THE ROLE OF VITAMIN A IN NUTRITIONAL ANAEMIA:

A STUDY IN PREGNANT WOMEN IN WEST JAVA, INDONESIA

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THE ROLE OF VITAMIN A IN NUTRITIONAL ANAEMIA:

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STELLINGEN

1. Sub-optimal vitamin A status is associated with nutritional anaemia in pregnant women (this thesis).

2. The effect of supplementation with iron and vitamin A on haemoglobin concentration in anaemic pregnant women is additive (this thesis).

3. The mechanism by which the administration of vitamin A increases haemoglobin concentration in anaemic pregnant women is poorly understood (this thesis).

4. An estimate of nutrient intake on one day is not sufficient to determine micronutrient status.

5. Iron supplementation needs to be closely monitored if it is to be effective in combatting nutritional anaemia (Schultink et al. Am J Clin Nutr 1993;57:135-9).

6. Tut wuri handayani [The future of students depends on the guidance of their mentors] (Ki Hadjar Dewantoro).

7. Human dignity is a basic right: it requires adequate food, freedom and equity.

8. Applied research is more valuable than research designed to satisfy the ego of researchers.

9. Those who consider that the third world exists should not forget that we have just one world in which to live.

10. Politically, health should be seen as central to human development: it pleads for a better use of science and technology (Nakajima H. Address to the World Health Assembly, 7 May 1991: WHA 44/1991/REC/2, p 18).

11. Families with educated mothers largely succeed in escaping severe ill-health and malnutrition, possibly because they can use optimally the meagre resources which are available (Gopalan C. Nutrition: problems and programmes in South-East Asia. New Delhi: WHO Regional Office, 1987 (SEARO Regional Health Papers No. 15), p 14).

12. Technology is not the limiting factor in addressing micronutrient deficiencies (Ramalingaswami V. Challenges and opportunities - one vitamin, two minerals. World Health Forum 1992;13:222-31).

13. In providing a steady flow of iron tablets to mothers, there is no doubt that patient compliance is important but the supply and distribution of tablets is critical.

14. The prevalence of nutritional anaemia in a population could perhaps be used as an indicator of vitamin A deficiency.

Stellingen behorende bij het proefschrift
'Vitamin A, iron and nutritional anaemia in pregnant women in West Java, Indonesia'
van Djoko Suharno, Wageningen, 17 juni 1994.

untuk Bapakku (alm)
Ibu dan saudara-saudaraku
serta guru-guruku

ABSTRACT

Nutritional anaemia affects 50-70% of pregnant women in the developing world where vitamin A deficiency is also a problem. Since previous studies have indicated that vitamin A deficiency can be involved in the aetiology of nutritional anaemia, the role of vitamin A deficiency in nutritional anaemia in pregnant women has been investigated in West Java, Indonesia. Of the 318 normal pregnant women examined in a cross-sectional study, 49% were anaemic and, according to multiple criteria, 43% had iron-deficiency anaemia, 22% had iron-deficient erythropoiesis, and 7% were iron depleted. Based on serum retinol values, 2.5% of the women were vitamin A deficient and 31% had marginal vitamin A status. After adjustment for gestational stage, parity and subdistrict, serum retinol concentrations were significantly positively associated ($p<0.01$) with haemoglobin concentrations, haematocrit, and serum iron concentrations. In order to test whether the anaemia observed would respond to improvement in vitamin A supply, a randomised, double-masked, placebo-controlled field trial was carried out in which anaemic pregnant women in the same area were supplemented with vitamin A and iron. The women (n=251), aged 17-35 years, parity 0-4, gestation 16-24 weeks, and haemoglobin between 80 and 109 g/L were randomly allocated to four groups: vitamin A (2.4 mg retinol) and placebo iron tablets; iron (60 mg elemental iron) and placebo vitamin A; vitamin A and iron; or both placebos, all daily for 8 weeks. Maximum response in haemoglobin was achieved with both vitamin A and iron supplementation (12.78 g/L, 95% CI 10.86 to 14.70), with one-third of the response attributable to vitamin A (3.68 g/L, 2.03 to 5.33) and two-thirds to iron (7.71 g/L, 5.97 to 9.45). After supplementation, the proportion of women who became non-anaemic was 35% in the vitamin-A-supplemented group, 68% in the iron-supplemented group, 97% in the group supplemented with both, and 16% in the placebo group. In a subgroup (n=104) of the women in the intervention study, the intake of food and nutrients was also measured. The median daily intake of vitamin A, including provitamin A, was 1023 (10th and 90th percentiles; 65, 1914) μg retinol equivalent (RE). About 24% of the women had intakes below the daily intake of vitamin A recommended for pregnant women in Indonesia (700 μg RE). The suboptimal vitamin A status associated with nutritional anaemia suggests that measures to combat anaemia in pregnant women should involve improvement not only of iron status but also of vitamin A status.

CONTENTS

PREFACE

This thesis is the result of five years of intensive effort. The excellent collaboration between the Indonesian Ministry of Health, the Nutrition Research and Development Centre (NRDC) in Bogor, and the Department of Human Nutrition of Wageningen Agricultural University made the research work possible. This collaboration started more than a decade ago. In 1981, Dr R. Soebekti, the previous Director General of Community Health in Jakarta gave me the opportunity to participate in the Regional Course on Food and Nutrition Planning at the University of the Philippines, Los Baños (UPLB) which was founded under the auspices of the Department of Human Nutrition, Wageningen and funded by FAO, WHO and NUFFIC. There I met Professor Joseph Hautvast and Professor Jane Kusin and from them I got the feel for nutrition. Of course my teachers especially Dr Peter Dijkhuizen, Dr Martin Nube, Dr Josefa Eusebio, and Dr Cora Barba shared their knowledge on nutrition. To you all, I would like to express my sincere gratitude.

Quite often I met with Professor Hautvast at UPLB and in Jakarta. The idea of working towards a PhD from Wageningen often arose in our discussions. It was Dr Suyono Yahya, the successor to Dr R. Soebekti, who gave me the necessary support and arranged for my fellowship for the PhD programme. He also gave me an opportunity to go to Wageningen for a short visit in early 1988 to have further discussions with Professor Hautvast and Professor Clive West. Dr Frits van der Haar, then at the International Courses in Food Science and Nutrition, played an important role in making the discussions more fruitful. Professor Anton Beynen also inspired me to enrol for a PhD. I am grateful for the role played by my superiors, Dr Ignatius Tarwotjo and Dr Abdulrachman Soerono.

Professor Hautvast, I am grateful to you not only for encouraging me to come but also to keep going. I remember your visit to Indonesia between my two studies when you spent considerable time making arrangements for me to continue. You also taught me a lot about nutrition and provided guidance in designing the studies and writing articles. To you Clive, I would like to thank you for your unlimited support not only in Wageningen but also in Indonesia. You called in to Bogor en route from Australia, from Nepal and from Vietnam to keep an eye on things. From beginning to end, you helped with the design of the studies,

arranged the logistic support and assisted with the preparation of articles for publication and with this thesis. From you I have learned much about hard work and being open. I do believe that our research cooperation should be sustained and our institutional links strengthened. I am also grateful to your wife Helen, for allowing me to work with you during your 'time off'. I would also like to thank my second co-promotor, Dr Muhilal. Pak Muhilal, you provided excellent scientific advice and leadership especially to the laboratory staff. Without your guidance, this work could not have been done.

My first stay in Wageningen for 6 months in 1988-89, deepening my knowledge of nutrition and preparing for the field work, was very hard indeed to switch from the atmosphere of being a government officer and a medical practitioner, the work which I had done since graduating from medical school. I had to move from dealing with policy, administrative and practical matters to an academic and scientific world.

At the NRDC in Bogor, I received magnificent support not only from Dr Muhilal but also from Professor Darwin Karyadi. NRDC provided an excellent base for carrying out the field work and for performing the laboratory analyses. I am very much indebted to Ance Murdiana, Dewi Permaesih, Sukati and their co-workers in Bogor for their help in the field and in the biochemical analyses. Frouwkje de Waart and Margo Logman, students from Wageningen, made an invaluable contribution to the cross-sectional study. I very much appreciate being able to share their ideas and experience at that time. My special thanks go to Dr Adjo Sukardjan, Bogor District Health Officer, who gave me official permission. To Dr Petilman in Bantarjaya, Dr Yettie in Sindangbarang, Dr Willy Katili in Ciherang, Dr Suhanda in Sukamanah, and their staff member in the Health Centres, for their help and field arrangement for this research. Special thanks also devoted to all of the health volunteer 'Kaders' in 'Posyandu' in the villages in the study areas. Of course, the study would have not been possible without participation and cooperation of the pregnant women in the study areas to whom I am very grateful.

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Wageningen, May 1994 Djoko Suharno

CHAPTER 1

GENERAL INTRODUCTION

The Second Report on the World Nutrition Situation in 1992 (1) showed that protein-energy malnutrition and micronutrient malnutrition continue to affect large numbers of people in the developing world. Vitamin A, iron and iodine are essential for the normal processes of growth, development, maintenance and resistance to infection. In their absence, individuals as well as families suffer serious consequences expressed as increased mortality, morbidity and disability rates; and communities and nations suffer losses in human potential, the social and economic costs of which no country can afford. Micronutrient deficiencies constitute today a major constraint on future human development. Pre-conceptual nutrition (nutrition in anticipation of pregnancy) may be considered as a key concept in dealing with micronutrient deficiencies, as micronutrient malnutrition is a pervasive phenomenon and its consequences are felt at all stages of the human life cycle, starting from the moment that the fertilized ovum settles in the mother's womb or even before. Thus seeking to underscore the urgency of prevention and control of micronutrient malnutrition in pregnancy may exert a great impact on the future generation.

Indonesia

The Republic of Indonesia is a nation of 13,677 islands and stretches 3,200 miles across the equator, covering an area of 5 million square kilometres, geographically dynamic with about 400 volcanoes, 100 of which are still active (2). The country has 27 provinces with a total of 241 districts, 55 municipalities, 3,601 subdistricts and 67,033 villages all over the country. Based on the 1990 census, the population of Indonesia is about 180 million (2). The life expectancy is estimated at 58 years for males and 61 years for females (3). The rate of population growth is 1.9% per annum, while the crude birth and death rates are about 25.0 and 9.1 per 1000 respectively. Over 70% of the people live in rural areas with agriculture being the main activity while the literacy rate is 82% (3). Since 1969, the

Government of Indonesia has adopted a development strategy, which has been pursued through successive five-year plans. The plans are based on a commitment to broad-based economic growth, equity, and national dynamic stability to ensure the economic growth and equity. Indonesia's Food and Nutrition Policy, which has been pursued since the second Five Year Plan (1975-1980) has the following objectives:

* to achieve food self-sufficiency, price stabilization, improve income, and equitable food distribution;
* to diversify production and consumption of staple foods, and not to rely mainly on rice;
* to improve nutritional status by reducing macronutrient and micronutrient deficiencies; and
* to pursue continually the acceptance of the "small, happy and prosperous family" norm, through family planning programs.

The principal nutrition problems in Indonesia at the present time are protein-energy malnutrition, nutritional anaemia, vitamin A deficiency and endemic goiter (4). Since nutritional status has an important role in the development of equity of life, the prevention and control of poor nutrition have been given high priority by the government.

Vitamin A deficiency

Originally discovered as a growth nutrient, vitamin A soon became recognized as an essential factor for normal vision. Its role in the prevention of blindness is widely recognized. It is also involved in reproduction, in the maintenance of differentiated epithelia, in the formation of specific glycoproteins, in mucous secretion, and in disease resistance, and may have a protective role in specific cancers (5). The most obvious result of vitamin A deficiency is progressive damage to the eye. The general term for this is "xerophthalmia", which ranges from the mildest form, night blindness, through reversible signs in the eye, to ulceration and destruction of the cornea, and blindness. WHO reported that in 1991 nearly 14 million preschool children had eye damage due to vitamin A

deficiency and that around 10 million of these children are located in Asia (6). Each year it is estimated that between 250,000 to 500,000 preschool children go blind from vitamin A deficiency with about two-thirds of these children dying within months of going blind. Although the eye is the most observable tissue damaged by vitamin A deficiency, progressive damage to other epithelial tissues also occur.

Increased ill health and mortality have long been associated with vitamin A deficiency, and in recent years intervention trials have established with increasing certainty that providing vitamin A to young children in areas where the deficiency exists, has a significant effect on mortality. A recent review of eight trials has estimated that a 23 % reduction in mortality can be expected when children in vitamin A-deficient areas are supplemented with vitamin A (7). In addition, there is strong evidence that improving vitamin A status would also improve children's growth, raise haemoglobin levels, and have other benefits (8-14).

Over the past 15 years, Indonesia has evolved and implemented a multi-pronged attack on the problem of vitamin A deficiency particularly in preschool children, employing capsule distribution, nutrition education and the targeting of high-risk communities. The latest data from a malnutrition prevalence survey in the Eastern provinces of Indonesia revealed that xerophthalmia is no longer a public health problem. However, the results of a 1991 survey of 4 provinces in the Eastern islands on blood serum vitamin A levels indicate that marginal vitamin A deficiency is still a public health problem in these provinces (15). Data on vitamin A deficiency in women in Indonesia are rather scarce. Kusin et al (16) reported that in a study of pregnant women in rural East Java, 23% had serum vitamin A levels regarded as deficient (<0.35 μmol/L or 10 μg/100 mL) compared with 13% of non-pregnant controls. More recently, Stoltzfus et al (17) reported a level of serum retinol levels in women who were breast feeding ranging from 1.08 to 1.30 μmol/L in a study in rural Central Java. However, there is sufficient evidence to show that in the communities where malnutrition is prevalent among preschool children, women are undernourished as well.

Vitamin A is present in food in two main forms. The various carotenoids (predominantly beta-carotene) that serve as provitamins occur mainly in plant material, such as carrots, green leafy vegetables, red palm oil, yellow and orange vegetables and fruits. Preformed

retinol (usually in the ester form) is found naturally only in foods of animal origin, notably liver and dairy products. While in theory, world food supplies contain enough vitamin A to satisfy global requirements, great differences exist in the available sources and per caput consumption of the vitamin A among countries, age categories and socioeconomic groups (1,18). In addition, individual differences in the distribution of intake relative to need almost certainly exist. In most cases, per caput consumption ranges between 300 and 500 μg retinol equivalent per day, of which the intake of children may be lower. It is known that during pregnancy, additional vitamin A is needed for the growth of the foetus, for its maintenance, for building some reserves in the foetal liver and for maternal tissue growth. However, no reliable figures are available for the specific vitamin A requirements of these processes but FAO/WHO recommended 600 μg retinol equivalent per day (18). A figure of recommended daily requirement of vitamin A for pregnant women in Indonesia is around 700 μg retinol equivalent (19).

Iron deficiency

Iron deficiency is not only the most common micronutrient deficiency but the most common nutritional disorder in the world and affects over one billion people, particularly women of reproductive age and preschool children in tropical and sub-tropical zones; it also has a serious impact on school children and working males (1). Although the problem is much more severe in developing countries, developed countries are by no means unscathed: nearly one third of women in developing countries suffer from anaemia while 11% of women in developed countries are confronted with the problem. If uncorrected it leads to anaemia of increasing severity, reduced work capacity (20-25), diminished learning ability (26,27), increased susceptibility to infection (28,29) and greater risk of death associated with pregnancy and childbirth (30).

The main causes of iron deficiency are insufficient available iron in the diet, increased needs at various stages of the life cycle, chronic iron losses, and impaired iron utilization (31). These factors operate concurrently. The dominant factor is dietary; low levels of iron intake and significantly low iron absorption in cereal and legume-based diets because of

inhibitors in foods and beverages such as tea and coffee (32). Importantly, absorption of iron improves with intake of vitamin C-rich fruits and vegetables (33), and meats, as well as with certain food preparation methods. For example, steeping of maize in water before milling can result in the destruction of the iron absorption inhibitor, phytate. Increased iron needs during pregnancy and rapid growth in small children and adolescents partially explain their increased risk of iron deficiency and anaemia (30). Cause of anaemia other than iron deficiency from the diet often include malaria, intestinal parasites, other nutrient deficiencies such as folate and vitamin B12, and genetically determined haemoglobinopathies such as thalassaemia (34). It is generally held that at least half of the anaemia worldwide is directly due to iron deficiency.

Since there are multiple causes of anaemia, and since iron deficiency can exist without haemoglobin being lowered, there are potentially four different situations, or populations: those anaemic and iron deficient; those iron deficient but not (yet) anaemic; those anaemic not due to iron deficiency; and those iron replete and with normal haemoglobin (35). These criteria are useful in classifying populations or groups. Anaemia prevalence, however, can generally be taken as an indicator of the extent and trends of iron deficiency. Estimates of the extent of anaemia in women in the developing world in 1988 was 44%; in pregnant women (with a cut off of 110 g/L haemoglobin) the prevalence is estimated at 56%, and in nonpregnant women at 43% (cut off 120 g/L), with variations between regions from around 64% in South Asia to 23% in South America (1). Nearly half the total number of anaemic women are in South Asia. The current prevalence of anaemia in pregnant women in Indonesia according to the last survey varies from 40% to 63% with the national average about 52% (36). Comparison with the previous prevalence of anaemia rate of approximately 70% before 1987, indicates a significant improvement. The prevalence of anaemia among low income female workers is 30-40%, and among adult non-lactating and non-pregnant women is 20-45%. Many studies have reported that anaemia in Indonesia is primarily due to iron deficiency and that it improved significantly after iron treatment for at least a two-month period (36).

The principal factor in body iron homeostasis is considered to be the control of absorption of ingested dietary iron from the intestinal lumen. The main factors influencing the

proportion of ingested dietary iron which is absorbed are the level of body iron stores, the amount of iron presented to the mucosa, and bioavailability factors (18). In a normal person, the level of body iron stores regulates the basal level of iron absorption (37). Within certain bands determined by the basal level of iron absorption, absorption will vary inversely with the amount of iron presented to the intestinal mucosa which regulates the amount of iron absorbed (38). Iron from animal food sources (haem iron) is absorbed to a greater extent than is the iron from plant derived food (non-haem iron) (39). The iron intake patterns in the various regions of the developing world show the dominance of non-haem iron from plant sources (30). The diet typically contains cereals, roots and/or tubers and only small quantities of meat, fish or ascorbic acid-rich foods. Thus, cereals and roots/tubers account for a large proportion of iron in their diet; the rest of the sources of iron comes from pulses, and green leafy vegetables. Those food sources contain factors which inhibit absorption of iron, such as phytates, polyphenols, dietary fibres, soy protein, and calcium. Iron supply from animal sources is extremely low. Such a combination of foods in the diet is conducive to very low absorption of iron.

Average iron consumption based on national figures generally hide the distribution across various locations and socioeconomic groups (40). Iron consumption is more income elastic, owing to the fact that as income increases consumers will purchase more meat and fish; this means that iron consumption is more likely to be inadequate for poorer than for richer households.

Vitamin A and iron interrelationships

The evidence that vitamin A has specific relationships with iron comes from studies both in laboratory animals and humans. Findlay and Mackenzie (41) in 1922 reported that rats fed a diet deficient in vitamin A developed patches of gelatinous degradation in their bone marrow and that in animals which had survived longest, most of the haemopoietic tissue was replaced with fibrous stroma. A subsequent study by Wolbach and Howe (42) in 1925 did not show marked changes in the bone marrow, although in a few cases, reduction in haematopoietic cells was observed. In 1926, another study by Koessler et al (43) observed

anaemia in early vitamin A-deficient rats but haematocrit and haemoglobin levels increased as the severity of vitamin A deficiency developed. Similar changes were also observed by Sure et al in 1929 (44), by Frank in 1934 (45), and later by Corey and Mejía and co-workers in 1970s (46-48). Other authors did not see the early depression of haemoglobin levels in vitamin A deficient rats and reported only increased values (49). Staab et al (50) have demonstrated an accumulation of iron in the liver in vitamin A-deficient rats. Studies to date have shown an increase of iron absorption in rats fed on diets containing marginal amounts of vitamin A (51). Studies in horses (52) and rhesus monkeys (53) showed a reduction of haemoglobin concentrations with vitamin A deficiency, while increased levels have been reported in chickens (54). The anaemia in Rhesus monkeys was refractory to dietary liver, iron and whole blood but responsive to treatment with vitamin A. From these animal studies it could be concluded that vitamin A deficiency does produce anaemia but as the deficiency progresses, the process of haemoconcentration leads to increased concentrations of haemoglobin in the blood.

Studies in children in the mid-1930s showed that vitamin A deficiency results in anaemia and haemosiderosis in the liver and spleen (55,56). It was also noted that the correction of vitamin A deficiency is followed by regeneration of bone marrow, disappearance of haemosiderin from spleen and liver, and an outburst of erythroblast activity. This would suggest that vitamin A deficiency interferes with some step in the transfer of iron from storage in liver to incorporation in haemoglobin in haematopoietic tissues. In 1940 Wagner (57) reported a study of experimental vitamin A deficiency in ten human subjects. After 188 days the subjects showed signs of vitamin A deficiency and during this period haemoglobin values and erythrocyte counts fell, and poikilocytosis and anisocytosis were noted. Wagner concluded that haematopoiesis was impaired. A similar study was conducted by Hodges and Kolder (58) in 1971 with 8 human volunteers maintained on very low vitamin A intakes for 357 to 771 days. Moderate anaemia developed which was refractory to medicinal iron but responsive to vitamin A.

As a result of this finding, Hodges et al. (59) reviewed the results of eight studies carried out by the US Interdepartmental Committee for National Defense on non-pregnant and non-lactating women in developing countries where the intake of iron was 14 mg per day or

more. Most intakes were below 18 mg per day except in Ethiopia where it was almost 30 times higher at 471 mg per day. They found no relationship between haemoglobin levels and iron intake but a strong relationship between serum levels of vitamin A and blood levels of haemoglobin. In a similar analysis of data from studies carried out at INCAP in Guatemala, Mejía et al (60), found a positive correlation between serum vitamin A and blood haemoglobin levels in children with adequate intakes of iron but not in those with inadequate intakes. To investigate whether an increased vitamin A intake by a community with a high prevalence of vitamin A deficiency would have effects on iron status, Mejía et al evaluated data of a national program of vitamin A fortification in sugar in Guatemala (61,62). After 6 months of fortification, elevation of serum levels of iron was accompanied by simultaneous elevation of total-iron-binding capacity. It was also observed that serum ferritin levels declined during this period. The authors concluded that vitamin A deficiency increases stores of iron and that administration of vitamin A stimulates the synthesis of transferrin which would increase the availability of iron for haematopoiesis. After two years of fortification, however, there was an overall improvement of the iron nutritional parameters. As may be expected in normal conditions of iron metabolism, the level of serum transferrin, percent transferrin saturation, and serum ferritin rose significantly, while the levels of total-iron-binding capacity decreased.

More recent studies in humans in Thailand, Ethiopia and Indonesia have revealed correlations of vitamin A indexes with those of iron. A study in Thailand (63) showed no significant correlation of vitamin A levels with haemoglobin although there was with haematocrit, while a study in Ethiopia in children (64) revealed a significant correlation of serum retinol levels with haemoglobin. Several trials have demonstrated the beneficial effects of vitamin A on anaemia. A study in India by Mohanram et al (65) reported a significant increase in haemoglobin, haematocrit and serum iron in a group of vitamin A-deficient children given daily supplementation of 8 mg retinyl palmitate for two to three weeks. In a subsequent study in pregnant women (66), it was observed that the rise in haemoglobin levels at 26-28 weeks was higher for supplementation with iron plus vitamin A than for iron alone. Another study was carried out by Mejía and Chew (67) in children receiving vitamin A, iron, both of these, or a placebo. A significant elevations in serum retinol levels, haemoglobin, haematocrit, erythrocyte counts, serum iron, and percent

saturation of iron was observed in group supplemented with vitamin A, but have no effect on total-iron-binding capacity.

Bloem and colleagues carried out two studies in which children with haemoglobin levels less than 7.5 mmol/L were given a single dose of 200.000 IU of vitamin A. In the first study (63), in 166 children one to six years old, there were significant differences between the two groups in the levels of serum iron and transferrin saturation one and two months after supplementation. However, after four months, there were no differences between the two groups in haemoglobin and haematocrit possibly because the effect of the single vitamin dose had worn off. In their second study (9), 134 children aged 6 to 9 years were examined two weeks after receiving a massive dose of vitamin A. There were significant increases in haemoglobin, haematocrit, serum iron and saturation of transferrin but not in serum ferritin. A recent study in Indonesia (12) in children supplemented with vitamin A showed increases in haemoglobin levels.

However, regardless of the mechanisms involved, vitamin A deficiency would appear to contribute to the development of anaemia, particularly in regions where dietary intakes of both vitamin A and iron are low. Thus it should be investigated whether supplementation programmes to improve iron status should involve supplementation not only with iron, but also with vitamin A.

Prevention of vitamin A and iron deficiency

In general, prevention of micronutrient deficiencies may be addressed by appropriate combinations of the following strategies: diversification of diets as a long-term measure; massive dosing or more frequent supplementation, and food fortification or enrichment (34). The choice of any one or combination of strategies depends upon the urgency of the problem, its prevalence and distribution within the country, the existing infrastructure, and the availability or potential for producing foods rich in vitamin A, iron or iodine.

In the context of vitamin A and iron deficiency, diversification of diets so as to provide the

required quantities of vitamin A and iron on a daily continuing basis is a long-term measure. Promotion of breast feeding and improving maternal nutrition are of critical importance. Improved production, availability and access of vitamin A-rich and iron-rich foods at affordable prices are also vital. Attention should be directed towards increasing the intake of haem iron from animal products and or enhancers of iron absorption. Creating awareness as a part of education goal must be converted into initiative and action on the part of the target group.

Perhaps the most popular strategy to increasing vitamin A intake utilized to date has been the periodic distribution of massive doses of vitamin A (200,000 IU) every four to six months to children six months through five years of age (100,000 IU for children under one year of age) (68,69). Much of the vitamin is stored in the liver and slowly released over time. The advantage of this periodic massive dosing is the speed with which it can be initiated but cost, coverage and sustainability, however, are potential problems. While massive dosing every 4-6 months appears to be a safe and effective schedule for children six months and older, the most effective way for dealing with infant deficiency is at yet unclear. To ensure that infants are born with adequate liver stores and receive optimal amounts in breast milk, pregnant and lactating women should be supplemented with vitamin A. Fear of teratogenic effects, particularly during the first trimester (70), requires that massive doses be restricted to late pregnancy and the first few months following delivery (69). Alternatively, women of child-bearing age should receive small daily supplements not exceeding 10,000 IU per day (71). Newborns can probably safely receive 50,000 IU (70).

Among the three micronutrient problems, nutritional anaemia is the one that has low indication of improvement. The elimination of this single micronutrient deficiency can do more than any other single programme to achieve the goal of development, because all age groups and sexes are affected (71). In clinical practice, those suspected of being anaemic are treated with medicinal iron supplementation. In a public health setting, however, it is impossible to test each patient and therefore the approach is to give supplements to entire high-risk groups, mainly pregnant women. There are good delivery mechanisms which have been tried for supplementation (31). And some of these are now being used in developing countries. There is no doubt that iron supplementation during pregnancy is effective and has

fundamental benefits to mothers and babies and can prevent long lasting undesirable effects (72). However, many programmes have limited effectiveness and sustainability partly because of the need for frequent administration in contrast to vitamin A and iodine. Up until recently, daily dosing has been recommended but trials are now under way to see whether weekly dosing is just as effective.

Iron supplementation programmes have been implemented in Indonesia since 1970 particularly for pregnant women, through antenatal activities at the Posyandu (Integrated health services post) level (36). Supplementation is started during the first visit for antenatal care. The daily dose is 60 mg elemental iron (i.e., 200 mg ferrous sulphate with 250 μg folate); each pregnant women should take at least 90-100 tablets during her pregnancy. In order to improve the coverage and compliance, pregnancy monitoring cards have been used. A study indicated that adding nutrition education in combination with iron tablet distribution and pregnancy monitoring cards is even more effective in decreasing the number of anaemic women (73). A number of problems were encountered in the distribution of iron pills. These include low compliance, inadequate distribution and delivery mechanisms, low coverage of pregnant women, and lack of monitoring and evaluation of the programme. Infection control, particularly for malaria and intestinal parasites, should be part of an anaemia control strategy. Like supplementation and dietary modification, infection control can be successful only if the proper links are established with relevant elements of primary health care.

Food fortification and enrichment are widely used in developed countries but is not yet in developing countries. Fortification is addition of a nutrient to a food vehicle does not contain appreciable quantities of the nutrient while in enrichment, the vehicle contains some of the nutrient being added. Adoption of an effective food fortification or enrichment program requires a long-term commitment. A suitable food vehicle should be centrally processed; have no change of taste, texture, colour, or appearance; frequently used by the target groups; and made available through an effective distribution system (74). In Guatemala, sugar was the vehicle of choice for vitamin A fortification, while in the Philippines and Indonesia, monosodium glutamate (MSG) is currently being very seriously considered (8,75-77). Pursuing efforts on further development of MSG fortification and

feasibility of vitamin A fortification with other vehicles such as noodles which are becoming popular among low income people and children is already on the agenda of the Indonesian government. A number of approaches to fortification with iron have been used. For example, wheat flour and bakery products have been used successful in Europe and North America while more targeted approaches such as acidified milk with added iron and ascorbic acid and haemoglobin-fortified cookies targeted at children have been successful in Chile (31). In Indonesia, iron-fortified salt was tried out in a pilot study in one plantation between 1977 and 1981. Due to administrative and processing constraints, the fortification was not continued. Fortification of foods with iron has been proposed to the Indonesian Government but has been hindered by ecologic factors, economic constraints, and the use of foods unsuitable for fortification.

Motivation and the aims of the present study

Improvement of health and nutrition constitutes an investment in human capital for national development. Appropriate strategies may vary according to needs and opportunities. Alleviating one constraint while ignoring others may do little for nutrition. In the Maternal and Child Health services of most developing countries more emphasis is put on the child than on the mother. Maternal nutrition is often neglected. Within the framework of "Safe Motherhood", one may expect a balance of approaches towards the intricate mother-infant relationship for better quality of life.

High prevalence of iron deficiency in pregnancy with its negative effects on the mothers and infants continues to be a problem particularly in the developing world. Strategies for combatting deficiencies of iron and vitamin A have been established and widely implemented with various degrees of success and progress. Deficiencies of iron and of vitamin A are often approached in isolation. It has been shown that vitamin A deficiency contributes to anaemia but there are few studies in pregnant women regarding the role of vitamin A in anaemia. The question arises whether supplementation with vitamin A either or not in combination with iron leads to an increase in the iron status of pregnant women in areas where the prevalence of vitamin A and iron deficiencies are high.

These are the reasons why this study was undertaken with the following objectives: first, to study the prevalence of anaemia and vitamin A deficiency in pregnant women through a cross-sectional study; secondly, to investigate the relationship between vitamin A status and iron indices in pregnant women; thirdly, to investigate the effect of supplementing anaemic pregnant women with iron and/or vitamin A; and finally, to examine the intake of vitamin A and of iron and their relationship with the status of these nutrients.

The findings of these studies will hopefully bridge a gap in a panorama of investigations and contribute to a new programme approach.

Outline of the thesis

In the following chapters in this thesis, results from studies in pregnant women conducted in Bogor, West Java, Indonesia are presented.

Chapter 2 reports the results of a cross-sectional study, illustrating the prevalence of vitamin A deficiency and nutritional anaemia, and examines the relationship between vitamin A and iron status. **Chapter 3** describes the effects of vitamin A and iron supplementation on iron indexes in anaemic pregnant women. **Chapter 4** deals with the nutritional intake of anaemic pregnant women and the relationship of the intake of vitamin A and of iron with the status of these nutrients. In **Chapter 5**, the findings of the various studies and their practical implications are discussed.

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CHAPTER 2

CROSS-SECTIONAL STUDY ON THE IRON AND VITAMIN A STATUS OF PREGNANT WOMEN IN WEST JAVA, INDONESIA

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Abstract

A cross-sectional study of the prevalence of iron and vitamin A deficiency in normal pregnant women in West Java, Indonesia, was carried out. Of the 318 women studied, 49.4% were anaemic and, according to multiple criteria, 43.5% had iron- deficiency anaemia, 22% had iron-deficient erythropoiesis, and 6.6% had iron depletion. Serum retinol values revealed that 2.5% of the pregnant women were vitamin A deficient and 31% had marginal vitamin A status. The relative-dose-response test carried out on 45 women showed that 4 (8,9%) had deficient vitamin A liver stores. After gestational stage, parity, and subdistrict were adjusted for, serum retinol concentrations were significantly positively associated ($p < 0.01$) with haemoglobin concentrations, haematocrit, and serum iron concentrations. The suboptimal vitamin A status associated with nutritional-deficiency anaemia suggests that pregnant women in the area should be supplemented not only with iron but also with vitamin A. This proposal should be tested in an intervention study.

Introduction

Human deficiency of vitamin A and nutritional anaemia continue to be serious problems in developing countries (1,2). Deficiency of iron in the diet is regarded as the most important factor by far in the etiology of nutritional anaemia. Several studies in humans and animals have shown that vitamin A deficiency is associated with abnormalities of iron metabolism (3-7), and the supplementation with vitamin A may increase iron status as measured by haematological indexes (8-13). In human volunteers maintained on very low vitamin A intakes for 1-2 y, moderate anaemia developed, which was refractory to medicinal iron but responsive to vitamin A (14). As a result of the finding, Hodges et al (15) reviewed the results of studies carried out on nonpregnant and nonlactating women in developing countries where the intake of iron was $\geq$ 14 mg/d. They found a strong relationship between serum concentrations of vitamin A and blood concentrations of haemoglobin.

In a similar analysis of data from studies carried out at the Instituto de Nutrición de Centro América y Panamá (INCAP) in Guatemala, Mejía et al (3), found a positive correlation between serum iron and blood haemoglobin concentrations in children with adequate intakes of vitamin A but not in those with inadequate intakes. In a study of preschool children in Guatemala, Mejía and Chew (9) found that changes in serum retinol showed a positive correlation with total iron-binding capacity and transferrin saturation and a negative correlation with serum ferritin after 6 mo of fortification with vitamin A. Significant associations of serum retinol with haematocrit, serum iron, serum ferritin, and transferrin saturation have been reported by Bloem et al (11) in a cross-sectional study of children in Northeast Thailand.

Liver iron stores have been shown to be increased in vitamin A-deficient rats (4,6), and this increase is associated with decreased concentrations of iron in serum (4) and femur (6). Thus it would seem likely that decreased utilization of iron for haemoglobin synthesis may be related to an inability to mobilize iron from hepatic stores.

In pregnant women, nutritional anaemia is often a serious refractory problem. As far as we are aware, only one study has been carried out on the role of vitamin A in nutritional anaemia in pregnant women, but the results were not very conclusive (15). We present the results of a cross-sectional study in which we determined the prevalence of vitamin A and iron deficiency in pregnant women in the Bogor district of West Java, Indonesia. We also examined whether there is an association between vitamin A and iron indexes in this population.

Subjects and methods

The study was carried out in September and October 1989 and involved the examination of 333 clinically normal pregnant women aged 20-35 y in the second or third trimester of pregnancy, in 13 villages in 3 subdistricts of Bogor District, which is situated about 70 km south of Jakarta in the West Java Province of Indonesia. Much of the district comprises fertile land that is being used for rice and other crops, horticulture and plantations, whereas the southern part (28%) is a mountainous area. The average elevation of the western part of Bogor district is 150-250 ms. Bogor District has an area of 0.3 million hectares, and a population of $\approx$ 3.5 million people. More than 70% of the pregnant women use the Mother and Child Health Service for antenatal and nutritional care (16).

The women in this study came from the middle to lower socio-economic classes and usually make use of the community health services. The pregnant women were requested to come to the Posyandu (integrated service post) or to the 'Balai Desa' (community centre) in the village for examination. The stage of gestation and estimated date of delivery were calculated from the first day of their last period and compared with results of the obstetric examination.

Every third women was questioned about food consumption, socioeconomic status, and history of previous pregnancy (data from these questionnaires are not reported in this paper). Of the 124 women questioned, every second women was chosen for the relative-dose-response (RDR) test for measuring vitamin A stores. Subjects requiring medical

attention were treated or referred elsewhere if necessary.

The study was carried out following the ethical standards of the Ministry of Health in Indonesia.

Anthropometry and blood collection

Anthropometric measurements comprising height, weight, and midupper-arm circumference (MUAC) were performed. Information on age, parity, chronic diseases, and interval since the last pregnancy was recorded by using mother and child health cards. Venous blood samples of ≈ 5 Ml were taken on the same day as the antenatal examination, and stored on ice for transport to the laboratory. Haematocrit and haemoglobin concentration were measured and serum was separated from blood by centrifugation at 2000 X g for 15 min at room temperature on arrival. Samples of serum were stored separately at -20° C in the dark for analysis of ferritin, vitamin A, and iron; blood samples were analyzed for free erythrocyte protoporphyrin. All analyses were carried out within 3 mo after blood collection.

Biochemical analysis

Haemoglobin concentration was measured by the cyanomet-haemoglobin method and haematocrit by the micromethod (17). Measurements of ferritin were performed by using the enzymelinked immunosorbent sandwich assay (ELISA) as described by the manufacturer (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). Serum iron and total-iron-binding capacity were measured spectrophotometrically as modified by Cook et al (17) and transferrin saturation was calculated by dividing the serum iron concentration by the total-iron-binding capacity. Free erythrocyte protoporphyrin was measured by fluorometry according to Orfanos et al (18). Retinol was measured by HPLC (19). The RDR test for vitamin A was carried out by using a dose of 4000 IU retinyl acetate (1200 μg retinol)(20). The RDR was calculated from the absolute rise in plasma retinol concentration between 0 and 5 h postdosing, which was divided by the concentration reached at 5 h and expressed as a percentage. Thus,

$$RDR = 100 \times (A_5 - A_0)/A_5$$

where A_5 is the plasma retinol concentration (μmol/L) at 5 h after dosing and A_0 is the plasma retinol concentration (μmol/L) in the fasting subject before dosing.

Iron status was assessed by using multiple criteria (21). By this approach, iron-deficiency anaemia is diagnosed in pregnant women when the haemoglobin concentration is <110g/L (22) and at least two of the following three indexes are abnormal: serum ferritin (<12 μg/L)(21), transferrin saturation (<16%)(21), and free erythrocyte protoporphyrin [>1.25 μmol/L(700 μg/L) red blood cells](22). When the haemoglobin concentration is normal, but there are abnormal concentrations of at least two of these iron indexes, iron status is categorized as iron deficient. Iron depletion is defined by a serum ferritin concentration <12 μmol/L while the rest of the iron indices are normal (21).

Vitamin A status was based on the serum retinol concentration: marginal, <0.70 μmol/L (20 μg/L); and deficient, <0.35 μmol/l (10 μg/L), and on RDR values (deficient when the RDR was >20%)(23).

Statistical analyses

Statistical analyses comprised single and multiple regression and Student's *t* test. Multiple-regression analyses were carried out with the different iron indexes as dependent variables and serum retinol, gestational age, parity, and subdistrict as independent variables. Dummy variables were used to represent parity and subdistrict in the models whereas gestational age in weeks was included as a continuous variable. Because the distribution of serum ferritin and transferrin saturation was skewed, natural log transformed (ln) values for these biochemical variables were used in the regression models. All data were analyzed with the *SPSS/PC* statistical package (24).

Results

After the data were examined, those of 15 women were not analyzed. Of these, 12 women had anaemia characteristic of chronic infection (25,26) because they had a haemoglobin

concentration <110 g/L and a serum ferritin concentration ≥ 300 μg/L, accompanied by reduced transferrin saturation and/or elevated free erythrocyte protoporphyrin. Data from the remaining three women were inconsistent: they had abnormal free erythrocyte protoporphyrin values when compared with the rest of the population [>3.50 μmol/L (2000 μg/L) red blood cells], whereas all other indices were normal. Thus, the data from 318 women were analyzed.

Table 1 shows characteristics and indexes of iron and retinol status. The average gravidity of the pregnant women was 3.9 ± 2.3 ($\bar{x}$ ± SD), of which one of five pregnancies did not result in a live birth.

Vitamin A status based on serum retinol concentrations was as follows: 8 women (2.5%) were categorized as deficient (< 0.35 μmol/L), 99 women (31.1%) as marginal (<0.70 μmol/L), and 211 (66.4%) as adequate (>0.70 μmol/L). Of the 62 women selected, data were obtained on the RDR test from 45 women who returned to the examination centres for the test. The values found ranged from -27.8% to +41%, with a median of 1.7%. On the basis of an RDR >20%, four women (8.9%) had deficient liver retinol stores, two of whom had marginal and two adequate concentrations of vitamin A in serum. Of the 41 women with an RDR <20%, 15 had marginal and 26 adequate concentrations of vitamin A in serum.

Haemoglobin concentrations <110 g/L were found in 157 pregnant women (49.9%). A low serum ferritin concentration (<12 μg/L) was observed in 160 women (50.3%), and elevated free erythrocyte protoporphyrin [>1.25 μmol/L (700 μg/L)] in 237 women (74.5%). Iron status based on multiple criteria is shown in **Table 2**. According to this method, 143 (45.0%) women had iron-deficiency anaemia whereas 71 (22.3%) women had deficient erythropoiesis and 21 women (6.6%) had iron depletion. Forty-six women (14.5%) could not be classified by this method: 33 had normal haemoglobin and serum ferritin concentrations but abnormal free erythrocyte protoporphyrin (n=32) or abnormal transferrin saturation (n=1); 13 women had haemoglobin concentrations <110 g/L but only one of the three indexes (serum ferritin, transferrin saturation, and free erythrocyte protoporphyrin) was abnormal; one women had a haemoglobin concentration <110 g/L and all other indexes were normal.

Table 1

Characteristics and biochemical indexes of the pregnant women*

		Percentiles		
	Value	5th	50th	95th
Age (y)	25.9 ± 4.4	20.0	25.0	35.0
Height (cm)	149.5 ± 4.9	141.1	149.5	157.7
Weight (kg)	50.2 ± 6.5	41.1	49.5	61.2
Midupper-arm circumference (cm)	24.0 ± 2.1	21.0	23.9	27.6
Systolic blood pressure (mm Hg)	112.1 ± 9.6	100.0	110.0	120.0
Diastolic blood pressure (mm Hg)	74.9 ± 7.1	60.0	80.0	80.0
Gestational stage (wk)	26.2 ± 5.8	16.0	28.0	36.0
Haemoglobin (g/L)	109 ± 10	92	110	125
Haematocrit L/L)	32.9 ± 2.8	28.0	33.0	37.3
Serum ferritin (μg/L)	13.8 ± 11.4 (11.0)	4.2	10.3	33.0
Free erythrocyte protoporphyrin (μmol/L RBC)	1.56 ± 0.44	0.83	1.55	2.30
Serum iron (μmol/L)	10.1 ± 2.40	6.05	10.2	14.2
Total-iron-binding capacity (μmol/L)	59.9 ± 11.2	41.9	59.6	80.4
Transferrin saturation (%)	17.8 ± 6.1 (16.8)	9.5	15.9	29.1
Serum retinol (μmol/L)	0.87 ± 0.35 (0.80)	0.43	0.80	1.56
Relative-dose-response test (%) (n=45)	2.5 ± 13.0	-17.2	1.7	27.0

* Mean ± SD (geometric mean; n=318, except where otherwise noted.

Multiple-regression analysis was used to adjust for effects of gestational stage, parity, and subdistrict. After adjustment for these effects, serum retinol remained significantly positively associated with haemoglobin, haematocrit, and serum iron (**Table 3**). Thus an increase of 1 μmol/L in the serum retinol concentration was associated significantly (p <0.01) with an increase of 4.30 g/L haemoglobin, 1.37 L/L haematocrit, and 1.18 μmol/L serum iron and almost significantly (p=0.065) with a decrease of free erythrocyte protoporphyrin of 0.132 μmol/L red blood cells. In the multiple-regression models, gestational stage had a significant negative association with haemoglobin (p <0.05), haematocrit (p <0.01), serum ferritin (p <0.001), and transferrin saturation (p <0.05).

Discussion

Anaemia in pregnant women is a public health problem particularly in developing countries, and the common type is iron-deficiency anaemia (27,28). Severe anaemia during pregnancy is associated with an increased risk of maternal and fetal morbidity and mortality. Even mild anaemia is associated with an increased risk of premature delivery, low birth weight, placental hypertrophy, and reduced oestriol excretion (29). Low availability of iron in the diet, poor iron absorption, repeated and closely spaced pregnancies, and prolonged lactation are common factors in developing countries that place a constant drain on the iron stores of pregnant women.

The prevalence of nutritional anaemia is highest (65%) in South Asia (2). In general, anaemia studies in pregnant women conducted in Indonesia have revealed that anaemia in pregnant women is mainly due to malnutrition (nutritional anaemia)(30-33). Surveys in West and Central Java and in Bali by Martoadmodjo et al in 1973 (30) showed that the highest prevalence of anaemia (77%) among pregnant women was found in the rice-eating area of West Java, compared with the rice and sweet potato-eating area of Bali (56%) and the rice-and cassava-eating area of Central Java (46%). Kusin et al (34) observed a prevalence of anaemia of 24.5% in a poor rural community in East Java. A study by Husaini et al in 1989 (35) showed that the prevalence of anaemia in pregnant women was lowest in Central Java (39.7%), 42.6% in East Java, 54.2% in South Sumatra, and highest

Table 2

Iron status of pregnant women on the basis of multiple criteria*

	Normal	Iron depletion	Iron-deficient erythropoiesis	Iron-deficient anaemia
	n=37	n=21	n=71	n=143
Haemoglobin (g/L)	120 ± 6	121 ± 4	115 ± 4	110 ± 6
Serum ferritin (μg/L)	27.2 ± 19.5 (22.9)	7.8 ± 2.7 (6.7)	10.4 ± 6.9 (8.9)	10.1 ± 5.2 (8.9)
Free erythrocyte protoporphyrin (μmol/L RBC)	1.00 ± 0.20	1.00 ± 0.20	1.50 ± 0.25	1.83 ± 0.38
Transferrin saturation (%)	23.5 ± 4.4 (23.1)	25.1 ± 5.4 (24.6)	17.9 ± 5.5 (17.2)	13.8 ± 3.5 (13.3)

* Mean ± SD (geometric mean). n=318; 46 women were unclassified.

(62.9%) in the rice-eating area of South Kalimantan. In our study in West Java the higher prevalence could possibly be due to the consumption of rice as a staple food compared with maize in the area studied by Kusin et al in East Java and with rice and cassava in the area studied by Martoadmodjo et al in Central Java.

In our study 2.5% of the pregnant women had vitamin A concentrations <0.35 μmol/L, which was regarded as a deficiency. This prevalence is <5%, which is regarded as indicating a problem of public health significance (23), but it should be considered that this cut-off point is based on that for children at risk of xerophthalmia (36). Because xerophthalmia is an extreme form of vitamin A deficiency, interference with metabolism, for example with that of iron, probably arises in individuals before this prevalence is reached in the population. Pregnant women are particularly prone to iron deficiency, and perhaps even mild vitamin A deficiency interferes with their iron

Table 3

Multiple-regression coefficients with iron indexes as dependent variables and serum retinol (μmol/l) as the independent variable*

Dependent variable	Independent variable†
Haemoglobin (g/L)	4.30 ± 1.432‡
Haematocrit (L/L)	1.37 ± 0.430‡
Serum ferritin (μmol/L)§	0.070 ± 0.097
Free erythrocyte protoporphyrin (μmol/L RBC)	-0.132 ± 0.071
Transferrin saturation (%)§	0.095 ± 0.057
Serum iron (μmol/L)	1.18† ± 0.385‡
Total-iron-binding capacity (μmol/L)	2.10 ± 1.898

* Mean ± SE.

† Coefficient adjusted for subdistrict, parity, and gestational age.

‡ $P<0.01$

§ Transformed to natural logarithm.

metabolism. In addition, extra vitamin A is needed during pregnancy for growth and maintenance of the fetus, for building vitamin A reserves in the fetal liver, and for maternal tissue growth. In our study we found that 31% of the pregnant women had vitamin A concentrations that could be regarded as marginal. The International Vitamin A Consultative Group (IVACG;19) has proposed that when the proportion of the population with retinol values <0.70 μmol/L (20 μg/L) is >15%, vitamin A deficiency can be regarded as a public health problem. When measured by the RDR method, a somewhat higher proportion of women (8.9%; 4 of the 45 women studied) was found to have deficient vitamin A liver stores. This method might be useful in distinguishing subclinical vitamin A status from other factors that depressed plasma retinol concentrations (37). Of the 17 women who had

marginal serum vitamin A concentrations, 2 had vitamin A-deficient liver stores according to the RDR test. Although some women showed negative RDR values and the test dose of 4000 IU was not very high (10 000 IU/d being the maximum acceptable supplementation for pregnant women)(38), negative RDR test and failure to show a transient rise after a small test dose are not found in individuals known to have depleted liver stores (20,39,40). Regardless of the method used, the extent of vitamin A deficiency found in the present study indicates the existence of a public health problem although it is much less than that observed in a comparable population in East Java by Kusin et al (34), in whom 23% of pregnant women compared with 13% of nonpregnant control subjects had serum vitamin A concentrations <0.35 μmol/L.

There are several confounding factors that can influence the various results found. Anaemia is not only caused by iron deficiency but also by other factors such as vitamin B-12 deficiency, folate deficiency (which, after iron deficiency, is the most common cause of anaemia in pregnant women), infectious diseases, and haemoglobinopathies. Measuring haemoglobin concentrations is useful for screening of anaemia but, because of its lack of specificity and the marked overlap in concentrations between normal and anaemic individuals, its usefulness is somewhat limited (17). An additional problem in pregnant women is the considerable variation in the increase in plasma volume that occurs during pregnancy (28). Predicting iron-deficiency anaemia by the existence of two or three abnormal indexes of iron status (serum ferritin, transferrin saturation, and free erythrocyte protoporphyrin) is more specific than use of haemoglobin concentrations alone (41). By these criteria, 45% (143 of 318) of women with haemoglobin concentrations <110 g/L had two or more abnormal iron indexes and were classified as being iron-deficient anaemia. When abnormal iron indices were first considered, 59% of the 122 pregnant women with two, and 77% of the women with three abnormal iron indexes had haemoglobin values <110 g/L. This increase in specificity, albeit with some loss of sensitivity, was also reported by other authors (21,41,42).

Prevalence of anaemia is associated with gestational stage, with the highest prevalence being found in the last trimester of pregnancy (28,34,43,44). In our study the overall prevalence of anaemia (haemoglobin <110 g/L) was 49.4% and the prevalence of anaemia

of women in the third trimester of pregnancy was 58.1% (data not shown). In addition, mean values of iron indexes (haemoglobin, haematocrit, serum ferritin, serum iron, and transferrin saturation) and mean serum retinol values were significantly lower and mean values of free erythrocyte protoporphyrin and total-iron-binding capacity were significantly higher in the third trimester than in the second trimester. Therefore, in the multiple-regression analysis examining the effect of serum vitamin A on iron indexes, correction was made for gestational age in weeks by applying regression analysis.

In this study, infection and infestation in the women were not investigated. Thus to decrease the effect of such confounding factors, 12 women were not included in the data analysis because of suspected infection. They had low concentrations of haemoglobin and high serum ferritin concentrations together with reduced transferrin saturation and/or elevated free erythrocyte protoporphyrin. Both infection and iron deficiency are characterized by a depression in serum iron and transferrin saturation whereas free erythrocyte protoporphyrin is increased (17,25,27). Serum ferritin is often elevated in chronic infections and depressed in iron deficiency.

As indicated in the introduction, several studies have paid attention to the possible association between indexes of iron and vitamin A status. In our study serum retinol concentration was associated significantly with haemoglobin, haematocrit, and serum iron when adjusted for effects of gestational stage, parity, and subdistricts. In a comparable cross-sectional study carried out by Bloem et al (11) with children in northeast Thailand, an association between serum retinol and haematocrit and serum iron was found. They also found an association between serum retinol and transferrin saturation, transferrin, and serum ferritin, but no association between serum retinol and haemoglobin. In intervention studies carried out subsequently, the observed effect of vitamin A supplementation on iron indexes depended on the time elapsed since the supplementation was given. In the first study, children with a haemoglobin concentration <7.5 g/L were selected. A random subsample received vitamin A capsules, whereas the remaining children served as control subjects. Two months after supplementation, significant differences for retinol, retinol-binding protein, serum iron, and transferrin saturation were found between the supplemented and the control groups (11). In a following study they found significant differences in retinol,

retinol-binding protein, haemoglobin, haematocrit, serum iron, and transferrin saturation after 2 wk of supplementation, which excluded seasonal influences (12). In India, Panth et al (15) have shown that the decline in haemoglobin occurring at one of the time points studied (26-28 wk of gestation) was prevented when pregnant women were given 1800 μg vitamin A/d for >12 wk.

Because the present cross-sectional study has shown a relationship between the metabolism of vitamin A and that of iron, an intervention study with vitamin A and iron supplementation is now planned. Groups will receive either iron, vitamin A, or both and if the association of vitamin A with iron turns out to be causal, supplementation of pregnant women with vitamin A will exert an even greater impact on public health by simultaneously reducing nutritional anaemia among pregnant women and providing the additional vitamin A required during pregnancy.

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CHAPTER 3

SUPPLEMENTATION WITH VITAMIN A AND IRON FOR NUTRITIONAL ANAEMIA IN PREGNANT WOMEN IN WEST JAVA, INDONESIA

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Abstract

Nutritional anaemia, thought to be caused by iron deficiency, affects 50-70% of pregnant women in the developing world. The influence of vitamin A and iron supplementation was studied in anaemic pregnant women in West Java, in a randomised, double-masked, placebo-controlled field trial. 251 women aged 17-35 years, parity 0-4, gestation 16-24 weeks, and haemoglobin between 80 and 109 g/L were randomly allocated to four groups: vitamin A (2.4 mg retinol) and placebo iron tablets; iron (60 mg elemental iron) and placebo vitamin A; vitamin A and iron; or both placebos, all daily for 8 weeks. Maximum haemoglobin was achieved with both vitamin A and iron supplementation (12.78 g/L, 95% CI 10.86 to 14.70), with one-third of the response attributable to vitamin A (3.68 g/L, 2.03 to 5.33) and two-thirds to iron (7.71 g/L, 5.97 to 9.45). After supplementation, the proportion of women who became non-anaemic was 35% in the vitamin-A-supplemented group, 68% in the iron-supplemented group, 97% in the group supplemented with both, and 16% in the placebo group.

Improvement in vitamin A status may contribute to the control of anaemic pregnant women.

Introduction

Over 50 % of pregnant women in developing world are anaemic and the cause for more than half is iron deficiency (1,2). In Indonesia, the proportion of pregnant women with nutritional anaemia is 50-70% (3). Several studies, mainly in children, show an association between vitamin A status and iron indices (4-9), and that supplementation with vitamin A may increase iron status (10-16). However, few studies have been done in pregnant women (7,15) and as it is difficult to combat nutritional anaemia in such women, it is important to know whether other nutrients, such as vitamin A, can alleviate the problem. Sommer et al (17) showed that in Indonesia vitamin A deficiency has a deleterious effect on childhood mortality and morbidity from respiratory disease and diarrhoea (18). We report an intervention study in pregnant women in an area where vitamin A deficiency is associated with nutritional anaemia (7).

Subjects and methods

Subjects

The study was conducted from April to September, 1992, in 20 rural villages in three subdistricts of Bogor, West Java. The women involved were from the middle and low socioeconomic groups, were aged 17-35 years, were parity 0-4, and were 16-24 weeks' pregnant. Any with clinical manifestations of chronic or infectious disease or an abnormal pregnancy were excluded.

For our study, four groups of women with haemoglobin between 80 and 109 g/L were required. This was based on: an expected increase in haemoglobin of 8 g/L in the iron intervention group (as observed in a pilot study in 1983 by Muhilal in Kuningan, West Java), and of 12 g/L in the group supplemented with iron and vitamin A; a between-subject standard deviation of the differences of haemoglobin of 10 g/l; a 5% significance level; a power of 0.80; and a drop-out rate of 20%. 572 pregnant women met the selection criteria and were evaluated to determine their packed cell volume (micromethod) and haemoglobin

concentration (19). From this screening, 305 participated in the study. Consent was obtained from the participants and the study was approved by the Indonesian Ministry of Health whose ethical standards were followed.

Design and interventions

Before supplementation, each women had an antenatal examination and height, weight, and mid-upper-arm circumference were measured (20). A blood sample was drawn from an antecubital vein, stored on ice in the dark until transported to the laboratory, and then centrifuged. Serum was stored at -20^0 C until analysis of serum ferritin by an enzyme-linked immunoabsorbent assay (Boehringer Mannheim). Iron and total-iron-binding capacity were measured spectrophotometrically (19) and retinol was assayed by high-performance liquid chromatography (21). Transferrin saturation in serum was calculated by dividing iron concentration by the total-iron-binding capacity.

For the double-masked study, subjects were randomly assigned to four groups: one group received vitamin A (2.4 mg retinol as retinyl palmitate) and placebo iron tablets; the second group, iron (60 mg elemental iron as ferrous sulphate) and placebo vitamin A; the third group, vitamin A and iron; and the fourth group, both placebos. All preparations were given daily for 8 weeks. The vitamin A and placebo vitamin A preparations were provided by Hoffman-La Roche in oil-soluble form, while the coated iron and iron placebo tablets were supplied by Lomapharm Medicine (Emmerthal, Germany). Subjects were allocated a sequential number from 1 to 305. An independent researcher randomly labelled the iron and placebo preparations red or blue, and the vitamin A and placebo vitamin A preparations green or yellow. In order of admission to the study, each subject was allocated to one of the red-green, red-yellow, blue-green or blue-yellow combinations. The code was revealed once the data or all analyses had been entered in the computer and cleaned up.

The supplementation were administered every day by trained village workers under strict supervision. To ensure the preparations were swallowed, a small cup of water was given after treatment. Antenatal care was provided with the usual schedule but none of the subjects had received treatment with vitamin A or iron 6 months before, or extra

supplements during the study. Background socioeconomic information was recorded during home visits. A second blood sample was drawn between 2 and 7 days after the last supplements were given, and analyzed as before. All subjects were treated with iron tablets until 3 months after delivery.

Analysis

The effect was analyzed by subtracting the increase after treatment of the placebo group from the increase in the other treatment groups, and significance was calculated with a *t* test.

Results

Of the 305 pregnant women recruited, complete data were available on 251 (83%). 54 women did not complete the trial for the following reasons; moved to other villages, 11 (placebo group, 5; vitamin A group, 0; iron group, 4; vitamin A and iron group, 2); supplements taken for less than 8 weeks, 23 (5, 7, 4, 7): refused to provide a blood sample, 10 (1, 4, 2, 3); and not available to provide a second blood sample, 10 (3, 2, 3, 2).
There were no differences between the women at baseline in age, height, weight, gravidity, parity, and gestational stage (**Table 1**). There was also no difference between the groups in mid-upper-arm circumference (mean 24.4 [SD 2.2] cm; n=251), body mass index (21.9[2.5] kg/m^2), and systolic and diastolic blood pressures (114.4 [9.2] and 75.4 [7.9] mm Hg).

The indices of vitamin A and iron status at baseline are shown in **Table 2**. The range of blood haemoglobin was 85 to 109 g/L. All pregnant women had transferrin saturation values less than 0.16% (0.118 [0.014], while all but 2 of the women had serum iron levels under 8.95 μmol/L. 65 women (25.9%) had low serum ferritin levels (>12 μg/L); because serum ferritin levels were not distributed normally (range 2-78), values were transformed to natural logarithms before statistical analysis. No women were vitamin A
deficient (serum retinol <0.35 μmol/L), but 25 women (10%) had marginal vitamin A status

Table 1

Characteristics of subjects*

	All (n=251)	Intervention			
		Placebo (n=62)	Vitamin A (n=63)	Iron (n=63)	Vitamin A +iron (n+63)
Age (y)	23.7 ± 4.9	23.0 ± 4.8	23.4 ± 4.2	24.2 ± 5.5	23.3 ± 4.9
Height (cm)	150.2 ± 4.6	149.8 ± 4.8	159.9 ± 4.3	150.0 ± 4.6	149 ± 4.5
Weight (kg)	49.5 ± 6.3	49.2 ± 5.7	50.4 ± 6.2	49.6 ± 6.0	48.7 ± 6.8
Gravidity	2.3 ± 1.2	2.4 ± 1.3	2.3 ± 1.3	2.5 ± 1.2	2.2 ± 1.2
Parity	1.2 ± 1.2	1.1 ± 1.1	1.3 ± 1.2	1.3 ± 1.2	1.1 ±1.1
Gestational stage (wk)	19.1 ± 2.8	19.2 ± 2.3	19.2 ± 2.3	19.1 ± 3.1	19.0 ± 3.0

* Mean ± SD.

Vitamin A and iron supplementation significantly increased haemoglobin to 12.78 g/L (95% CI 10.86 to 14.70), one-third (3.68 g/L) of which could be attributed to vitamin A supplementation and two-thirds (7.71 g/L) to the supplementation with iron. The changes in packed cell volume were similar to those in haemoglobin. After supplementation, the proportion of women who became non-anaemic (haemoglobin > 110 g/L) was 35% in the vitamin-A-supplemented group, 68% in the iron-supplemented group, 97% in the group supplemented with both vitamin A and iron, and 16% in the placebo group (**Table 3**). Supplementation with iron resulted in an increase in serum ferritin and a decrease in total-iron-binding capacity although there was no effect of vitamin A supplementation on these variables. The concentration of iron in serum and transferrin saturation increased with iron supplementation, but to a lesser extent with vitamin A; and the combined effect of both (serum retinol 0.35-0.70 μmol/L) based on the criteria generally used for children.

TABLE 2
Effect of supplementation with vitamin A and iron on parameters of iron and vitamin A status[a]

Intervention		Blood		Serum				
		Haemoglobin concentration	Haematocrit	Ferritin	Total iron binding capacity	Iron concentration	Transferrin saturation	Retinol concentration
		g/L		ln µg/L	µmol/L	µmol/L		µmol/L
All (n=251)	Baseline	103(4.7)	0.31(0.013)	2.9(0.6)	57.8(4.0)	6.82(1.00)	0.118(0.014)	1.08(0.31)
Placebo	Baseline	103(5.4)	0.31(0.014)	2.8(0.7)	58.1(3.7)	6.92(1.01)	0.119(0.014)	1.11(0.31)
(n=62)	8 weeks	105(5.1)	0.32(0.015)	2.6(0.5)	58.0(3.8)	7.02(0.90)	0.121(0.013)	1.10(0.33)
Vitamin A	Baseline	103(3.9)	0.31(0.010)	3.0(0.6)	57.6(3.7)	6.89(0.92)	0.120(0.015)	1.07(0.29)
(n=63)	8 weeks	109(5.0)	0.33(0.016)	3.0(0.6)	56.8(3.8)	7.21(0.83)	0.127(0.010)	1.25(0.30)
Iron	Baseline	103(5.0)	0.31(0.014)	2.7(0.6)	57.6(3.9)	6.71(0.97)	0.117(0.013)	1.10(0.33)
(n=63)	8 weeks	113(5.2)	0.34(0.018)	3.3(0.5)	56.2(3.2)	7.62(0.77)	0.135(0.013)	1.10(0.32)
Vitamin A +	Baseline	103(4.6)	0.31(0.012)	2.9(0.6)	58.0(4.6)	6.75(1.08)	0.117(0.014)	1.04(0.30)
Iron (n=63)	8 weeks	118(5.5)	0.36(0.015)	3.3(0.5)	55.8(3.3)	8.46(0.80)	0.154(0.010)	1.27(0.30)
Effect of intervention[b]								
Vitamin A		3.68‡ (2.03;5.33)	0.01† (0.004;0.015)	0.3 (-0.1;0.5)	-0.6 (-1.7;0.5)	0.22 (-0.02;0.46)	0.006† (0.003;0.009)	0.20‡ (0.12;0.28)
Iron		7.71‡ (5.97;9.45)	0.02‡ (0.018;0.030)	0.8‡ (0.6;1.0)	-1.3* (-2.3;-0.3)	0.81‡ (0.57;1.05)	0.017‡ (0.013;0.021)	0.02 (-0.08;0.12)
Vitamin A + Iron		12.78‡ (10.86;14.70)	0.04‡ (0.034;0.046)	0.6‡ (0.4;0.8)	-3.0‡ (-4.2;-1.8)	1.62‡ (1.32;1.92)	0.036‡ (0.032;0.040)	0.25‡ (0.15;0.35)

[a] Values are mean (SD). For serum ferritin, values were transformed logarithmically (ln).
[b] Increase in treatment group minus increase in control group, mean (confidence interval).
Statistical significance of effect of intervention using student's t-test: *,p<0.05;†,p<0.01;‡,p<0.001

Table 3

Proportion of women who become non-anaemic*

	Women without anaemia	
	No (%)	95% CI
Placebo (n=62)	10 (16%)	7 to 29
Vitamin A (n=63)	22 (35%)	22 to 48
Iron (n=63)	43 (68%)	54 to 79
Vitamin A + iron (n=63)	61 (97%)	88 to 99

* Haemoglobin ≥ 110 g/L

nutrients was even greater than the individual effects. Supplementation with vitamin A increased serum retinol levels but, as would be expected, iron supplementation had no effect.

Discussion

Nutritional anaemia is a very serious problem world-wide and pregnant women and those living in developing countries are at particular risk. At both the World Nutrition Conference in Rome, December, 1992 (22), and the Montreal Conference on Nutrition, Montreal, October, 1992, participants pledged to reduce deficiencies of vitamin A and iron by the end of the millennium. Because nutritional anaemia is so difficult to control, it is important to look at interventions other than iron supplementation.

Vitamin A deficiency is an important cause of nutritional anaemia (23). In 1971 Hodges et al (10) studied 8 volunteers on very low vitamin A intakes for 357-771 days. Moderate anaemia developed which did not respond to medicinal iron, but did to vitamin A. They reviewed the conclusions of eight studies by the US Interdepartmental Committee for

National Defence on non-pregnant and non-lactating women in developing countries where the intake of iron was 14 mg per day or more. There was no relation between haemoglobin and iron intake but a strong relation (r=0.78, $p<0.05$) between serum vitamin A and haemoglobin. In a similar analysis of data from studies at INCAP in Guatemala, Mejía et al (4), found a positive correlation between serum iron and blood haemoglobin in children with adequate intakes of iron but not in those with inadequate intakes. There is also a positive correlation between retinol in serum and haemoglobin in the blood of children in Ethiopia (9) and in pregnant women in Indonesia (7). In an evaluation of a vitamin A fortification programme, Mejía and Arroyave (11) observed an improvement of iron status. In a controlled intervention trial in children with commercially marketed monosodium glutamate fortified with vitamin A, Muhilal et al (12) reported an increase in haemoglobin. Significant increases in haemoglobin in anaemic children have also been reported after daily dosing with vitamin A (equivalent to 3 mg retinol) for 2 months and after dosing with vitamin A (3.3 mg retinol) for 2-3 weeks (5,13).

Single massive doses are often not as effective in increasing haemoglobin concentrations in anaemic children. In two studies, children with haemoglobin levels below 7.5 mmol/L were given a single dose of vitamin A (60 mg retinol). In the first study (6), there were significant differences in iron status between the two groups 1 and 2 months after supplementation. However, this ceased after 4 months and there were no differences in haemoglobin between the two groups at any time. In the second study (14), haemoglobin was increased 2 weeks after supplementation. In another study (16) haemoglobin in preschool children was increased 3 weeks after a single dose of vitamin A.

Although supplementation with vitamin A increases haemoglobin in children, little attention has been paid to pregnant women. However, in one study (15) haemoglobin at 26-28 weeks was increased in pregnant women supplemented with iron plus vitamin A than in those with iron alone.

Our study was a randomised, double-masked, placebo-controlled field trial of anaemic pregnant women. There were enough subjects to answer whether vitamin A supplementation should be done, either alone or in combination with iron to control nutritional anaemia. An

approach involving an improvement in vitamin A status is a useful adjunct to one directed to the problem of the intake of iron alone. The increase in haemoglobin was more than 50% greater when vitamin A was supplied with iron and sufficient to eliminate anaemia in 97% (95% CI 88-99) of those anaemic women who received both vitamin A and iron.

Two important questions remain. The first concerns the mechanism by which vitamin A increases haemoglobin. From our study, no conclusions can be drawn since there were no significant changes in total-iron-binding capacity, which is an indicator of transferrin, iron, or ferritin. The elucidation of the mechanisms involved will probably need to involve animal studies. Studies to date have shown that absorption of iron is not decreased in vitamin A deficiency but increased (24). Haemoglobin synthesis is reduced but whether this is due to a decreases supply of iron to the bone marrow or to inhibition of erythropoiesis is unknown (23). The second question is related to intervention strategies in the long term. Our study has shown that supplementation can virtually eliminate nutritional anaemia, but is it feasible through the use of locally available and acceptable foods to achieve the same results? Other investigations are required because the food approach is probably the only sustainable target in the long term.

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CHAPTER 4

VITAMIN A AND IRON INTAKE AND STATUS OF ANAEMIC PREGNANT WOMEN IN WEST JAVA, INDONESIA

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Abstract

The intake of food and nutrients together with parameters of vitamin A and iron status were measured in 104 anaemic pregnant women (haemoglobin levels 85-109 g/L), aged 17-35 y, parity 0-3, 16-24 wk gestation in a rural area of West Java, Indonesia. Vitamin A status was marginal (serum retinol concentration, 0.35-0.70 μmol/L) in 11% of the women and just adequate (serum retinol, 0.70-1.05 μmol/L) in 42 % of the women respectively. The median daily intake of vitamin A, including provitamin A, was 1023 (10th and 90th percentiles; 65, 1914) μg retinol equivalent (RE), of which 906 (617, 1606) RE was derived from provitamin A carotenoids especially green leafy vegetables. This estimate, based on the best available data, is about half of that based on the current data for the (pro)vitamin A content of foods in the Indonesian food composition tables. About 24% of the women had intakes below the daily intake of vitamin A recommended for pregnant women in Indonesia (700 μg RE). The concentration of serum retinol was correlated significantly (p<0.001) and positively with the intake of total vitamin A (r=0.36) and with the intake of carotenes (r=0.37). The mean intake of iron was 15.5 mg/d, which was provided mainly by non-haem iron. There was no relationship between measures of iron status and iron intake.

Introduction

Nutritional anaemia and vitamin A deficiency are major nutritional deficiency problems in developing countries (1). As over 50% of pregnant women in such countries suffer from anaemia, it is one of the most prevalent public health problems (2). This is true for Indonesia where the prevalence of nutritional anaemia among pregnant women has been estimated to be between 40% and 63%, with the national average about 52%. Deficiency of iron is regarded as the most important cause (3). In a number of studies of Indonesian pregnant women, marginal vitamin A status has been found to be associated with anaemia especially in those with low incomes (4-5). Thus this group is confronted with deficiency of not one micronutrient but two. Inadequate or imbalanced dietary intake, intestinal parasites, malabsorption, low bioavailability and individual variation play important roles in the aetiology of the deficiency of both micronutrients (6).

Up until now, programs to combat vitamin A deficiency and nutritional anaemia have been directed largely towards the use of vitamin A and iron supplements and much less effort has been directed towards using locally available foods. Most people agree that the use of such foods is the method of choice for combatting deficiency of vitamin A and of iron in the long term, however there are only few studies to relate dietary intake and status of individuals or of particular groups. A community-based intervention study of anaemic pregnant women in West Java, Indonesia (7), provided an opportunity to study the relationship between status and intake of vitamin A and those of iron in anaemic pregnant women.

Subjects and methods

Subjects

The study was conducted between April and September 1992 in 20 rural villages in three subdistricts in Bogor District, West Java, Indonesia, as a part of an intervention trial in which 251 anaemic pregnant were supplemented with vitamin A and/or iron (7). Prior to

entry in the study, the women were given an antenatal and physical examination which also included anthropometry and collection of a blood sample as described earlier. The women selected for the intervention study were 16-24 wk pregnant and had a blood haemoglobin concentration between 85 and 109 g/L. From the total population available, 104 women were selected using a table of random numbers. Informed consent was provided by the women and the study protocol was approved by the Indonesian Ministry of Health whose ethical standards were followed.

Biochemical analysis

Biochemical parameters comprising haemoglobin, haematocrit, ferritin, total iron-binding capacity, serum iron, transferrin saturation and serum retinol, were measured in all subjects. Haematocrit was measured using the micromethod and haemoglobin by the cyanmethaemoglobin method (8). Measurements of ferritin were performed using the enzyme-linked immunoabsorbent (ELISA) sandwich assay (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). Serum iron and total iron-binding capacity were measured spectrophotometrically (8) and transferrin saturation was calculated by dividing the serum iron concentration by the total iron-binding capacity. Retinol was measured by HPLC (9).

Dietary intake

Information on food consumptions of all subjects was collected on home visits. Dietary intake of vitamin A was assessed with a self-made quantitative food frequency questionnaire (10) which includes 60 food sources of vitamin A commonly consumed in the study area. Frequency of consumption of fruits and vegetables was recorded per week or per month in terms of portion sizes or portion servings. Portion sizes of other food items other than fruits and vegetables were estimated in terms of number of items, fraction of a whole, prices, purchase units, number of household utensils, and converted into weight (g). Dietary intake of iron was assessed with a three-day 24-h food recall (10). Consumption of rice and tea were estimated by subjects' weighing. Real food models and weighing of duplicate samples were used to improve accuracy of the estimation of food portions. For

mixed or ready-made dishes purchased, subjects were asked to express their consumption in prices rather than purchased unit, because the price/weight-ratio was more constant than household-unit/weight-ratio. A conversion list of units and weights of local foods was drawn up based on a local market survey. Food consumption data were converted into energy and nutrients using the Indonesian food composition tables (11-13). Revised values on the provitamin A content of fruits and vegetables based on several references (14-20) were also used.

Statistical analysis

All data were tested for normality and for data not normally distributed, the 10th, 50th (median) and 90th percentiles were calculated. The intake of vitamin A and iron were related to biochemical parameters using Pearson's correlation co-efficient. Data not normally distributed was transformed into natural logarithms. All statistical tests were two-tailed and *P*-values of less than 0.05 were considered statistically significant. The computer package SPSS was used for all statistical analysis (21).

Results

Subject characteristics

Characteristics of the subjects, vitamin A status and iron indices are presented in **Table 1**. As it was used as a basis for selection, the blood haemoglobin levels were between 85 and 109 g/L, the mean being 102.0 g/L. All women had a transferrin saturation <16% and serum iron 8.95 μmol/L. Low serum ferritin values (<12 μg/L) were observed in 24 women (23%). No women were vitamin A deficient (serum retinol <0.35 μmol/L) but 11 women (10.6%) were marginal (serum retinol 0.35-0.70 μmol/L), 44 women (42.3%) were just adequate (serum retinol 0.70-1.05 μmol/L), while 49 women (47.1%) were adequate with respect to vitamin A status (serum retinol >1.05 μmol/L).

Table 1
Characteristics of pregnant women (n=104)

Variable	Mean	SD
Age (y)	23.5	4.4
Height (cm)	150.4	3.9
Weight (kg)	50.9	6.5
Gravidity	2.4	1.1
Parity	1.2	1.0
Gestational stage (wk)	19.4	2.9
Haemoglobin (g/L)	102.0	5.1
Packed cell volume	0.30	0.02
Ferritin (μg/L)	22.5	14.4
Total iron-binding capacity (μmol/L)	54.7	4.4
Serum iron (μmol/L)	6.8	1.0
Transferrin saturation	0.12	0.01
Serum retinol (μmol/L)	1.02	0.27

Food pattern

A meal was defined as food consisting of rice as the common staple food and side dishes. The most salient feature of the diet is its monotony and the irregularity of consumption of foodstuffs other than rice. The majority of women (75%) consumed breakfast and two meals. Consumption of breakfast delayed the first meal from around 11.00 to around 13.00 while the second meal was usually consumed just after sunset (18.00 - 19.00). Between-meals, snacks were consumed more than three times a week and varied from cooked or fried tubers, fried banana to Indonesian cookies. The habitual diet consisted of rice, 'tempe' (fermented soybeans), 'tahu' (soybean curd), pulses, salty fish and vegetables. Soy sauces and red chilies were commonly used for flavouring the dishes and snacks. Vegetables formed part of the daily menu; only 60% of women consumed green leafy vegetables

Table 2
Daily dietary intake of energy and macronutrient

Variable		Mean	SD
Energy	(cal/d)	1959	278
	(MJ/d)	8.2	1.2
Protein	(g/d)	54	13
	(energy, %)	11.1	1.3
Fat	(g/d)	45	16
	(energy, %)	20.6	6.9
Carbohydrate	(g/d)	334	61
	(energy, %)	68.2	5.7

three times a week or more. Leafy vegetables were usually cooked by boiling in water, boiling in coconut cream, steaming, stewing or frying. Boiled or steamed vegetables were often served with seasoned grated coconut or peanut sauce. Some leafy vegetables were eaten raw with chilies sauce as a part of the main dishes. The most commonly consumed fruits were bananas, ripe papaya, ripe jackfruit, and oranges. Mangoes were not in season during the study while pineapples are avoided during pregnancy. Milk, mainly in the form of milk powder or sweetened condensed milk, meat and eggs were rarely consumed. Salted fish was consumed more commonly than fresh fish and coconut oil was commonly used for frying and stewing.

Energy and macronutrients intake

Table 2 shows the daily energy and macronutrients intake. Based on the Indonesian recommended daily allowance for pregnant women (22), 95% consumed less than the recommended energy intake of 10.5 MJ/day and 55% consumed less than the recommended protein intake of 60 g/day. The proportion of energy from protein, fat and carbohydrate was 10%, 21%, and 68% respectively.

Vitamin A intake

Because of the concern for the quality of data on the provitamin A content of foods, we have recently collected all available data on the carotenoid content of foods (18). These new data are compared with data currently presented in the Indonesian food composition tables (**Table 3**). As can be seen, the new data are generally much lower than those currently in use. Estimates of vitamin A intake based on the two sets of data are presented in **Table 4** and it can be seen that the revised estimate is about half of that based on the data previously available. The revision of the estimate increases the population of women with vitamin A intakes below that recommended for pregnant women in Indonesia (700 RE/day) from 2% to 24%. As can be seen from **Table 5**, the difference in the estimate in intake is mainly attributable to the difference in the estimated provitamin A content of green leafy vegetables, which are the major source of vitamin A. The median estimate of vitamin A from this source is reduced by more than half from 1493 RE/day to 682 RE/day. As shown in Table 6, the major source of vitamin A from animal origin, primarily preformed vitamin A, is liver followed by eggs.

Intake of iron and factors affecting iron absorption

Table 7 shows the daily intake of iron, calcium and ascorbic acid. The mean daily iron intake was 15.5 mg which is very low compared with the Indonesian recommended daily allowance of 55 mg/day for pregnant women (22). The recommended intake corresponds to the FAO/WHO requirement for iron from diets for which iron bioavailability is intermediate (23). On this basis, 53% of women would have an adequate iron intake. The bioavailability of non-haem iron is affected markedly by a variety of enhancing and inhibiting food factors. The mean intake of vitamin C, uncorrected for losses during cooking and storage, was about 120 mg/day which is twice the recommended allowance of 60 mg/day. Unfortunately, we have no information on losses of vitamin C, and these can be quite high. Reduced absorption of iron could come about because of the polyphenols in tea which was consumed in reasonable quantities (720 g liquid/d). The contribution of food groups to the dietary iron intake is shown in Table 8. The major iron sources were plant in origin of which soy and soy products were the largest contributors (30%). From animal

Table 3
Comparison of provitamin A content of foods as listed in Indonesian food composition tables and current table
(RE/100 g edible portion)*

Name			Indonesian tables	Revised data
English	Indonesian	Scientific		
Fruits				
Banana	Pisang	*Musa sapientum*	45[a]	12[d]
Jack fruit	Nangka	*Artocarpus heterophyllus*	51[a]	10[d]
Orange	Jeruk	*Citrus nobilis*	29[a]	8[d]
Papaya, ripe	Papaya	*Carica papaya*	56[a]	201[d]
Watermelon	Semangka	*Citrullus vulgaris*	91[a]	55[d]
Vegetables				
Carrot	Wortel	*Daucus carota*	1800[a]	1000[d]
Cassava, leaf	Daun singkong	*Manihot utilissima*	1369[a]	1261[f]
Chili, red	Cabai merah	*Capsicum annum*	71[a]	150[d]
Chili, green	Cabai rawit	*Capsicum annum*	1658[a]	65[e]
Chinese cabbage	Petsai	*Brassica chinenses*	499[a]	390[e]
Egg plant	Terong	*Solanum melongena*	6[c]	10[d]
Jointfir spinach leaf	Daun melinjo	*Gnetum gemon*	1500[a]	608[g]

Table 3
(continued)

Holly basil leaf	Daun kemangi		750[a]	390[e]
Locust bean	Petai	*Parkia speciosa*	30[b]	121[d]
Maize	Jagung	*Zea mays*	65[c]	42[g]
Mungbean	Kacang hijau	*Vigna radiata*	20[a]	0[d]
Papaya, leaf	Daun papaya	*Carica papaya*	1369[b]	1261[g]
Pumpkin	Waluh kuning	*Cucurbita moschata*	27[b]	374[i]
Spinach	Bayam	*Amaranthus viridis*	914[a]	207[f]
Sweet potato, yellow	Ubi rambat kuning	*Ipomoea batatas*	963[a]	1467[h]
Sweet shoot leaf	Daun katuk	*Sauripus androgenus*	1555[a]	608[d]
Tomato, ripe	Tomat	*Lycopersicum sculentum*	225[a]	60[d]
Water spinach	Kangkung	*Ipomoea aquatica*	945[a]	214[f]
White cabbage	Kobis	*Brassica oleracea*	10[a]	13[j]
Yard long bean	Kacang panjang	*Vigna unguiculata*	50[a]	24[f]
Yard long bean, leaf	Daun kacang panjang	*Vigna unguiculata*	786[a]	608[d]

* RE, μg retinol equivalent.
[a] reference 13; [b] reference 12; [c] reference 11; [d] reference 18;
[e] reference 14; [f] reference 15; [g] reference 16; [h] reference 17;
[i] reference 19; [j] reference 20.

Table 4

Vitamin A intake (RE/d)*

	Mean	SD	Percentiles		
			10th	50th	90th
Estimate I[a]					
Total	2059	918	1199	1880	2713
Carotenes	1854	504	1195	1819	1575
Retinol	205	506	0	38	317
Estimate II[b]					
Total	1271	972	625	1023	1914
Carotenes	1067	570	617	906	1606
Retinol	205	506	0	38	317

* RE, μg retinol equivalent.

[a] Based on Indonesian food composition tables (see Table 3).

[b] Based on revised data (see Table 3).

Table 5
Contribution of food groups to dietary intake of vitamin A (RE/d)* from plant origin

Food groups	Mean	SD	Percentiles		
			10th	50th	90th
Estimate I[a]					
Green leafy vegetables	1453	359	956	1493	1917
Non-leafy vegetables green	54	50	11	36	128
Non-leafy vegetables yellow	167	180	4	108	403
Fruits	36	53	0	19	97
Staples, yellow	55	154	0	0	289
Cereals, yellow	4	12	0	0	18
Legumes	3	3	0	0	6
Total	1770	444	1113	1761	1351
Estimate II[b]					
Green leafy vegetables	726	26	413	682	1173
Non-leafy vegetables green	33	56	7	18	83
Non-leafy vegetables, yellow	105	320	7	26	207
Fruits	30	71	0	6	105
Staples, yellow	84	234	0	12	140
Cereals, yellow	3	8	0	2	23
Legumes	3	3	0	0	6
Total	984	505	583	852	1504

* RE, μg equivalent
[a] Based on the Indonesian food composition tables (see Table 3).
[b] Based on revised data (see Table 3).

Table 6
Contribution of food groups to dietary intake of vitamin A (RE/d)* from animal origin

Food groups	Mean	SD	Percentiles		
			10th	50th	90th
Liver	198	797	0	16	851
Eggs	42	74	1	12	162
Meat, poultry	16	47	1	5	36
Fish	15	11	0	0	26
Milk	13	24	0	1	35
Fats	6	20	0	0	22
Total	291	809	10	42	898

* RE, μg retinol equivalent

Table 7
Daily intake of iron, calcium and ascorbic acid

Variable	Mean	SD	Percentiles		
			10th	50th	90th
Total iron (mg/d)	15.5	4.8	8.8	15.2	22.2
density (mg/4.18 MJ)	7.9	2.4	4.9	7.9	11.3
Non-haem iron (mg/d)	13.8	4.9	7.2	14.3	19.8
density (mg/4.18 MJ)	7.2	2.5	3.9	7.2	10.3
Haem-iron (mg/d)	1.7	1.9	0.1	1.2	3.6
density (mg/4.18 MJ)	0.9	1.0	0.1	0.6	1.8
Calcium (mg/d)	463	198	200	458	735
Ascorbic acid (mg/d)*	121	63	55	143	279

* Estimated from raw foods and not adjusted for losses during preparation and storage.

Table 8
Contribution of food groups to dietary intake of iron (mg/d)

Food groups	Mean	SD	Percentiles		
			10th	50th	90th
Plant origin					
Leafy vegetables	1.4	1.5	0.0	1.0	3.3
Non-leafy vegetables	1.1	0.7	0.4	0.8	2.0
Fruits	0.6	0.9	0.0	0.2	1.7
Staple	0.3	0.5	0.0	0.2	1.2
Cereals grain products	2.8	0.8	1.8	2.6	3.8
Nuts, seeds, legumes	0.3	0.9	0.0	0.0	0.5
Soy and soy products	4.6	4.9	0.2	4.0	8.8
Dishes	1.5	2.7	0.0	0.2	6.0
Snacks	1.2	1.0	0.2	0.6	3.0
Total	13.8	7.1	6.8	12.2	22.5
Animal origin					
Meat	0.1	0.3	0.0	0.0	0.3
Poultry	0.1	0.3	0.0	0.0	0.6
Fish	1.1	0.5	0.3	0.8	1.5
Eggs	0.4	0.8	0.0	0.2	1.7
Total	1.7	1.2	0.5	1.3	3.5

Table 9
Median (percentiles 10th & 90th) of vitamin A intake (RE/day)* and quintiles of serum retinol concentrations.

Vitamin A intake	Quintiles of serum retinol concentration (μmol/L)					r
	0.67 (0.49-0.75)	0.84 (0.78-0.93)	1.04 (0.94-1.07)	1.16 (1.09-1.23)	1.38 (1.30-1.57)	
	n=19	n=22	n=21	n=21	n=21	
Total vitamin A	905 (447-2079)	800 (525-1799)	935 (697-1556)	1120 (804-2046)	1377 (514-5395)	0.36†
Carotenes	765 (442-1358)	738 (505-1601)	856 (688-1446)	1006 (745-2046)	1261 (514-2701)	0.37†
Retinol	32 (0-721)	41 (0-204)	32 (0-186)	57 (0-319)	68 (0-2815)	0.14

* Median (10th,90th percentiles) of daily vitamin A intake expressed in RE (μg retinol equivalent).
r Correlation coefficient of Ln vitamin A intake against serum retinol concentrations.
† $p<0.001$.

sources, fish was the most important, contributing about 60% to the haem iron intake.

As can be seen from the correlation co-efficient, there is a relationship between total vitamin A intake and vitamin A status expressed as serum retinol concentration. This relationship is attributable to the intake of carotenes. There were no correlation between the intake of iron (haem and non-haem) and parameters of iron status. Neither with consumption of factors enhancers and inhibitors and iron status. However, significant adverse correlation between tea and total-iron-binding capacity observed ($r=-0.25$, $p<0.01$).

Relationship between status and intake of vitamin A and iron

Table 9 shows the median daily dietary vitamin A intake classified by the quintiles of serum retinol concentrations. Serum retinol concentration was correlated ($p<0.001$)

significantly with the intake of total vitamin A and with the major component of carotene intake, carotenoid intake. However no relationship was found between serum retinol concentration and retinol intake.

Discussion.

In general, deficiencies of micronutrients may be addressed by appropriate combinations of the following strategies: dietary diversification or food fortification as long-term measures and massive dosing or supplementation as short-term measures.
Up until now, programs to combat vitamin A deficiency and nutritional anaemia have been directed largely towards the use of vitamin A and iron supplements and much less effort has been directed towards using locally available foods. Long-term intervention through dietary modification of intake of foods rich in (pro)vitamin A is regarded as more effective (24). The importance of this approach is underscored by the recent commitment of 159 countries to develop food-based strategies to meet micronutrient needs as a set forth in the World Declaration and Plan action that has adopted at the International Conference of Nutrition (25) and embodied in the policy statement of FAO on the use of nutrient-rich foods to prevent micronutrient deficiencies (26).

Of the 104 anaemic pregnant women in the present study, 10.6% had marginal vitamin A levels. This figure was practically identical to that in the total population from which the sample was drawn (7).

The energy intake of the women was low being on average 8.2 MJ/d which is 78% of the recommended daily allowance (RDA) for Indonesian pregnant women. Only 5% of the women reached the RDA. Of the energy, 68% was derived from carbohydrate. The average protein intake was 45 g/day or 75% of RDA. More than half of the women consumed less than the protein RDA. Although intakes were low in the study, they were higher than those reported by Martoadmodjo (27) in 1973, also in pregnant women in Bogor district. Intakes of energy and protein were 30% higher and fat was 50% higher while carbohydrate intake was about the same.

In this study, two sets of food composition data were used to calculate vitamin A intake: one based on Indonesian food composition tables (11-13) and the other on revised values (14-20). The estimated vitamin A intake based on the Indonesian tables was 2059 RE/day while that based on the revised values was 1271 RE/day. Both values are higher than the RDA established by FAO/WHO of 600 RE (23) or in Indonesia of 700 RE (22). The discrepancy between the two intake estimates was mainly due to the difference in the estimated carotenoid content of leafy vegetables. This stresses the need for more analytical work to be carried out on the carotenoid content of food in Indonesia and in other countries.

The reduction in the estimate of vitamin A intake arising from the downward revision of the carotene intake of foods resulted in an increase the proportion of women estimated to be consuming less than the RDA for vitamin A from 2 to 24% based on the Indonesian RDA. The influence of the database used on nutrient intake has been highlighted earlier (28). The question arises as to whether other factors may make it necessary to revise the estimated intake further. Three factors could be mentioned: firstly, the validity of the estimate of food intake; secondly, the natural variation in the carotenoid content of foods; and thirdly, the bioavailability of carotenoids. Inadequate information from the subjects and problems associated with dietary portions which are generally overestimated (10) may influence the estimate of nutrient intake. Such overestimation is very hard to overcome (29) and contributes not only to random error but also to systematic error (30-32).

Natural sources of variation, notably soil pH, rainfall, seasonality, genetic diversity, and stage of maturation, can lead to large differences in estimates of dietary intake of preformed vitamin A and provitamin A (33-36).

The absorption and utilization of carotenoids and vitamin A are enhanced by dietary fat, protein, and vitamin E and depressed by the presence of peroxidized fat and other oxidizing agents in food. Large doses of α-tocopherol also inhibit β-carotene uptake by the intestine (37). The absorption of carotenoids and vitamin A is markedly reduced when diets contain very little (<5 g/d) fat (38). Deficiencies of a variety of other nutrients, including protein, α-tocopherol, iron, and zinc also adversely affect vitamin A transport, storage, and utilization (39-41). In addition, gastrointestinal diseases, such as diarrhoea and sprue, reduce

the absorption of vitamin A and carotenoids (41), Infection might disrupt the absorptive surface of the gut limiting absorption (42), and intestinal parasites may compete with the body for carotenoids and vitamin A (43).

Some studies focusing on the bioavailability of carotenoids have suggested that large inter-individual variations may exist within normal populations in plasma concentrations of β-carotene (44-47) and seasonal variation may also influence carotenoid bioavailability (48).

Effect of processing can also have consequences on the (pro)vitamin A content of foods: the content may be reduced during food preparation (15,49-55). Changes in (pro)vitamin A content have been documented when traditional methods have been used. As a general rule, food boiled in an open container showed the greatest losses (50,56,57).

In this study, a significant correlation was found between serum retinol concentration and intake of total vitamin A and of carotene but not of retinol. However we have been unable to confirm this in a subsequent study carried out in the same area with women who are breastfeeding (58). In this latest study, serum retinol levels in the 73 women studied was not related to provitamin A carotenoid intake but significantly and positively related to retinol intake.

At this stage, the possible contribution which dietary retinol and ß-carotene can make to serum retinol levels is unclear. Several studies in children from India (59,60), Thailand (61) and Indonesia (62) have demonstrated significant increases in serum retinol levels when green leafy vegetables are consumed. Recently Rankins and coworkers (63) have reported a positive correlation between the concentration in serum of total carotenoids and of retinol in children aged 24 to 48 months which would indicate that the ingestion of carotenoids is a determinant of vitamin A status. A number of epidemiological studies in children in Indonesia have shown a negative association between fruit and vegetable intake and xerophthalmia (64,65). However, results from other studies point to a stronger relationship between serum retinol concentrations with retinol intake rather than carotenoid intake. In a case-control study in Malawi, Keith West and colleagues (66) found no difference in the consumption pattern of fresh green leaves and mango/papaya between children with and

without xerophthalmia. However, they did find that children with xerophthalmia began weaning onto porridge and ceased breastfeeding earlier, had a shorter weaning interval and were fully weaned from the breast for a longer duration than the controls. This suggests that retinol from breast milk is the most important source of vitamin A for protection against xerophthalmia. In Tanzania, a smaller case-control study was carried out in children with and without xerophthalmia (67) and the most important difference found was that cases consumed more skimmed than full-cream milk and less butter than controls thus suggesting that retinol from dairy products is protective against xerophthalmia. A recent study from Nepal has shown that family ownership of a goat was strongly protective against xerophthalmia in children suggesting that milk was an important source of vitamin A for the children (68). In the Ghana VAST study, age at weaning and consumption of mangoes and red palm oil were not associated with increased serum retinol levels while supplementation with solid/semi-solid foods and dried beans were (69).

In a cross-sectional study carried out in the same area two years previously (5), 53% of the pregnant women were anaemic and a further 26% and 8% had either iron-deficient erythropoiesis or iron depletion. Although the major cause is probably nutritional and the area is malaria-free, infestation with intestinal parasites should be taken into account. In addition, pregnancy itself results in haemodilution (70). The mean haemoglobin value of the subjects, selected on the basis of being anaemic, was 102.0 g/L while the daily iron intake was 15.5 mg/d which is very low; it is less than 30% of the Indonesian RDA for pregnant women of 55 mg/d (22) which corresponds to the FAO/WHO requirement for iron from diets in which the bioavailability of iron is intermediate (23). On this basis, 55% of women would have inadequate iron intake.

Overall, in any given diet the quantity of iron habitually ingested is relatively constant and difficult to modify, but some types of diet are inherently denser in iron than others. Iron density is actually higher in the diets of the developing world than in those typical of industrialized countries. Typical Western diets usually contain 6 mg/1000 kcal (71) while in the present study, iron intake was 7.9 mg/1000 kcal. All too often, however, the advantage of higher iron density is offset by the inadequacy in developing countries of energy intake. The average energy intake reported here was low so the most straight

forward way of increasing iron intake would be to increase total energy intake. Pregnant women are also a special case since, because of their lower energy needs, they tend to consume less food than other groups. Thus it is more difficult for them to meet their iron needs. Dietary iron intake can be increased in two ways. The first is to insure that women consume larger amounts of their habitual foods so that their energy needs are fully met. How much additional iron ingested will depend on how fully the existing energy gap is bridged. Since no qualitative changes in the diet are needed, this approach may appear to be simple but it involves increasing the purchasing power of households, which to some extent is somewhat beyond their capacity. Supplementation with medicinal iron is also commonly used to increase iron intake but, as pointed out by Schultink (72), this needs careful supervision.

A factor just as important as the total iron content of the diet is the proportion absorbed or the bioavailability which depends on the nature of iron in the diet and a number of host factors such as intestinal mucosal behaviour, iron status and health status. Concerning the nature of dietary iron, most is non-haem iron. Although the bioavailability of haem iron is little affected by the nature and composition of a given meal, the absorption of non-haem iron is highly variable and depends on other foods eaten with the meal, especially on the balance between foods which promote and those which inhibit iron absorption (73).

In our study a high prevalence of anaemia could possibly be due to the consumption of rice as a staple food, as rice has the potential to inhibit iron absorption (74). It is well known that the polyphenol constituents of tea and other beverages (75,76), calcium (77), soy protein (78) and dietary fibre (79) are regarded as inhibitors of iron absorption while ascorbic acid, meat, fish and poultry are regarded as enhancers of iron absorption (80,81). However, the only correlation found between food/nutrient intake and parameters of iron status was the negative relationship between tea intake and total iron-binding capacity which is possibly not particularly meaningful.

No correlations were found between meat, poultry and fish intake possibly because of the low intake which varied little. Although the daily intake of vitamin C in this study was double the RDA, the estimate of intake was based on raw foods and not adjusted for losses

during storage and cooking. Approximately 50-80% of the vitamin C originally present in food can be lost during cooking. Moreover, the vitamin C content of food cooked and left standing decreases considerably and reheating reduces it still further. In many rural households, food for the whole day is cooked at one time, providing two meals that may be eaten as long as 12 hours apart. Under such circumstances, it is difficult to ensure that enough vitamin C is retained in the food unless a good source of ascorbic acid is added or the food has a sufficiently high vitamin C content to begin with. Encouraging the community to minimize nutrient losses through proper food preparation and cooking methods would benefit nutritional status.

As reported earlier by others (82-85) and from our laboratories (5,7), vitamin A deficiency contributes to anaemia. In the study population from which a sub-group for the present investigation was drawn, we found a correlation of serum retinol concentration with haemoglobin, haematocrit and serum iron (5). In addition we found an effect of vitamin A supplementation on these parameters (7).

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CHAPTER 5

GENERAL DISCUSSION

The studies presented in this thesis were aimed at investigating the relationship between vitamin A and iron indices in pregnant women and the effect of vitamin A and/or iron supplementation on nutritional anaemia. In addition, the intake of vitamin A and iron and the relationship of these parameters with the status of these micronutrients was also investigated. As shown in studies both in animals and man, vitamin A deficiency contributes to the development of anaemia (1). Thus the possible involvement of vitamin A deficiency should always be taken into consideration in programmes to control anaemia.

The problem of vitamin A deficiency

Deficiency of vitamin A is seen in at least 40 countries throughout the world, and results each year in an estimated 14 million people with eye damage of whom half a million are children, mainly of preschool age, go blind (2). A recent review has concluded that improving vitamin A status in children in deficient populations reduces mortality by about 23% (3). Studies in children have shown that improving vitamin A status reduces severity of infection, improves growth and raises haemoglobin levels (4-9).

Over the past 15 years, there has been a multi-pronged attack on the problem of vitamin A deficiency in Indonesia, particularly in preschool children. Based on massive dosing with vitamin A and on nutrition education targeted at high-risk communities, it has contributed to the present situation where xerophthalmia is no longer a public health problem (10). The prevalence in preschool children of Bitot's spots (0.33%), corneal xerosis/ulceration/keratomalacia (0.005%) and corneal scars (0.033%) are now less than the criteria (0.5%, 0.01% and 0.05% respectively) laid down by WHO (11) for regarding vitamin A deficiency as being a problem of public health significance. Thus the measures

taken have possibly contributed to reducing the problem of vitamin A deficiency to the present levels. However, there are still some areas where there is a problem, with prevalences of clinical signs of vitamin A deficiency above the WHO criteria for preschool children.

Compared with preschool children, data on vitamin A status in women are limited. A study by Kusin on women in rural East Java (12) showed that vitamin A deficiency was found in 23% of pregnant women compared to 13% of non-pregnant controls, while a study by Stoltzfus (13) of women in rural Central Java who were breast feeding showed levels of serum retinol ranging from 1.08 to 1.30 μmol/L. A high prevalence of vitamin A deficiency was found in the study of the population reported here although there were no clinical signs of vitamin A deficiency (Chapter 2). Of the 318 pregnant women studied, 2.5% were vitamin A deficient (serum retinol concentration <0.35 μmol/L) 31% had marginal vitamin A levels (0.35-0.70 μmol/L), and 66.4% had adequate levels (>0.70 μmol/L). There are accepted criteria, based on serum retinol levels, for regarding vitamin A deficiency as a problem of public health significance in preschool children: >5% of children with serum retinol concentrations <0.35 μmol/L (11) and >15% of children with serum retinol concentrations <0.70 μmol/L (14). Although these criteria may not be applicable to pregnant women, the women studied would appear to be vitamin A deficient as we found a response not only in levels of retinol in serum but also of haemoglobin when the women were supplemented with vitamin A (Chapter 3). Using the relative-dose-response (RDR) method in a sub-sample of women (45/318), 9% (4/45) had a RDR >20% indicative of deficient liver stores of vitamin A.

From the dietary study in selected anaemic pregnant women (Chapter 4), the median vitamin A intake was 1023 RE/day compared with the recommended intake for pregnant women in Indonesia of 700 RE/day (15). The intake of vitamin A was based on newly collected data (16). If the values in the Indonesian food composition tables were used, the estimated median intake would be 1880 RE/day. This difference is attributable mainly to the revision of the estimate of the content of dark green leafy vegetables which was reduced from a median daily intake of 1493 RE/day to 682 RE/day. Thus the need for more data, which is reliable, on the (pro)vitamin A content of foods, especially dark green leafy vegetables, is urgent in order to be able to assess the (pro)vitamin A intake of potentially

vulnerable groups. Using the revised data, the estimated proportion of women consuming <700 RE/day of vitamin A increased from 2% to 24%. The higher figure is more realistic because in the population of women in the intervention study, 11% had marginal serum retinol levels (0.35-0.70 μmol/L) and 42% had just adequate levels (0.70-1.05 μmol/L).

Apart from knowing the (pro)vitamin A content of the foods consumed which can vary immensely, estimates of vitamin A intake also depend on measurements of food intake which are highly variable and are often overestimated (17-19). Bioavailability should also be taken into consideration. The absorption of ß-carotene from carrots, for example, is just one third of that when dissolved in oil (20). In addition, lumen intestinal function and other factors are also likely to contribute to low levels of serum retinol. Thus, efforts need to be strengthened to increase not only vitamin A intake but also bioavailability and to control intestinal parasites and infections.

Plasma levels of retinol in pregnant women may be depressed not only by inadequate (pro)vitamin A intake but also by other causes such as the physiological stress of pregnancy itself, physical and social stress, and infection (21-28). McLaren (29) recommends the use of the host-agent-environment concept to describe the various factors playing a role in the pathogenesis of vitamin A deficiency: the human being as host, diet as the agent while the environment comprises the physiological, pathological (including infections), socioeconomic and psychological factors that influence vitamin A intake and requirement, and consequently vitamin A status.

Iron deficiency and anaemia

The national prevalence of anaemia in Indonesia is estimated at 52% while for pregnant women, estimates vary from 40% to 63% with iron deficiency as the primary cause (30). Indonesia is thus typical of many developing countries where in 1988, the overall prevalence of anaemia in pregnant women was estimated to be 44% (56% in pregnant women, and 43% in non-pregnant women) (2) with higher than average prevalences in South and East Asia. In the present cross-sectional study (Chapter 2), about 50% of the

pregnant women had haemoglobin levels <110 g/L. The prevalence is lower than that found in the same area in 1973 (31) when the prevalence was 77% and in the rice-eating area of South Kalimantan (32) where the anaemia prevalence was 63%. There is evidence that, where potatoes or cassava are eaten with rice, the prevalence of anaemia is lower. Consumption of rice as a staple food possibly contributes to a higher prevalence of anaemia, as rice has the potential to inhibit iron absorption (33).

The fact that supplementation of anaemic pregnant women with iron (Chapter 3) resulted in improved iron status indicates that the iron deficiency was nutritional in nature (34-36). As there was no malaria in the study area, this disease could be ruled out as a cause of anaemia. However it is quite possible that intestinal parasites, particularly hookworm, could have contributed to the anaemia. The nutritional nature of the anaemia was also borne out by the low intake of iron, mainly non-haem in nature, together with a considerable intake of inhibitors of iron absorption, such as tea and foods containing phytates, polyphenols and soy protein, observed in the food intake study (Chapter 4). The low intake of iron can be attributed mainly to the low energy intake in the pregnant women studied as the intake of iron relative to energy was higher than that seen in western diets (Chapter 4). No relationship was found between iron intake and iron status but this could possibly be attributed to the fact that insufficient correction was made for the effects of inhibitors and enhancers of iron absorption.

Vitamin A and iron intake and status.

The role of vitamin A deficiency in anaemia has been reported by others earlier, especially in children (8,37-39) and in pregnant women (40). We found not only correlations of serum retinol concentration with haemoglobin, haematocrit and serum iron (Chapter 2) but also an effect of vitamin A supplementation on these parameters in anaemic pregnant women (Chapter 3). Maximum haemoglobin was achieved with both vitamin A and iron supplementation with one-third of the response attributable to vitamin A and two-thirds to iron. The proportion of women who became non-anaemic after supplementation was 35% in the vitamin-A-supplemented group, 68% in the iron-supplemented group, 97% in the

group supplemented with both, and 16% in the placebo group. This is convincing evidence that improvement in vitamin A status, in a vitamin A-deficient population of pregnant women, can contribute to the control of anaemia. The period of supplementation was eight weeks. Thus the question arises whether the effect observed was related to the period of intervention. Further studies will need to be carried out to see whether the same effect can be achieved with a shorter period of intervention or whether larger effects would be seen if supplementation continued for a longer period of time.

The intervention study reported here was carried out using pharmaceutical preparations of iron and vitamin A. However the long-term solution to the problem of vitamin A deficiency will come from the use of foods rich in (pro)vitamin A and iron in a bioavailable form. Such an approach may be more sustainable and eliminate the possibility of overdosing with vitamin A which may produce teratogenic effects (41). In this study, the administration of vitamin A was well controlled but the approach used would not be particularly suitable for use on a wider scale. If it is possible to change food habits, compliance is more likely to be higher with a food approach than with a pharmaceutical approach. A food approach should be seen in the context of a wider approach involving public health measures such as the control of intestinal parasites and, where necessary of malaria. However, the effort required to change food and other habits should not be underestimated.

Conclusions and recommendations

1. Vitamin A deficiency and nutritional anaemia have been shown to be public health problems in pregnant women in a rural area in Bogor, West Java.

2. Vitamin A deficiency contributed to nutritional anaemia in the population studied.

3. Supplementation with vitamin A improved not only vitamin A status but also iron status. The maximum increase in haemoglobin levels was achieved when both iron and vitamin A were provided to the women.

4. Estimates of intake of (pro)vitamin A and iron were below the recommended daily intake in 24% and 53% respectively of the women studied.

5. Although a sharp reduction in the prevalence of anaemia could be achieved using a pharmaceutical approach, problems related to supply, logistics, compliance and monitoring would remain serious issues. Thus a food-based approach should be considered. This should be introduced along side other measures aimed at controlling infections and infestations, improving education particularly in health and nutrition matters, improving environmental sanitation, improving antenatal care and encouraging breast feeding. High-risk areas should be targeted.

6. The production and consumption of foods rich in (pro)vitamin A and iron, which are readily bioavailable, should be encouraged.

7. In order to introduce effective food-based approaches, attention will need to be given at the global and national level to generating, evaluating and distributing improved data on food composition, particularly on the (pro)vitamin A content of foods. The present data are inadequate.

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SUMMARY

Nutritional anaemia, for which iron deficiency is the most important cause, affects 40-63% of pregnant women in Indonesia and this high prevalence is typical of the developing world. Studies from animals and humans have shown that vitamin A can play a role in the aetiology of nutritional anaemia. In order to investigate this, two studies have been carried out in Bogor District, West Java, Indonesia, with the following objectives: 1), to study the prevalence of anaemia and vitamin A deficiency in pregnant women through a cross-sectional study; 2), to investigate the relationship between vitamin A status and iron indices in pregnant women; 3), to investigate the effect of supplementing anaemic pregnant women with iron and/or vitamin A; and 4), to examine the intake of vitamin A and of iron and their relationship with the status of these nutrients.

The prevalence of anaemia and vitamin A deficiency were examined in a cross-sectional study involving 318 healthy pregnant women of low to middle socioeconomic class, aged 20-35 y, in the second or third trimester of pregnancy. The mean value of haemoglobin in this population was found to be 109 (SD 10) g/L. About 49% of the women (157/318) had haemoglobin values below 110 g/L, that could be regarded as anaemic according to the World Health Organization standard for pregnant women. Based on multiple criteria, 44% had iron-deficiency anaemia, 22% had iron-deficient erythropoiesis, and 7% were iron depleted. The median serum retinol concentration of the women studied was 0.80 μmol/L. Based on serum retinol concentrations, 2.5% were categorized as deficient and 31% as marginal. Of 45 women for whom data on the relative-dose response (RDR) test were available, the values ranged from -27.8% to +41%, with a median value of 1.7%. On the basis of an RDR >20%, 9% (4) of women had deficient liver retinol stores.

Significantly positive correlations ($p<0.01$) of serum retinol with haemoglobin, haematocrit and serum iron were observed. From a regression analysis, an increase of 1 μmol/L in serum retinol concentration was associated significantly ($p<0.01$) with an increase of 4.30 g/L in haemoglobin, 1.37 L/L in haematocrit, and 1.18 μmol/L in serum iron. Gestational stage was found to be significantly associated in a negative way with haemoglobin, haematocrit, serum ferritin, and transferrin saturation indicating the stress produced by

pregnancy on iron status.

To investigate whether the association of vitamin A with iron was causal, an intervention study was conducted in the same area. In this study, 251 anaemic pregnant women (haemoglobin values between 80 and 109 g/L) aged 17-35 years, parity 0-4 and gestation 16-24 weeks, were selected for a randomised, double-masked, placebo-controlled field trial. The subjects were randomly allocated to four groups: vitamin A (2.4 mg retinol) and placebo iron tablets; iron (60 mg elemental iron) and placebo vitamin A; vitamin A and iron; or both placebos, daily for 8 weeks. Maximum haemoglobin response was achieved with both vitamin A and iron supplementation (12.78 g/L, 95% CI 10.86 to 14.70), with one-third of the response attributable to vitamin A (3.68 g/L, 2.03 to 5.33) and two-thirds to iron (7.71 g/L, 5.97 to 9.45). After supplementation, the proportion of women who became non-anaemic was 35% in the vitamin-A-supplemented group, 68% in the iron-supplemented group, 97% in the group supplemented with both, and 16% in the placebo group. Supplementation with iron resulted in an increase in serum ferritin and a decrease in total-iron-binding capacity although there was no effect of vitamin A supplementation on these variables. The concentration of iron in serum and transferrin saturation increased with iron supplementation, and to a lesser extent with vitamin A. Serum retinol concentrations increased in women supplemented with vitamin A but not in those supplemented with iron.

A dietary study was also carried out in a subgroup of 104 anaemic pregnant women in the intervention study. Vitamin A status was found to be marginal in 11% and just adequate in 42% of the women. Based on the best available data of carotenoid content of Indonesian foods, the median estimate of daily vitamin A intake, including provitamin A, was found to be 1023 μg retinol equivalent (RE), of which 906 μg RE was derived from provitamin A carotenoids especially green leafy vegetables. These values were about half of those estimated using current data for the (pro)vitamin A content of foods in the Indonesian food composition table thus stressing the need for more analysis of foods in Indonesia especially with respect to vitamin A. About 24% of women had intakes below the daily intake of vitamin A recommended for pregnant women in Indonesia (700 μg RE). There were significant positively correlations between serum retinol concentrations with intake of total

vitamin A ($p<0.001$, r=0.36) and intake of carotenes ($p<0.001$, r=0.37) but not with intake of retinol. The mean intake of iron was 15.5 mg/d, mainly non-haem iron, and this is far below the recommended daily allowance for iron. No correlations were found between iron intake and iron indices except a negative correlation between tea and total-iron-binding capacity ($p<0.01$, r=-0.25).

In summary, the present study shows:

1. The prevalence of iron deficiency anaemia was high among pregnant women in rural area in West Java, Indonesia. Marginal vitamin A status was found although no clinical signs were detected.
2. Serum retinol concentrations were positively associated with haemoglobin, haematocrit and with serum iron concentrations.
3. Supplementation with vitamin A and iron increased haemoglobin levels and the effect of the two nutrients was additive.
4. The median intake of vitamin A, including that from carotenes, was higher than the recommended daily allowance (RDA) but 24% of women had intakes below the RDA.
5. The mean iron intake was below the RDA in 53% of the women.
6. Serum retinol concentrations were correlated with intakes of total vitamin A and carotene but not retinol.
7. There was no relationship between iron intake and iron status.

These findings suggest that measures to combat anaemia in pregnant women should involve improvement in nutritional status not only with respect to iron but also to vitamin A.

SAMENVATTING

Voedingsanaemie, waarvan ijzerdeficiëntie de belangrijkste oorzaak is, treft 40-63% van de zwangere vrouwen in Indonesië wat vergelijkbaar is met andere ontwikkelingslanden. Studies met proefdieren en mensen hebben laten zien dat vitamine A een rol kan spelen in de etiologie van voedingsanaemie. Om dit te onderzoeken zijn in Bogor district (West Java, Indonesië) twee studies uitgevoerd, met de volgende doelstellingen: 1), het bestuderen van de prevalentie van anaemie en vitamine A deficiëntie bij zwangere vrouwen door een cross-sectionele studie; 2), het bestuderen van het verband tussen vitamine A status en parameters van de ijzer status bij zwangere vrouwen; 3), het bestuderen van het effect van supplementatie met ijzer en/of vitamine A bij zwangere vrouwen; en 4), het verband tussen de inname van vitamine A en ijzer en de lichaamsstatus van deze nutriënten te bestuderen.

De prevalentie van anaemie en vitamine A deficiëntie is onderzocht door middel van een cross-sectionele studie, uitgevoerd bij 318 gezonde vrouwen. De vrouwen, 20 tot 35 jaar oud, behoorden allen tot de lage of middelbare sociaal-economische klasse in het tweede of derde trimester van de zwangerschap. De gemiddelde haemoglobine-waarde in deze populatie bedroeg 109 (SD 10) g/L. Ongeveer 49% van de vrouwen (157/318) had haemoglobine-waarden beneden de 110 g/L, een grenswaarde waar beneden volgens de normen van de wereld gezondheidsorganisatie sprake is van anaemie bij zwangeren. Op basis van meerdere criteria had 44% ijzerdeficiëntie anaemie, 22% had ijzerdeficiëntie erythropoiese en bij 7% was de ijzervoorraad uitgeput. De mediane concentratie van retinol in het serum van de onderzochte vrouwen was 0,80 μmol/L. Op basis van de concentratie van retinol in het serum werd 2,5% geklassificeerd als vitamine A-deficiënt en 31% als marginaal. Van de 45 vrouwen waarvan gegevens bij de relative dose response (RDR) test beschikbaar waren, varieerden de waarden van -27,8% tot 41%, met een mediaanwaarde van 1,7%. Op basis van een RDR>20%, had 9% (4) van de vrouwen een deficiënte levervoorraad vitamine A.

De serum retinolconcentraties waren positief gecorreleerd ($p<0.01$) met de haemoglobine-waarden, haematocriet en serumijzer-waarden. Uit regressie-analyse bleek dat een stijging van 1 μmol/L in serum retinolconcentratie gepaard ging met een significant toename van

4,30 g haemoglobine/L, 1,37 L haematocriet/L en 1,18 µmol serumijzer/L (p<0.01). De zwangerschapsperiode was significant negatief gecorreleerd met de haemoglobine-waarden, haematocriet, serum ferritine-waarden en transferrineverzadiging.

Om te onderzoeken of er tussen vitamine A en ijzer status een causaal verband was, werd in hetzelfde gebied een interventiestudie uitgevoerd. Voor deze studie worden 251 anaemische, zwangere vrouwen geselecteerd (haemoglobine-waarden tussen 80 en 109 g/L, 17 tot 35 jaar oud, pariteit van 0 tot 4, 16 tot 24 weken zwanger). voor deelname aan een gerandomiseerde, dubbel-blind, placebo gecontroleerde veldstudie. De proefpersonen werden 'at random' verdeeld over vier groepen: dagelijks, gedurende acht weken vitamine A (2,4 mg retinol) en placebo ijzertabletten (groep 1); ijzer (60 mg ijzer) en placebo vitamine A (groep 2); vitamine A en ijzer (groep 3); of voor beide placebo's (groep 4). De maximale respons in haemoglobinewaarde werd bereikt bij suppletie met zowel vitamine A àls ijzer (12,78 g/L, 95% betrowbaarheidsinterval 10,86 tot 14,70). Éénderde van de respons was toe te schrijven aan vitamine A (3,68 g/L, 95% b.t.b.h.i. 2,03 tot 5,33) en tweederde aan ijzer (7,71 g/L, b.t.b.h.i. 5,97 tot 9,45). Na suppletie werd de proportie van niet anaemiesche vrouwen 35% in de vitamine A-gesupplementeerde groep, 68% in de ijzer-gesupplementeerde groep, 97% in de ijzer- en vitamine A-gesupplementeerde groep en 16% in de placebogroep. Suppletie met ijzer resulteerde in een stijging van serum ferritine en in een daling van in totale ijzer-bindende capaciteit, terwijl er geen effect was van vitamine A suppletie op deze variabelen. Bij ijzersuppletie, en in mindere mate bij vitamine A suppletie, stegen de ijzer concentratie in serum en de transferrineverzadiging. De serum retinol concentratie steeg in de met vitamine A gesupplementeerde vrouwen, maar niet bij de vrowen die met ijzer gesupplementeerd waren.

Bij een subgroep van 104 anaemische, zwangere vrouwen uit de interventiegroep werd een voedingsstudie uitgevoerd. De vitamine A status bleek bij 11% van de vrouwen marginaal en bij 42% net adequaat. Op basis van de best beschikbare gegevens over het carotenoïden gehalte van Indonesische voedingsmiddelen werd geschat dat de mediane dagelijkse vitamine A inname, inclusief provitamine A, 1023 µg retinol equivalenten (RE) bedroeg, waarvan 906 µg RE afkomstig was van provitamine A carotenoïden, vooral groene bladgroenten. Deze waarden waren de helft van de waarden die verkregen worden bij

gebruik van de huidige gegevens voor (pro)vitamine A gehalte van voedingsmiddelen in de Indonesische voedingsmiddelentabel. Dit duidt op de noodzaak van meer voedingsmiddelenanalyses in Indonesië, vooral met betrekking tot vitamine A. Ongeveer 24% van de vrouwen had een inname beneden de in Indonesië voor zwangere vrouwen aanbevolen dagelijkse inname van vitamine A (700 μg retinolequivalenten). Er waren significante, positieve correlaties tussen de serum retinol concentraties en respectivelijk de inname van totaal vitamine A (p<0,001, r=0,36) en de inname van carotenen (p<0,001, r=0,37), maar niet met de inname van retinol. De gemiddelde inname van ijzer was 15,5 mg/d, hoofdzakelijk non-haem ijzer, dit is ver beneden de aanbevolen dagelijkse inname voor ijzer. IJzerinname en parameters van de ijzer status waren niet gecorreleerd, met uitzondering van een negatieve correlatie tussen thee consumptie en totale ijzer-bindende capaciteit (p<0,01, r=-0,25).

Samengevat laat de huidige studie het volgende zien:

1. De prevalentie van anaemie ten gevolge van ijzer deficiëntie was hoog onder zwangere vrouwen in een plattelandsgebied in West Java, Indonesië. Een marginale vitamine A status is gevonden zonder aanwijsbare klinische verschijnselen.
2. Retinolconcentraties in serum waren positief gecorreleerd met haemoglobine, haematocriet en ijzerconcentratie in serum.
3. Suppletie met vitamine A en ijzer verhoogde de haemoglobine-niveaus en het effect van de twee nutriënten was additief.
4. De mediane inname van vitamine A, inclusief die uit carotenen, was hoger dan de aanbevolen dagelijkse inname, 24% van de vrouwen had echter een inname beneden de aanbevolen dagelijkse inname.
5. De gemiddelde ijzerinname was beneden de aanbevolen dagelijkse inname in 53% van de vrouwen.
6. Retinolconcentraties in serum waren gecorreleerd met innames van totaal vitamine A en caroteen, maar niet met retinol.
7. Er was geen verband tussen ijzerinname en ijzerstatus.

Deze bevindingen wijzen erop dat maatregelen om anaemie bij zwangere vrouwen te bestrijden niet allen gericht moeten zijn op de ijzerstatus; ook verbetering van de vitamine A status moet hierin betrokken worden.

RINGKASAN

Anaemia gizi merupakan salah satu masalah gizi utama di Indonesia dan di negara berkembang lainnya. Saat ini, di Indonesia diperkirakan sekitar 40-63% wanita hamil menderita anaemia gizi, terutama anaemia defisiensi besi. Hasil penelitian-penelitian pada hewan percobaan dan pada manusia menunjukkan bahwa vitamin A berperan dalam etiologi anemia defisiensi besi. Untuk mengkaji peran tersebut lebih lanjut, dilakukan dua penelitian pada ibu hamil di Kabupaten Bogor, Jawa Barat, yang bertujuan untuk: 1), Mendapatkan gambaran tentang prevalensi anaemia dan kurang vitamin A pada ibu hamil; 2), Mengetahui hubungan antara status vitamin A dan status besi pada ibu hamil hamil; 3), Mempelajari efek suplementasi besi dan vitamin A pada ibu hamil; dan 4), Menelaah masukan vitamin A dan zat gizi besi serta mengkaji hubungan kedua zat gizi tersebut.

Penelitian pertama dilakukan pada 318 ibu hamil dari golongan sosio-ekonomi menengah dan rendah, umur 20-35 tahun dan pada kehamilan trimester kedua atau ketiga. Didapatkan nilai rata-rata haemoglobin sebesar 109(SD 10) g/L. Sekitar 49% (157/318) mempunyai kadar haemoglobin dibawah 110 g/L, yang menurut standard WHO digolongkan sebagai anaemia untuk wanita hamil. Berdasarkan *multiple criteria*, 44% termasuk *iron-deficiency anaemia*, 22% tergolong *iron-deficient erythropoiesis*, dan 7% tergolong *iron depleted.* Didapatkan median kadar serum retinol sebesar 0.80 μmol/L. Sekitar 2.5% ibu hamil mempunyai kadar serum retinol yang digolongkan sebagai *deficient* dan 31% sebagai *marginal.* Dari 45 ibu hamil yang diperiksa dengan menggunakan metoda *relative-dose response* (RDR) *test*, didapatkan nilai rentang antara -27.8% dan +41%, dengan median sebesar 1.7%. Berdasarkan nilai RDR >20%, ditemukan 9% (4) ibu hamil mempunyai *deficient liver retinol stores.*

Ditemukan hubungan bermakna ($p<0.01$) antara kadar serum retinol dengan kadar haemoglobin, haematokrit dan kadar serum besi. Dari analisa regresi, peningkatan kadar serum retinol sebesar 1 μmol/L setara dengan peningkatan 4.30 g/L haemoglobin dan 1.18 μmol/L serum besi. Didapatkan pula hubungan negatif bermakna ($p<0.01$) antara umur kehamilan dengan kadar haemoglobin, haematokrit, serum ferritin, dan jenuh transferrin yang menunjukkan adanya pengaruh kehamilan terhadap kadar zat gizi besi dalam tubuh.

Untuk mempelajari apakah hubungan vitamin A dengan zat besi merupakan hubungan sebab-akibat, penelitian kedua dilakukan di daerah yang sama. Sebanyak 251 wanita hamil yang anaemia (kadar haemoglobin antara 80 dan 109 g/L), umur 17-35 tahun, paritas 0-4, dan umur kehamilan 16-24 minggu, secara acak dibagi dalam empat kelompok perlakuan: kelompok vitamin A, diberikan vitamin A (2.4 mg retinol) dan placebo tablet besi; kelompok besi, diberikan tablet besi (60 mg besi elemental) dan plasebo vitamin A; kelompok vitamin A dan besi, diberikan vitamin A dan tablet besi; dan kelompok plasebo, diberikan plasebo vitamin A dan plasebo tablet besi. Suplementasi ini diberikan *secara double-masked dan placebo-controlled,* setiap hari selama 8 minggu. Setelah perlakuan, ditemukan kenaikan kadar haemoglobin dalam darah: kenaikan tertinggi ditemukan pada kelompok vitamin A dan besi (12.78 g/L, 95% CI 10.86 - 14.70), sepertiganya karena pemberian vitamin A (3.68 g/L, 2.03 - 5.33) dan dua pertiganya karena suplementasi dengan tablet besi (7.71 g/L, 5.97 - 9.45). Terdapat 35% ibu hamil menjadi tidak anaemia pada kelompok vitamin A, 68% pada kelompok tabet besi, 97% pada kelompok vitamin A dan besi, dan 16% pada kelompok plasebo. Kadar serum ferritin dan *total-iron-binding capacity* meningkatpada kelompok besi, sedangkan hal ini tidak terlihat pada kelompok vitamin A. Kadar besi dalam serum dan jenuh transferrin meningkat pada kelompok besi, dan terdapat juga sedikit peningkatan pada kelompok vitamin A. Kadar serum retinol meningkat pada kelompok vitamin A, tetapi tidak terjadi peningkatan pada kelompok besi.

Survai konsumsi zat gizi juga dilakukan pada 104 ibu hamil (subsampel dari penelitian kedua). Dari populasi ini, didapatkan 11% ibu hamil dengan kadar vitamin A yang *marginal*, dan 42% yang *just adequate.* Estimasi masukan vitamin A dengan memakai data yang lebih akurat untuk kandungan karotenoid bahan makanan di Indonesia, ditemukan estimasi median konsumsi vitamin A, termasuk provitamin A, sebesar 1023 μg *retinol equivalent* (RE) per hari, dan dari jumlah ini 906 μg RE berasal dari carotenoid provitamin A, terutama dari sayuran hijau. Estimasi ini kurang lebih setengah dari nilai estimasi jika menggunakan data (pro)vitamin A dari Daftar Komposisi Zat Gizi Indonesia. Oleh karena itu, usaha pengembangan analisa komposisi bahan makanan Indonesia, terutama untuk vitamin A, sangat diperlukan. Dari estimasi diatas, ditemukan sekitar 24% ibu hamil mengkonsumsi vitamin A perhari dibawah nilai kecukupan (*Recommended Daily Allowance*) yang dianjurkan (700 μg RE). Ditemukan pula hubungan positif antara kadar

serum retinol dengan masukan vitamin A (p<0.001, r=0.36) dan karoten (p<0.001, r=0.37) per hari, akan tetapi hubungan dengan masukan retinol tidak ditemukan. Mean masukan zat besi per hari adalah 15.5 mg/l, terutama berasal dari zat besi *non-haem*, dan jumlah ini jauh dari nilai kecukupan yang dianjurkan. Tidak ditemukan hubungan antara masukan zat besi dengan status besi. Terdapat hubungan yang negative antara konsumsi teh dengan *total-iron-binding capacity* (p<0.01, r=-0.25).

Kesimpulan :

1. Didapatkan prevalensi anaemia yang cukup tinggi pada ibu hamil di daerah penelitian. Didapatkan status vitamin A yang marginal tanpa gejala klinik.
2. Kadar serum retinol mempunyai hubungan bermakna dengan kadar haemoglobin, haematokrit dan kadar besi.
3. Suplementasi vitamin A atau besi meningkatkan kadar haemoglobin dan didapatkan efek aditif vitamin A dan besi.
4. Median masukan vitamin A per hari, termasuk karoten, lebih tinggi dari nilai kecukupan yang dianjurkan, walaupun demikian 24% ibu hamil yang diteliti mengkonsumsi vitamin A kurang dari nilai kecukupan yang dianjurkan.
5. Mean masukan zat besi per hari pada 53% ibu hamil lebih rendah dari nilai kecukupan yang dianjurkan.
6. Terdapat hubungan bermakna antara kadar serum retinol dengan masukan vitamin A dan karoten, akan tetapi tidak ada hubungan dengan masukan retinol.
7. Tidak didapatkan hubungan antara masukan zat besi dan status besi.

Berdasarkan hasil penelitian ini dapat dianjurkan bahwa dalam menanggulangi anaemia pada ibu hamil, perlu memperhatikan status vitamin A disamping status besi.

CURRICULUM VITAE

Djoko Suharno was born on 24 December 1944 in Malang, East Java, Indonesia, and attended elementary and secondary school in Malang and high school in Surabaya. He graduated as Medical Doctor from the Medical Faculty, Universitas Indonesia, Jakarta in 1973 and then was employed as a Government Official at the Ministry of Health. After serving almost 4 years as a rural doctor in East Kalimantan, he joined the Indonesian Nutrition Development Project/IBRD in the Ministry of Health in Jakarta, and served as the Head of the Monitoring and Evaluation Unit in 1978-83. He joined the Regional Course on Food and Nutrition Planning in the University of the Philippines at Los Baños in 1981 and obtained his Master's degree. He also participated the Planning and Appraisal of Health Sector Project Course at the University of Bradford, UK in 1982. From 1983 to 1987 he was Head of the Anaemia Control Section in the Directorate of Community Nutrition, Ministry of Health in Jakarta and Deputy Executive Secretary of the Second Nutrition and Community Health Project/IBRD in 1987-1990. From 1990 he joined the Directorate of Community Participation and served as Head of Subdirectorate of Institutional and Private Sector Health Services.

From October 1989 until September 1992, he carried out the work described in this thesis with the field work being carried out in Bogor, West Java, Indonesia. The preparatory and final phases of the work were undertaken in the Department of Human Nutrition of Wageningen Agricultural University.