan besi dan vitamin A meningkatkan konsentrasi hemoglobin, akan tetapi tidak menurunkan prevalensi anemia pada saat menjelang melahirkan. Hasil dari suplementasi besi secara mingguan tidak berbeda dari suplementasi besi secara harian dalam meningkatkan status besi selama kehamilan dan pasca melahirkan. Prevalensi anemia selama kehamilan dan pasca melahirkan tetap >40% meskipun ada suplementasi besi, baik dengan atau tanpa vitamin A. Intervensi yang diberikan tidak mempengaruhi berat dan panjang neonatus. Umur kehamilan, berat ibu pada waktu awal trimester kedua dan jenis kelamin adalah faktor-faktor penting yang mempengaruhi berat dan panjang neonatus. Ibu-ibu dari rumah tangga yang menunjukkan status sosial ekonomi yang lebih tinggi mempunyai status gizi yang lebih baik. Pemberian suplementasi besi-asam folat secara mingguan bukanlah suatu alternatif pemecahan untuk menanggulangi rendahnya efektifitas program nasional suplementasi besi yang sedang berjalan. Untuk program penanggulangan anemia dan defisiensi besi selama kehamilan, suplementasi besi harian perlu dilanjutkan dengan perbaikan kepatuhan dan penurunan efek samping sebagai akibat dari minum tablet besi. Selain itu, penyebab anemia yang lain perlu diteliti dan ditinjau lebih jauh. Disarankan untuk menambahkan vitamin A dalam program suplementasi besi.

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About the Author

Siti Muslimatun was born on 20 November 1967 in Karanganyar, Indonesia. In 1986 she completed high school at the SMAN 60 in Jakarta. She graduated from Gadjah Mada University, Yogyakarta, Indonesia in 1991 with an Ingenieur (BSc) degree in Food Technology and Nutrition Science. From 1991 to 1994 she worked as an Office Administrator in a forestry and agriculture consultancy company in Jakarta. From 1994 to 1996, she received a scholarship from German Technical Cooperation (GTZ) to study for her MSc degree in Community Nutrition at the South East Asian Ministers of Education Organization – Tropical Medicine (SEAMEO TROPMED), Regional Center for Community Nutrition at the University of Indonesia, Jakarta, Indonesia. From January 1997 until December 2001, she carried out the work described in this thesis within the framework of collaboration between SEAMEO TROPMED, GTZ and the Division of Human Nutrition and Epidemiology, Wageningen University. She was accepted to participate in the European Nutrition Leadership Program in Luxembourg in June 2000. Upon completion of her PhD work, she will return to SEAMEO TROPMED as a member of the teaching staff.

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Research papers

1. **Muslimatun, S.**, Schmidt, M.K., Schultink, W., West, C.E., Hautvast, J.G.A.J., Gross, R. & Muhilal (2001) Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J. Nutr. 131: 85-90.
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3. **Muslimatun, S.**, Schmidt, M.K., West, C.E., Schultink, W., Gross, R. & Hautvast, J.G.A.J. (2001) Determinants of weight and length of Indonesian neonates. Submitted for publication.
4. **Muslimatun, S.**, Schmidt, M.K., West, C.E., Schultink, W. & Hautvast, J.G.A.J. (2001) 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 Internation workshop on Socio-economic research as a tool for improving household food security and nutrition, July 8-12, 2001, Bogor, Indonesia.

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1. **Muslimatun, S.**, Schmidt, M.K., Schultink, W., Karyadi, D., Hautvast, J.G.A.J. The effect of weekly iron and vitamin A supplementation during pregnancy on infant growth. INACG Symposium, Durban, 12 March 1999.
2. **Muslimatun, S.**, Schmidt, M.K., West, C.E., Schultink, W., Hautvast, J.G.A.J. Iron and vitamin A status during pregnancy and lactation determine iron and retinol concentrations in breast milk, INACG Symposium, Hanoi, 15 –16 February 2001.
3. **Muslimatun, S.**, Schmidt, M.K., West, C.E., Schultink, W., Hautvast, J.G.A.J. & 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.
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