FOOD-BASED APPROACHES FOR CONTROLLING VITAMIN A DEFICIENCY: STUDIES IN BREASTFEEDING WOMEN IN INDONESIA

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Stellingen

1. De bijdrage die donkergroene bladgroenten kunnen leveren aan het verbeteren van de vitamine A status is veel kleiner dan tot nu toe werd aangenomen. (*dit proefschrift*)

2. Een voedselvragenlijst is ongeschikt om op individueel nivo het risico op vitamine A gebrek te schatten, zowel wanneer de vitamine A vooral uit plantaardige voedingsmiddelen komt als wanneer andere factoren zoals infectie ook een belangrijke invloed op vitamine A status hebben. (*dit proefschrift*)

3. De uitspraak dat het retinolgehalte van moedermelk beter uitgedrukt kan worden per gram vet in de moedermelk dan per volume moedermelk (*Stoltzfus et al, Int J Epidemiol 1993; 22: 1111-18*) geldt in ieder geval niet voor vrouwen die borstvoeding geven aan een kind van 3 tot 18 maanden oud. (*dit proefschrift*)

4. Bij een gemiddelde serum retinol concentratie van minder dan 0.85 μmol/L kan een verandering van de vitamine A status beter bepaald worden aan de hand van het serum retinolgehalte dan met behulp van de "modified relative dose response" (MRDR) methode. (*dit proefschrift*)

5. De conclusie dat donkergroene bladgroenten xeroftalmie kunnen genezen (*A. Sommer & K.P. West in Vitamin A deficiency, health, survival and vision, 1996, Oxford University Press*) is gebaseerd op resultaten van studies met een zwakke onderzoeksopzet en kan om ethische redenen niet meer getoetst worden.

6. Er dient meer onderzoek gedaan te worden naar de interactie tussen parasitaire infecties en voedingsstatus.

7. De procedures die sommige financiers hanteren voor het verkrijgen, besteden en verantwoorden van het onderzoeksgeld rechtvaardigen een budget-verhoging van 10-30%.

8. Het doen van veldwerk in ontwikkelingslanden vereist een bewuste aanpassing aan de locale cultuur zowel van de buitenlandse onderzoeker als van de nationale onderzoeker.

9. Artsen in ontwikkelingslanden hebben meer aandacht voor de invloed van voeding op de gezondheid dan hun collega's in westerse landen.

10. Een Nederlandse universiteit die haar promovendi niet toestaat om een dankwoord in het proefschrift op te nemen, doet daarmee tekort aan de inspanningen die door velen aan een promotieonderzoek worden geleverd.

11. De sociale interactie in het economisch verkeer vermindert wereldwijd, wanneer in ontwikkelingslanden vaste prijzen het afdingen overbodig maken en in westerse landen de chipkaart ervoor zorgt dat er geen wisselgeld meer nageteld hoeft te worden.

12. De validiteit van de niet-invasieve, bio-energetische functiediagnostiek, ook wel bekend als meting volgens Voll of "Vega test", verdient wetenschappelijke toetsing.

13. De hedendaagse beschikbaarheid van communicatiemiddelen, zoals fax en E-mail, vergroot niet alleen het aantal mogelijke contacten, maar ook het aantal mogelijke misverstanden.

14. Het combineren van twee internationale carrières binnen één relatie vereist veel flexibiliteit.

15. Never let sleeping dogmas lie. (*Bertrand Russell*)

Stellingen behorend bij het proefschrift

Food-based approaches for controlling vitamin A deficiency: studies in breastfeeding women in Indonesia

Saskia de Pee
Wageningen, 21 juni 1996

For everyone striving towards
the elimination of vitamin A deficiency

Abstract

Food-based approaches for controlling vitamin A deficiency: studies in breastfeeding women in Indonesia

PhD thesis by Saskia de Pee, Department of Human Nutrition, Wageningen Agricultural University, Wageningen, the Netherlands. June 21, 1996.

Micronutrient deficiencies seriously hinder mental and physical development and are still an important cause of death in developing countries. Therefore, goals have been set worldwide for the year 2000: to eliminate deficiencies of vitamin A and iodine and to reduce prevalence of iron deficiency anemia in women by one-third of 1990 levels. Food-based approaches for the control of micronutrient deficiencies, using foods naturally rich in micronutrients and/or fortified foods, are preferable, because a pharmaceutical approach can only reach a selective group and is less sustainable. This thesis describes research on the role of foods for improving vitamin A status of breastfeeding women in Indonesia.

In developing countries, people derive 80-85% of vitamin A from plant sources in the form of provitamin A carotenoids, which the body converts to vitamin A. Preformed vitamin A, retinol, exists only in animal foods and accounts for 15-20% of vitamin A intake. Our questionnaire confirmed that dark-green leafy vegetables are the most important source of vitamin A. However, feeding a portion of dark-green leafy vegetables daily for 12 weeks did not improve vitamin A status, while it improved after feeding a wafer enriched with the same amount of β-carotene. This, as well as results from other carefully controlled studies, contradicts the assumption that vegetables rich in provitamin A carotenoids can play an important role in the control of vitamin A deficiency. Causes of poor bioavailability of vegetable carotenoids could include: complex matrix of leaves; absorption inhibitors, such as fibre which entraps carotenoids, or other carotenoids which may compete for absorption; parasitic infestation; and genetic and/or dietary factors.

A very limited effect on vitamin A status should not lead to the conclusion that promoting the consumption of dark-green leafy vegetables is no longer necessary, but food approaches should be based on a variety of foods. Also, factors to calculate vitamin A activity of carotenoids should be reconsidered. In addition, we conclude that vitamin A intake questionnaires, which could be very simple, should only be used to estimate the risk of poor vitamin A status at community level. Vitamin A status and changes in status are best assessed by measuring serum retinol. The measurement of serum β-carotene is recommended for evaluating food-based interventions, because it is very responsive.

Contents

CHAPTER 1

General introduction

Background

Protein energy malnutrition and micronutrient deficiencies are the major causes of death and disability in the developing world. It has been estimated, based on weight-for-age data from 53 developing countries, that 56% of child deaths could be attributed to the potentiating effects of malnutrition in infectious disease [1]. The enormous impact of micronutrient deficiencies can be grasped from the following example. A country with a population of 50 million and a prevalence of micronutrient deficiencies that exists in South Asia today suffers each year, due to inadequate nutriture of vitamin A, iron and iodine [2]:

- 20,000 deaths;
- 11,000 children born as cretins or blinded as preschoolers;
- 1.3 million person-years of work lost due to lethargy and/or more severe disability;
- 360,000 student-years wasted.

At the World Summit for Children in 1990, goals were set for the turn of the century, which were affirmed at the Ending Hidden Hunger Conference in 1991 and the International Conference on Nutrition in 1992. These goals read as follows:

- virtual elimination of vitamin A deficiency;
- virtual elimination of iodine deficiency;
- reduction of iron deficieny anaemia in women of reproductive age by one-third compared with 1990 levels.

Apart from those three micronutrients, many other micronutrients and trace elements, such as zinc and selenium [3], also have essential functions in the human body. More and more attention is currently being paid to deficiencies of those elements which have until now been poorly understood.

The research described in this thesis deals with the role of naturally occurring and locally available and acceptable foods, for controlling vitamin A as well as iron deficiency in breastfeeding women in rural West Java, Indonesia. In this introduction, a short description of micronutrient deficiencies in general is followed by information about micronutrient deficiencies in Indonesia and an outline of the research done.

Vitamin A deficiency

In 1995 it was estimated that 254 million children of preschool age were at risk of vitamin A deficiency [4], and, as a consequence, had a higher risk of morbidity and mortality. Around 13.8 million people suffered from xerophthalmia, the clinical sign of vitamin A deficiency. Vitamin A is involved in the maintenance of differentiated epithelia, in the prevention of blindness, in the formation of specific glycoproteins, in mucous secretion, in disease resistance, in reproduction, and possibly also in preventing specific cancers [5]. Distribution of vitamin A supplements to children in populations with clinical signs of vitamin A deficiency has been shown to reduce child mortality by 23% [6] and to reduce signs and severity of illness episodes [7]. These anti-infection properties are due to its very selective effect on the immune system [8].

Vitamin A status can be assessed with several methods which all have advantages and disadvantages [9]. Because the gold standard of vitamin A status, amount stored in the liver, is very difficult to measure, most indicators have not been validated against this gold standard. This makes it difficult to choose a good indicator. In this thesis, the performance of several biochemical indicators of vitamin A status in women, especially breastfeeding women, is discussed.

Iron deficiency

The latest estimate is that 2,150 million people in the world suffer from iron deficiency *and/or iron deficiency anaemia* [10]. Iron is an essential component of haemoglobin, the oxygen transporting molecule in blood. A shortage of iron results in a shortage of haemoglobin which in turns leads to a shortage of blood, also called anaemia. Anaemia has a variety of symptoms such as pallor, listlessness and fatigue. Anaemia reduces work performance [11], and children suffering from iron deficiency anaemia have slower psychomotor and mental development than their peers who do not suffer from iron deficiency [12]. Iron deficiency anaemia can be reduced with iron supplements, and when it co-exists with vitamin A deficiency, adding vitamin A supplements can result in an additional improvement [13].

Iodine deficiency

It is estimated that 1,005 million people worldwide are at risk of iodine deficiency [10], and as a consequence, at risk of retarded intellectual and physical development. The developmental retardation is due to the lack of thyroid hormones, induced by the lack of iodine [14]. The number of people with an enlarged thyroid gland (goitre), which is the clinical sign of iodine deficiency, is estimated at 225 million.

Requirements of micronutrients and causes of deficiencies

The amount of (micro)nutrients needed to maintain adequate nurtriture depends on the person and his/her condition. Adults for example, need more than children, and pregnant and lactating women usually need more than men [15]. Infections can temporarily increase the need for (micro)nutrients. Micronutrient deficiencies can thus be caused by low intake and/or poor bioavailability of micronutrients as well as by increased losses and/or increased needs. Iron intake may for example be high, but when bioavailability is poor because of the presence of factors which inhibit absorption, or when losses are high due to malaria infection or menstrual bleeding, iron deficiency may develop. The most vulnerable groups for micronutrient deficiencies are infants and young children, and pregnant and breastfeeding women.

Controlling micronutrient deficiencies

There are several ways to prevent or combat micronutrient deficiencies [16, 17]. Direct measures, the pharmaceutical and the dietary approach, are aimed at increasing the intake and/or the bioavailability of micronutrients. Indirect measures can be aimed at increasing the intake as well as at reducing the loss and/or the need for micronutrients.

Through a pharmaceutical approach, high or low dose supplements can be provided at periodic intervals to at risk groups. The approach is highly effective, but needs a continuous supply of supplements and a good delivery system. A more

sustainable approach is the dietary approach, which can use foods naturally rich in micronutrients, including breastmilk, as well as fortified or enriched foods. It could also include foods with a relatively high content of absorption enhancers and/or a relatively low content of absorption inhibitors. Another advantage of an increased consumption of foods naturally rich in micronutrients, apart from being more sustainable, is that more than one (micro)nutrient can be provided at the same time.

Infectious diseases can reduce food intake, reduce intestinal absorption of nutrients and increase urinary losses of nutrients [16]. Prevention of infections can thus be an indirect measure to improve (micro)nutrient status. Measures to prevent infection include immunization, improved environmental sanitation, increased food safety, and good personal hygiene. Other indirect measures include those which increase the availability and affordability of micronutrient-rich foods, such as increased purchasing power, development of crops with better drought resistance, and increasing the distribution area for foods.

The choice of strategies to prevent or combat micronutrient deficiencies depends very much on the local situation: which micronutrient deficiencies exist, what are the major causes, what strategies are feasible etc. By now, salt iodization has been adopted by UNICEF as the major strategy to prevent iodine deficiency disorders [18]. The major strategy to combat iron deficiency is iron pill distribution and improvement of its coverage [17], while vitamin A capsules are distributed to areas with a vitamin A deficiency problem, and dietary approaches, including food fortification, are being advocated widely for prevention of vitamin A deficiency [16].

Prevalence of micronutrient deficiencies in Indonesia

The Republic of Indonesia comprises more than 13,000 islands with a surface area of almost 2 million square kilometres and a population of almost 190 million. With a fertility rate per woman of 2.8, infant mortality of 71/1000 and a life expectancy at birth of 62 years [19], the population is expected to grow to 260 million in 2020 [20]. Approximately 15% of the population lives in absolute poverty [19]. While food consumption patterns change and the prevalence of degenerative diseases such as cardiovascular disease and cancer increases,

micronutrient malnutrition and protein energy malnutrition are still major public health problems.

The national household survey carried out in 1992 has shown that the prevalence of xerophthalmia has decreased from 1.3% in 1978 to 0.3% in 1992 [21]. Therefore, according to WHO criteria [9], vitamin A deficiency is no longer a public health problem in Indonesia. This is a great achievement. Analysis of serum samples from 1255 children, collected within the framework of the household survey, however, showed that 51% had low serum retinol concentrations (<0.70 μmol/L) [22]. This means that efforts to improve vitamin A status of the population in Indonesia should continue.

The national survey also detected that 64% of the pregnant women and 55% of the children between 1-5 years old suffered from iron deficiency anaemia [19]. In addition, it has been estimated that 30-40% of non-pregnant non-breastfeeding women and 20-30% of adult men suffer from anaemia [23]. The national prevalence of goitre has decreased from 37% in 1980/1982 to 28% in 1987/1990 [19]. Data collected in the national household survey also showed that 46% of the boys and 38% of the girls younger than 5 years had weight-for-age below 80% of the NCHS-standard and that for 14% of the boys and 10% of the girls it was lower than 70% of the NCHS-standard [19].

Policies to combat micronutrient deficiencies in Indonesia

The aims with respect to combating vitamin A, iron, and iodine deficiencies in Indonesia are formulated in the national five year plan for 1994-1999 (Repelita VI), as follows [24]:

- elimination of the vitamin A deficiency problem;
- reduction of the prevalence of anaemia
  - in pregnant women: from 63.5% to 40%
  - in underfive children: from 55.5% to 40%
  - in low income workers: from 30% to 20%;
- reduction of the total goitre rate from 27.7% to 18.0%.

Specific strategies to prevent and control micronutrient deficiencies, include the following [24]:

Vitamin A deficiency:

1. Nutrition education to promote the consumption of vitamin-A-rich foods.
2. Further development of fortification of foods, especially noodles and rice.
3. Distribution of vitamin A capsules in specific areas.

Iron deficiency anaemia:

1. Development of fortification of foods such as noodles.
2. Nutrition education to promote the consumption of good food sources of iron.
3. Distribution of iron pills for pregnant women and improvement of the distribution system for the pills.

Iodine deficiency disorders:

1. Improvement of the salt iodization programme.
2. Nutrition education to promote consumption of iodized salt.
3. Development of techniques to iodize other food vehicles.
4. Distribution of iodized oil capsules in endemic areas.

At the same time, research on micronutrients focusses on the use of β-carotene for vitamin A capsules, effective ways of iron supplementation, the use of seafoods to tackle iodine deficiency disorders, the prevalence of other micronutrient deficiencies, and the use of traditional foods as vehicles for food fortification [24].

Those specific strategies and research questions to improve micronutrient nutrtion are complemented by other strategies which aim at a more general improvement of nutrition, health and socio-economic conditions. This includes the "Family Nutrition Improvement Programme (UPGK)" which provides a mixture of growth monitoring, nutrition education, promotion of breastfeeding, distribution of vitamin A capsules and iron/folate pills, referral to local health facilities, promotion of small scale local food production, supplementary feeding and nutrition surveillance based on growth monitoring [25]; the school feeding programme which is being expanded in 1996/1997; the poverty alleviation programme which is targeted at 15% of the population; and investment in human capacity building.

Foods and the prevention of vitamin A deficiency in Indonesia

Now that Indonesia has succeeded in reducing the prevalence of vitamin A deficiency so that it is no longer considered a public health problem, it has to be decided whether and/or how the capsule distribution programme should be continued. In 1994 and 1995, the capsule distribution was continued nationwide because low serum retinol concentrations were still found in a relatively high proportion of the population and the capsules could not yet be replaced by effective dietary strategies. In order to be able to replace the capsule distribution in the near future, food fortification of frequently eaten foods, such as noodles and rice, is currently being developed further and dietary diversification is being promoted. At the same time, the effectiveness of both approaches is being studied.

Vitamin A occurs as preformed vitamin A or retinol, and as provitamin A carotenoids. Provitamin A has to be converted into retinol by the body. Animal foods contain retinol and a small amount of provitamin A, while plant foods contain provitamin A only. Retinol-rich foods are liver, fish with liver intact, milk, butter, eggs and breastmilk. Foods rich in carotenoids are red palm oil, dark-green leafy vegetables, yellow and orange fruits, and red and orange roots and tubers such as carrots and red sweet potato. In Indonesia, as in most developing countries, plant sources provide approximately 85% of the dietary vitamin A intake [15]. Because dietary carotenoids are such an important source of vitamin A and because the evidence for their effectiveness has been based on small studies with often weak designs (see *Chapter 5* of this thesis), the topic of the research described in this thesis was:

> *The role of foods naturally rich in vitamin A to prevent vitamin A deficiency in breastfeeding women in Indonesia, and its concurrent impact on iron status.*

The studies were conducted in breastfeeding women because they are one of the groups vulnerable to vitamin A deficiency.

Outline of thesis

The research described in this thesis was conducted between August 1992 and February 1994 in four rural villages in Bogor District, West Java, Indonesia. This area is characterized by a relative abundance of vegetables.

Chapter 2 describes the development and performance of a vitamin A intake questionnaire for breastfeeding women. The aims of the questionnaire were to determine what the most important food sources of vitamin A were, what the vitamin A intake was and whether vitamin A intake was correlated with vitamin A status.

Chapter 3 reports the results of an intervention study in breastfeeding women with low haemoglobin levels. The aim was to examine the effectiveness in increasing vitamin A status of the most important source of vitamin A in the study area, dark-green leafy vegetables.

Chapter 4 cites and discusses the reactions which we received after publishing the results of the intervention study.

In *Chapter 5* all other intervention studies with carotene-rich fruits and/or vegetables in populations with vitamin A deficiency reported in literature are reviewed. The design and results of those studies have been compared with those of our study. The chapter includes recommendations for designing future intervention studies on the effectiveness of dietary carotenoids for improving vitamin A status.

Chapter 6 evaluates the performance of the following biochemical indicators of vitamin A status in breastfeeding and non-breastfeeding women: the modified relative dose response method and concentrations of serum retinol, breastmilk retinol, and serum retinol binding protein. In addition, serum β-carotene concentration was also evaluated, because this also changes when carotene-rich foods or synthetic β-carotene are given to improve vitamin A status.

Chapter 7 discusses the most important findings of the research described in this thesis. Recommendations are made for the development of effective dietary approaches to prevent vitamin A deficiency, for the use and development of questionnaires on vitamin A intake in areas with a large variety of vegetables, and for the choice of parameters for assessing vitamin A status of breastfeeding women.

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CHAPTER 2

Vitamin A intake of breastfeeding women in Indonesia: critical evaluation of a semi-quantitative food frequency questionnaire

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Submitted for publication

ABSTRACT

The aim of this study was to evaluate a semi-quantitative food frequency questionnaire for assessing vitamin A intake of breastfeeding women in West Java, Indonesia. Usually, food frequency questionnaires seek information on frequency of consumption. But because vegetables, the major source of vitamin A in this area, were prepared in so many different ways, our questionnaire began with questions on frequency of food preparation and methods used.

Total vitamin A intake was estimated at 1951 RE/d, when based on Indonesian food composition tables, and 1489 RE/d, when based on most recent data on carotene content of foods (n = 346). The rank correlation between two assessments of intake 6 weeks apart (n = 59) was lowest for fruits (0.27) and highest for fish (0.60) and meat (0.64). Daily vegetable consumption assessed with the food frequency questionnaire was twice as high as when assessed with the 24-h recall method (n = 175). The following correlations were found (n = 271-281): serum retinol with dietary retinol, 0.26 and with provitamin A, 0.07 (n.s.); serum β-carotene with dietary retinol, 0.24 and with provitamin A, 0.18; serum lutein with dietary retinol, 0.14 and with provitamin A, 0.18.

It should be possible to improve the questionnaire in order to reduce the overestimation of intake and to improve its reproducibility. Correlation of intake with status would however remain low, because the correlation is also influenced by such factors as quality of food composition data, bioavailability of carotenoids, and health and nutritional status. Therefore, estimates of risk of vitamin A deficiency based on a questionnaire should, if at all, only be made at a community level, which would possibly require a very simple questionnaire.

INTRODUCTION

It has been shown that improving vitamin A status of vitamin A deficient populations reduces child mortality by an average of 23% [1], decreases the duration and severity of illness episodes [2], prevents xerophthalmic lesions [3], and reduces the prevalence of anaemia when it co-exists with vitamin A deficiency [4]. Vitamin A can be obtained from natural foods, from fortified foods and from supplements. Plant foods contain provitamin A carotenoids which can be converted into retinol, while the vitamin A in animal foods is mainly retinol (preformed vitamin A) with approximately 10-30% as provitamin A carotenoids.

Vitamin A intake can be estimated with food consumption questionnaires. The aim of such a questionnaire can be to estimate vitamin A intake of a population, to identify important food sources of vitamin A, or to address the question whether a population might be vitamin A deficient [5]. Vitamin A intake has a very large day-to-day variation [6] because the vitamin A content of foods varies widely, even within food groups, and because rich sources of vitamin A, such as liver, are often consumed infrequently. Therefore, vitamin A intake can best be estimated using a food frequency questionnaire which includes the most important vitamin-A-rich foods and uses a reference period most appropriate for the specific patterns of food availability and food consumption. A questionnaire should be evaluated for the purpose for which it has been developed. If the data is to be used to assess the risk of vitamin A deficiency, intake should correlate well with status, estimated intake should approximate real intake, and random error of the intake estimate should be small, or in other words, reproducibility should be high. To ensure that the estimate of intake is precise, the questionnaire should be validated against a reliable food consumption method. In addition, the quality of food composition data should be adequate.

Very few questionnaires for estimating vitamin A intake in developing countries have been evaluated using these criteria. As concluded at the 1994 meeting of the International Vitamin A Consultative Group (IVACG) [7], correlation between vitamin A intake and serum retinol concentrations at the individual level has been found to be poor. The relationship between consumption of vitamin-A-rich foods assessed at community level and the proportion of children with marginal serum retinol levels has been found to be better [8]. It should be possible to develop a food frequency questionnaire which can distinguish between

populations with high and those with low vitamin A status, as long as intake can be classified either as high or as low, but it may be much more difficult to make this distinction at an individual level.

This paper reports data on the evaluation of a questionnaire which we used to estimate vitamin A intake of breastfeeding women in Bogor district, West Java, Indonesia. The questionnaire was developed in September 1992 as part of a cross-sectional study on vitamin A intake and vitamin A status (Bogor92) and it was used again in September 1993 to collect data before the start of an intervention study with dark-green leafy vegetables (Bogor93) [9]. The questionnaire was different from other food frequency questionnaires in that the first question for each vegetable concerned methods of food preparation and the frequency with which they were used. The reason for this was that it was found that each vegetable was prepared in so many different ways and in such different amounts, that a question on frequency of consumption and assuming a standard portion size would not provide an adequate estimate of vitamin A intake. The reproducibility of the questionnaire was evaluated by administering it a second time after an interval of 6 weeks to 60 breastfeeding women who did not participate in the Bogor92 or the Bogor93 study. The correlation between intake and status was calculated and corrected for attenuation, based on the assessment of reproducibility. At group level, the intake of vitamin-A-rich foods as assessed with the food frequency questionnaire was compared with that assessed with a 24-h recall questionnaire administered on 2 or 3 consecutive days. As mentioned above, the 24-h recall method should not be used to estimate vitamin A intake because of large day-to-day variation [6]. However, because of the relatively monotonous composition of the diet, the day-to-day variation of the consumption of vitamin-A-rich foods combined into food groups, is expected to be relatively small. The availability of more recent data on carotene content of foods enabled an evaluation of the impact of using different food composition data on the estimate of vitamin A intake.

Based on the evaluation of our questionnaire and on work published previously, recommendations on how to develop and use vitamin A questionnaires in developing countries are made.

SUBJECTS AND METHODS

Subjects

The data presented in this paper were collected in three seperate studies: the Bogor92 study, the Bogor93 study, and the reproducibility study. All studies were approved by the Medical Ethics Committee of the Ministry of Health, Indonesia and by the Indonesian Institute of Science (LIPI) and all participants gave written informed consent. The studies were conducted in four neighbouring rural villages, 25 km west of Bogor city, Bogor district, West Java. Most inhabitants are of middle and low socioeconomic class and are moslim. A common daily diet consists of two to three rice-based meals with vegetables and dried salted fish, soya products, or meat, and one or more snacks such as fried banana and noodles. Vegetable consumption in this area is characterized by a large variety of vegetables which are available the whole year through due to a nine-months-long rainy season, by many mixed vegetables dishes, and raw green leaves which are dipped in chili sauce ("sambal"). Approximately 10% of the vegetables are consumed in the latter way (personal observation): it is a custom typically found in West Java. Many breastfeeding women do not eat fruit in the first half year after delivery because they believe that it would be deleterious to their health.

Bogor92 study. Participants were randomly selected from four villages after stratification for village, age, breastfeeding or non-breastfeeding status, and, if breastfeeding, age of the breastfed child (1-24 mo). Women who refused participation were replaced by other randomly selected women from the respective strata. A total of 162 women participated in the study, but data of 30 women were excluded from data analysis because they were pregnant ($n = 12$), did not give a urine sample for the pregancy test ($n = 17$), or suffered from tuberculosis ($n = 1$). The data on breastfeeding women ($n = 75$) are reported in this paper. The study was conducted in the period October-December 1992.

Bogor93 study. Participants were selected from two of the four villages which joined the Bogor92 study. They were breastfeeding a child aged 3-17 mo old, had haemoglobin concentrations < 130 g/L, haematocrit < 0.38 L/L, and were to be enrolled in an intervention study [9]. The data collected in September 1993 before the start of the intervention ($n = 212$) were used for this paper. Some of the women were not enrolled in the intervention, because their haemoglobin

concentration did not meet the inclusion criteria.

Reproducibility study. While the intervention study was being conducted in two of the four villages, 60 breastfeeding women were recruited from the other two villages, to study the reproducibility of the food frequency questionnaire. The questionnaire was administered twice with a 6-wk interval in between. Data on 59 women were available for analysis, because one woman had moved before the second interview. The study was conducted between October and December 1993.

Food frequency questionnaire

The food frequency questionnaire was constructed to include the foods which contributed most to vitamin A intake. First of all an exhaustive list was made of foods consumed in the area by visiting local shops, markets and fields. The vitamin A content of those foods was checked in Indonesian food composition tables [10-12] and, if not found, in Malaysian [13] or East Asian [14] food composition tables. Focus-group discussions were held to ask whether any foods were missing from the list, whether foods were consumed often, sometimes, rarely or never, whether the foods were prepared alone or together with other foods, and how the foods were prepared (stir-fried, grilled, steamed etc.). After that, discussions focussed specifically on the list of 70 vegetables. Most of the vegetables were prepared both together with other vegetables and alone. This made it very difficult to make assumptions about the portion size per vegetable. Therefore, we decided to enquire about each vegetable, how often it was prepared mixed and how often not mixed, how much was prepared when mixed and when not mixed, with how many people the different dishes were shared, whether this included the respondent, and whether the dish was eaten with fat. This information was sought for 28 different vegetables, for one group of dark-green leafy vegetables which were only consumed frequently when grown by the respondent herself, and for purchased dishes of mixed vegetables (for example "gado-gado"). In addition we requested data on 11 fruits, 4 dairy products (powdered and condensed milk, butter, margarine), chicken egg, duck egg, chicken meat, cow meat, liver, fresh fish, conserved fish and salted fish. For those foods, respondents were asked how often they ate them and how much each time. The reference

period for all foods was the previous year and the question on frequency was open ended, respondents could answer per week, month or year. Vitamin A intake was calculated by taking edible portions, assuming equal portion sizes for all consumers of the dish, and, for fruits, correcting for seasonal availability.

24-h recall questionnaire

The 24-h recall questionnaire was administered to a subsample of women in the Bogor92 and the Bogor93 studies. First, respondents were questioned on foods and beverages consumed during the previous day. This was followed by questions on ingredients and amounts used for preparation of the dishes and the amount or part of the dish consumed by the respondent.

Food composition data

All data on nutrient content were taken from Indonesian food composition tables [10-12] and, if the food was not found, from Malaysian [13] or East Asian [14] food composition tables, except for data on vitamin A in fruits and vegetables. For those, we have used data available from the most recent analysis of individual carotenoids. Preference was given to analyses from the Department of Human Nutrition of Wageningen Agricultural University [15] or, when those were not available, to the table compiled by West and Poortvliet [16]. For 39% of the fruits and vegetables, which were not frequently consumed, data were not available from either of these two sources. For those foods we have used a value based on a similar fruit or vegetable for which data were available in the table [16]. **Table 1** shows the provitamin A content of the most important vitamin A rich fruits and vegetables according to the most recent sources of data in comparison with those in Indonesian food composition tables. All foods analysed in Wageningen have been included in the table. For calculation of the intake of retinol and provitamin A, the provitamin A content of milk, eggs and poultry was set at 30% of the vitamin A content and of liver, fish, meat and animal fat at 10% [20].

Table 1. Provitamin A content of frequently eaten Indonesian vegetables and fruits in different food composition tables

Foods			Provitamin A content		
Indonesian name	English name	Scientific name	Indonesian tables	Recent data in literature	Analysed[h]
			RE / 100 g food as eaten		
Bayam	Spinach	*Amaranthus viridis*	914[a]	207[d]	640
Daun katuk	Leaf sweet shoot	*Sauropus androgynus*	1556[a]	608[g]	1889
Daun melinjo	Jointfir spinach leaf	*Gnetum gemon*	1500[a]	608[d]	289
Daun pepaya	Papaya leaves	*Carica papaya*	1369[b]	1261[d]	992
Daun singkong	Cassava leaves	*Manihot utulissima*	1650[a]	1055[d]	1776
Kangkung	Water spinach	*Ipomoea aquatica*	945[a]	214[d]	492
Wortel	Carrot	*Daucus carota*	1800[a]	1000[c]	n.a.[i]
Cabe rawit	Green chili	*Capsicum annuum*	1658[a]	65[e]	n.a.
Cabe merah, besar	Red chili, large	*Capsicum annuum*	71[a]	150[c]	n.a.
Kacang Panjang	Yardlong beans	*Vigna Unguiculata*	50[a]	24[d]	n.a.
Mangga	Mango	*Mangifera indica*			
gedong-dong			2528[a]		545
indramayu			447[a]		275
harum manis			185[a]		185
golek			573[a]		210
Pepaya	Papaya	*Carica papaya*	56[a]	201[c]	148[j]
Waluh	Pumpkin	*Cucurbita moschata*	27[b]	374[f]	542[k]

[a] Hardinsyah, Briawan D. 1990 [10]
[b] Nio OK. 1992 (Vitamin A content divided by 2, because of a mistake in the table, personal communication) [12]
[c] West CE, Poortvliet EJ. 1993. [16]
[d] VITAL. 1993 [17]
[e] Sunpuag P. 1988 [18]
[f] Speek AJ, Temalilwa CR, Schrijver J. 1986 [19]
[g] No data available, therefore taken from values for vegetable resembling the vegetable in questions: daun melinjo
[h] Chao X et al. 1996 [15]
[i] n.a.: not analysed
[j] Analysis of papayas from Benin
[k] Analysis of pumpkins from the Netherlands

Biochemical indicators of vitamin A status

In both the Bogor92 and the Bogor93 studies we have measured serum concentrations of retinol, β-carotene and lutein, and in the Bogor93 study also breastmilk retinol concentration. All serum and breastmilk samples were collected after the collection of food consumption data. Serum retinol was analysed at the same laboratory, while serum carotenoids were measured at two different laboratories.

Serum retinol. Women were examined clinically by a medical doctor or a nurse, before drawing a blood sample from an antecubital vein (10 mL in the Bogor92 and 6 mL in the Bogor93 study). The blood was placed on ice protected from light, until centrifugation. Serum was collected after centrifugation and stored at -20°C until analysis of retinol. Serum retinol was analysed at the Nutrition Research and Development Centre (NRDC) in Bogor by HPLC (column, Bondapak C18, Waters, Milford, MA; detector, Shimadzu SPD-6AV, Tokyo, Japan) with methanol/water (90/10 v/v) as mobile phase [21]. For analysis of the samples from the intervention study within-run coefficient of variation was 5.1%.

Breastmilk retinol. Breastmilk was collected in a standardized way. Between 08.00 and 11.00 a.m., all milk from one breast, which had not been used to feed the child during at least the previous hour, was collected using a breast pump (White River Concepts, San Clemente, CA). The breastmilk was stored in dark brown glass bottles and transported to the field laboratory on ice. Breastmilk was stored at -20°C for 0.5-1.5 mo, and subsequently at -80°C, until analysis of retinol. Analyses were performed at Nutricia Research (Zoetermeer, the Netherlands) in a room illuminated with yellow light, using the following method. An aliquot of a thawed and well mixed sample (500 μL) was incubated overnight at room temperature with 500 μL ethanolic (50% v/v) potassium hydroxide (4 M). Then, 2 mL acetonitrile (containing 5% acetic acid) was added and mixed thoroughly. After the two phases had separated, 50 μL of the upper layer was injected onto an HPLC column (Merck, RP-18) which was eluted with a mobile phase of methanol/water (95/5 v/v). Vitamin A was detected spectrophotometrically at 325 nm and quantified by relating the peak area to a calibration curve constructed using standards analysed in the same run. Within- and between-run coefficients of variation were 3.4% and 4.5%, respectively. Recovery of the method was 95-105%.

Serum β-carotene and serum lutein. Within one month after collection, serum samples were transported to the Netherlands where they were stored at -80°C until carotene analysis. Samples from the Bogor92 study were analysed at the Institute for Applied Technology, TNO Voeding, Zeist [22]. The samples collected in the Bogor93 study were analysed at the Department of Human Nutrition, Wageningen [20]. Within- and between-run coefficients of the analyses in Wageningen were 3.4% and 8.2% respectively for β-carotene and 5.7% and 6.6% respectively for lutein.

Statistical methods

The Micronap programme (Northern Technical Data Inc, Winnipeg, Canada) was used to process the food consumption data, and the computer programme SPSS (SPSS Inc, Chicago, Illinois) was used for all statistical analyses.

Because almost all food consumption data were not normally distributed, values are reported as median and 10th and 90th percentiles. Non-parametric tests were used to compare groups: the Kruskal-Wallis test for the comparison of more than two groups, followed by the Mann-Whitney test for comparison of two groups. The Spearman rank-correlation-coefficient was calculated for the reproducibility of the food frequency questionnaire, with a *z*-transformation according to Fisher for its confidence interval [23]. To calculate the correlation between intake and status, their distributions were first normalized by taking the natural logarithm. When intake was 0, the value of the transformation was set at the nearest round figure below the lowest value of the transformation (for example, if 1.83 or -0.24 were the lowest, the values for those with 0 intake were set at 1 and -1 respectively). Pearson's correlation coefficient was calculated for the relationship between the transformed data on consumption and biochemical indicators. Correlations were corrected for attenuation caused by variation in the independent variable intake, as described by Liu and co-workers [24]. A p-value <0.05 was considered statistically significant.

RESULTS

Data on the carotene content of foods

Table 2 shows that the use of the most recent data on carotene content of foods gave an almost 25% lower estimate of vitamin A intake as compared to data from Indonesian food composition tables. This reduction in the estimate of vitamin A intake from plant foods increased the proportion of vitamin A from animal foods from 12 to 16%, and it doubled the proportion of women with an intake below the recommended daily allowance for breastfeeding women of 850 RE/d [25].

Table 2. Vitamin A intake calculated using Indonesian and more recent data on carotene content of foods (data collected with food frequency questionnaire)¶

	Indonesian tables	New data§
	RE/day	
Vegetables		
green leafy	1163 (417, 2479)†	843 (285, 1780)
non-leafy red and yellow	159 (40, 511)	112 (31, 341)
non-leafy green	135 (40, 309)	18 (6, 37)
Fruits	41 (9, 134)	38 (7, 148)
Animal foods‖	211 (37, 1199)	211 (37, 1199)
Total vitamin A	1951 (832, 4111)	1489 (587, 3206)
Retinol	167 (29, 1062)	167 (29, 1062)
Provitamin A	1711 (703, 3396)	1154 (478, 2342)
Vitamin A from animal foods (%)	12 (3, 39)	16 (4, 49)
Proportion <850 RE/d	11%	21%

¶ $n = 346$ (Bogor92 study, $n = 75$; Bogor93 study, $n = 212$; reproducibility study, $n = 59$)
§ Based on data from last column of Table 1 or, in the absence of such data and for foods not listed in Table 1, on data as given in the second last column of Table 1
† median (10, 90 percentiles)
‖ Includes eggs, dairy products, fish and meat

Table 3. Reproducibility of food consumption data collected with food frequency questionnaire ¶

	First assessment	Difference related to first assessment §	Classified into opposite quartile	Difference <20% of individual mean †	Correlation	σ_w^2/σ_b^2 ‡
	g/day	*%*	*% (n)*	*% (n)*		
Vegetables						
green leafy	119 (36, 216) ‖	13 (-54, 139)	7 (4)	29 (17)	0.48 (0.26, 0.66) *	1.09
non-leafy red and yellow	43 (17, 96)	-6 (-67, 230)	9 (5)	22 (13)	0.29 (0.07, 0,53)	2.11
non-leafy green	47 (16, 101)	8 (-45, 139)	9 (5)	19 (11)	0.32 (0.04, 0.51)	2.09
Fruits	69 (16, 225)	18 (-91, 297)	7 (4)	12 (7)	0.27 (0.02, 0.49)	1.86
Eggs and dairy	15 (2, 52)	13 (-76, 600)	5 (3)	20 (12)	0.37 (0.13, 0.58)	1.10
Fish	46 (16, 111)	-4 (-63, 143)	0 (0)	17 (10)	0.60 (0.40, 0.74)	0.78
Meat	7 (1, 40)	40 (-71, 186)	3 (2)	19 (11)	0.64 (0.46, 0.77)	0.50
	RE/day					
Total vitamin A	1516 (754, 2825)	14 (-59, 128)	10 (6)	31 (18)	0.37 (0.13, 0.58)	2.37
Retinol	260 (36, 1301)	33 (-84, 961)	3 (2)	9 (5)	0.31 (0.06, 0.52)	2.48
Provitamin A	1151 (256, 2223)	7 (-50, 119)	9 (5)	29 (17)	0.40 (0.16, 0.59)	1.59

¶ $n = 59$ (reproducibility study)
§ (Second assessment - first assessment) * 100% / first assessment
† Subjects with difference between first and second assessment < 20% of mean of first and second assessment
‡ $(\sigma_{\text{within-subjects}})^2/(\sigma_{\text{between-subjects}})^2$, based on natural logarithm of intake
‖ median (10, 90 percentiles)
* Rank correlation coefficient (95% CI)

Reproducibility of the questionnaire

Table 3 shows that there was no systematic difference between the first and the second assessment of the consumption of vitamin-A-rich foods and vitamin A intake. Less than 10% of the women was classified into the opposite quartile of intake at the second assessment as compared to the first, but only 10-30% of the women had a difference between the first and the second assessment which was smaller than 20% of the mean of the two assessments. The rank-correlation-coefficient between the two assessments ranged from 0.27 for fruits to 0.60 for fish and 0.64 for meat, and the within-subject variation was larger than the between-subject variation for all foods, except for fish and meat.

Comparison of food frequency and 24-h recall questionnaire

The daily consumption of vitamin-A-rich vegetables estimated with the food frequency questionnaire was around 200 g, while the consumption as estimated with the 24-h recall questionnaire was around 100 g (**Table 4**). The difference in vitamin A intake was almost three-fold. The data in the last column of **Table 4** should be interpreted with caution. Because 24-h recall data were used in the denominator, only data from subjects who consumed the respective foods on the days recorded with the 24-h recall questionnaire were included in this calculation.

Relationship between vitamin A intake and biochemical indicators

Serum retinol concentration was <0.70 μmol/L for 36% of the women, while vitamin A intake was <850 RE/d for 25% according to the food frequency questionnaire, and for 66% according to the 24-h recall questionnaire ($n=171$) (data not shown). An intake <500 RE/d was found for 8% and 46% of the women respectively (also not shown).

We have examined the relationship between intake and status at an individual level, by calculating intake for quintiles of status (**Table 5**) and by calculating the correlation between intake and status (**Table 6**). Making quintiles for status instead of for intake would reduce misclassification, because the

Table 4. Comparison of intake of vitamin-A-rich foods and vitamin A as collected with food frequency questionnaire (FFQ) and with 24-h recall questionnaire[¶]

	FFQ	24-h recall	FFQ - 24-h recall	FFQ / 24-h recall	
		g/day		(*n*)	%
Vegetables					
green leafy	118 (42, 287)[§]	49 (0, 146)	66 (-48, 231)	(146)	224 (53, 1299)
non-leafy red and yellow	47 (15, 109)	24 (6, 121)	21 (-49, 83)	(175)	198 (33, 895)
non-leafy green	45 (16, 88)	13 (0, 89)	24 (-47, 71)	(140)	170 (37, 3446)
Fruits	71 (14, 218)	0 (0, 104)	47 (-47, 187)	(71)	135 (20, 608)
Eggs and dairy	12 (2, 48)	0 (0, 27)	8 (-7, 46)	(49)	90 (18, 428)
Fish	40 (18, 100)	40 (12, 82)	1 (-47, 61)	(169)	102 (34, 327)
Meat	6 (1, 22)	0 (0, 16)	4 (-9, 20)	(31)	34 (4, 236)
		RE/day			
Total vitamin A	1447 (529, 3704)	553 (91, 1924)	662 (-354, 2833)	(175)	273 (63, 1853)
Retinol	143 (27, 847)	19 (0, 75)	117 (5, 797)	(154)	512 (96, 3320)
Provitamin A	1137 (408, 2469)	495 (59, 1753)	550 (-455, 1999)	(175)	240 (64, 1521)

¶ $n = 175$ (average of 2 consecutive 24-h recalls for 2 subjects from Bogor92 study and 130 subjects from Bogor93 study, and average of 3 consecutive 24-h recalls for 43 subjects from Bogor92 study)
§ median (10, 90 percentiles)

Table 5. Intake of total vitamin A, retinol and provitamin A calculated from food frequency questionnaires (FFQ) and 24-h recall questionnaires by quintiles of biochemical parameters

Biochemical parameter		Total vitamin A		Retinol		Provitamin A	
		FFQ	24-h recall	FFQ	24-h recall	FFQ	24-h recall
μmol/L	*n*			*RE/day*			
Serum retinol¶							
0.17-0.60§	34	1120 (333, 3456)†	487 (67, 1797)	104 (16, 1342)	26 (0, 84)	1049 (293, 2311)	454 (36, 1764)
0.60-0.74	34	1553 (540, 3430)	559 (185, 1874)	102 (24, 713)	18 (0, 58)	1293 (386, 2775)	545 (155, 1846)
0.74-0.90	35	1447 (585, 4239)	554 (20, 2800)	231 (37, 1159)	20 (0, 189)	1187 (463, 2878)	392 (13, 2442)
0.90-1.17	34	1649 (782, 4493)	511 (58, 2454)	187 (34, 1113)	16 (8, 68)	1487 (607, 3399)	496 (44, 2217)
1.17-2.57	34	1277 (414, 2704)	609 (198, 1191)	137 (14, 586)	19 (0, 78)	1041 (343, 2213)	582 (173, 1160)
Breastmilk retinol‡							
0.10-0.42	26	1158 (320, 3560)	637 (76, 2592)	115 (24, 946)	23 (0, 82)	1054 (253, 2275)	558 (21, 2571)
0.45-0.56	25	1647 (523, 4193)	518 (36, 1720)	147 (27, 1398)	20 (0, 113)	1228 (471, 3983)	472 (24, 1655)
0.59-0.87	27	1465 (495, 2881)	498 (81, 1769)	131 (23, 566)	17 (0, 68)	1250 (396, 2463)	433 (55, 1611)
0.91-1.12	24	1133 (616, 3092)	780 (180, 1677)	113 (19, 1020)	17 (3, 160)	1054 (375, 2138)	766 (133, 1639)
1.15-4.82	25	1356 (442, 4980)	636 (37, 3500)	156 (24, 2491)	12 (0, 58)	791 (291, 2883)	612 (27, 2800)
Serum β-carotene‖							
0.02-0.09	33	1039 (332, 3142)[a]	547 (28, 2739)	68 (19, 765)	16 (0,72)	974 (302, 2172)[a]	492 (18, 2729)
0.09-0.13	33	1099 (539, 2792)[a]	512 (77, 2384)	105 (45, 527)	20 (0, 98)	932 (369, 2578)[a]	483 (65, 1816)
0.13-0.19	32	1253 (559, 3354)[a]	427 (152, 2099)	153 (30, 1027)	29 (0, 224)	984 (400, 2320)[a]	347 (86, 1649)
0.20-0.28	31	1531 (485, 3657)[ab]	567 (111, 1201)	118 (33, 853)	18 (0, 64)	1198 (408, 2934)[ab]	539 (93, 1196)
0.28-2.01	36	1648 (684, 5739)[b]	703 (96, 2392)	220 (22, 1512)	22 (6, 69)	1499 (580, 3758)[b]	638 (58, 2344)
Serum lutein‖							
0.05-0.28	33	1063 (386, 3503)[ab]	553 (78, 2420)	158 (28, 995)	20 (0, 140)	987 (350, 2329)[ab]	538 (35, 1955)
0.28-0.37	33	1039 (447, 1867)[a]	284 (72, 2070)	74 (16, 676)	17 (0, 79)	914 (334, 1760)[a]	253 (57, 2042)
0.37-0.49	33	1579 (600, 3456)[b]	567 (118, 2404)	131 (45, 651)	19 (0, 250)	1333 (530, 2688)[b]	412 (89, 1751)
0.49-0.64	33	1447 (452, 4002)[ab]	591 (46, 1595)	156 (21, 860)	16 (0, 52)	1245 (367, 2529)[ab]	570 (35, 1578)
0.65-1.74	33	1649 (551, 4018)[b]	860 (180, 1821)	167 (29, 1278)	25 (2, 71)	1482 (506, 3013)[b]	839 (147, 1790)

¶ Bogor92 study, $n=44$; Bogor93 study, $n=127$ § min-max † median (10, 90 percentiles)
‡ Bogor93 study, $n=127$ ‖ Bogor92 study, $n=44$; Bogor93 study, $n=121$
[a,b] Values in the same column of a biochemical parameter with a different superscript are significantly different from each other: $p<0.05$ Kruskal-Wallis test (more than two groups), followed by $p<0.05$ Mann-Whitney test (2 groups)

number of possible answers. Reproducibility was lowest for fruits and vegetables and highest for fish and meat. Others have also reported low levels of agreement for consumption of fruits and vegetables at repeated administrations of a food frequency questionnaire and better agreement for infrequently and very frequently consumed foods [26, 27]. We have found only one other report on the reproducibility of a food frequency questionnaire for vitamin A intake from a developing country. The correlation between two assessments of vitamin A intake using a questionnaire with 39 food items and a reference period of 7 d in Guatemala, was 0.49 [28]. This was slightly higher than for our questionnaire, while the reference days for their two interviews were different, because they were taken at a 1-wk interval. Their higher correlation may be due to the smaller number of foods, the use of standard portion sizes and the shorter interval between the two interviews. Suggestions for improving the reproducibility of a food frequency questionnaire include, making categories for frequency of consumption [26], reducing the list of foods as well as the number of questions per food, and assuming standard portion sizes [29]. The first suggestions could be worked out for our questionnaire, but assuming standard portion sizes would be more difficult, because of the many different ways of preparing vegetables.

We found a much higher estimate of consumption of vitamin-A-rich foods with the food frequency questionnaire than with the 24-h recall questionnaire. This was in line with findings from others, ranging from a slightly higher [28] to an almost two times higher estimate of vitamin A intake with the food frequency questionnaire [30-32]. Our higher estimate is most likely an overestimate, because the 24-h recall method can give a better estimate of the average daily consumption of foods grouped into food groups than the food frequency questionnaire. The higher estimate appears to be due to the long list of foods and inclusion of rarely consumed foods. Other investigators in West- and Central-Java who also had to use long lists of foods, also reported a high vitamin A intake [32-34]. In addition, the fact that no specific reference period was used for our questionnaire, may also have contributed to the overestimate. An overestimate can be reduced in different ways, some of which will also improve the reproducibility of the questionnaire. It has been suggested to include questions on the frequency of consumption of food groups [35, 36]. Based on these questions, Tjonneland and co-workers computed a correction factor per food group by dividing the frequency for a food group by the sum of the frequencies of the individual foods of that food group [35]. Another

way would be to reduce the length of the list of foods. This could best be done on the basis of data on the relative contribution of individual foods to vitamin A intake. This requires the availability of detailed food consumption data [37]. On basis of those data, it could also be decided to ask questions on prepared dishes instead of on individual ingredients. Such questions would be easier for the respondents [38], and would thus also improve the reliability of the answers. When such food consumption data do not exist, it is still recommended to include more prepared dishes and fewer individual vegetables in the questionnaire. Including specific dishes also enables a shortening of the questionnaire because standard portion sizes can be set [39]. If more detail is required, the respondent could be asked whether the usual portion is equivalent, smaller or larger than a specified portion size [40]. Another, relatively easy, way to shorten the questionnaire is by making categories for frequency of consumption.

No relationship was found between retinol concentrations in serum or breastmilk and the intake of retinol, provitamin A, and total vitamin A, except for a correlation between serum retinol concentration and retinol intake, corrected for attenuation, of 0.26. The serum concentrations of β-carotene and lutein showed a positive relationship with intake of provitamin A and total vitamin A, and β-carotene also showed a correlation with retinol intake. All the correlations found were relatively low and were only found for intake assessed with the food frequency questionnaire.

Very few studies in developing countries have examined the relationship between vitamin A intake and vitamin A status. Humphrey and colleagues [33] reported that food frequency data collected on preschool children in Indonesia did not correlate with serum retinol concentrations or relative dose response values, but instead, that data collected with the 24-h recall method correlated with both these indicators of vitamin A status. Stoltzfus and colleagues [41] also found no correlation between vitamin A intake and vitamin A status, expressed as breastmilk retinol concentration. However, the vitamin A intake from three foods, which highly correlated with vitamin A intake, was correlated with breastmilk retinol levels. Suharno and co-workers found a correlation of 0.37 between vitamin A intake and serum retinol in pregnant women in Indonesia [34]. The IVACG simplified dietary assessment method to estimate vitamin A intake uses locally determined portion sizes and calculates two scores, one for consumption during the previous month and one for consumption during the previous day [5]. The

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SUMMARY

There is little evidence to support the general assumption that dietary carotenoids can improve vitamin A status. We investigated in Bogor District, West Java, Indonesia the effect of an additional daily portion of dark-green leafy vegetables on vitamin A and iron status in women with low haemoglobin concentrations (< 130 g/L) who were breastfeeding a child of 3-17 months.

Every day for 12 weeks one group (n = 57) received stir-fried vegetables, a second (n = 62) received a wafer enriched with β-carotene, iron, vitamin C and folic acid, and a third (n = 56) received a non-enriched wafer to control for additional energy intake. The vegetable supplement and the enriched wafer contained 3.5 mg β-carotene, 5.2 mg and 4.8 mg iron, and 7.8 g and 4.4 g fat, respectively. Assignment to vegetable or wafer groups was by village. Wafers were distributed double-masked. In the enriched-wafer group there were increases in serum retinol (mean increase 0.32 [95% CI 0.23-0.40] μmol/L), breast-milk retinol (0.59 [0.35;0.84] μmol/L), and serum β-carotene (0.73 [0.59;0.88] μmol/L). These changes differed significantly from those in the other two groups, in which the only significant changes were small increases in breast-milk retinol in the control-wafer group (0.16 [0.02;0.30] μmol/L) and in serum β-carotene in the vegetable group (0.03 [0.00;0.06] μmol/L). Changes in iron status were similar in all three groups.

An additional daily portion of dark-green leafy vegetables did not improve vitamin A status, whereas a similar amount of β-carotene from a simpler matrix produced a strong improvement. These results sugest that the approach to combating vitamin A deficiency by increases in the consumption of provitamin A carotenoids from vegetables should be re-examined.

INTRODUCTION

Vitamin A supplementation and food fortification have beneficial effects on child mortality and morbidity [1, 2]. Supplementation of children and pregnant women with anaemia and vitamin A deficiency increases not only serum retinol but also haemoglobin concentrations [3, 4]. Vitamin A supplements given to women shortly after delivery increase serum and breastmilk retinol concentrations [5].

Of the strategies to reduce vitamin A deficiency, the dietary approach is increasingly being emphasized because it is sustainable, provides nutrients other than vitamin A, and adds variety to the diet. In developing countries, fruit and vegetables provide 70-90% of total vitamin A intake from their high content of provitamin A carotenoids [6]. However, studies on the effectiveness of vegetables and fruits to prevent vitamin A deficiency are scarce [7]. One well-controlled study showed an increase in serum retinol after consumption of red sweet potato and dark-green leafy vegetables [8] but other intervention studies that have shown positive results were controlled poorly or not at all, while cross-sectional and case-control studies had weak designs [7].

We have examined the extent to which an additional daily portion of local dark-green leafy vegetables can improve vitamin A status in anaemic breastfeeding women in a rural area in West Java, Indonesia. The effect on iron status was also examined. The women receiving vegetables were compared with others given a wafer enriched with β-carotene, iron, vitamin C and folic acid, so that we could examine the effect of a similar amount of micronutrients in a simpler matrix with better bioavailability. A third group received a non-enriched (control) wafer to allow for effects of additional energy intake.

SUBJECTS AND METHODS

Subjects

The study was carried out from September, 1993 to January, 1994, in two neighbouring villages in Bogor district, West Java. Most inhabitants are of middle or low socioeconomic class. The area is free of malaria. A large variety of fruits, vegetables, and staples are available all year. The usual diet consists of two to

three rice-based meals with vegetables and dried salted fish, soya products, or meat, and one or more snacks (fried banana, noodles, and cookies). Many breastfeeding women do not eat fruits for 6 months after delivery, believing them to be harmful to their health.

Power calculations, based on within-individual changes from a previous study of pregnant women [4], showed that the number of subjects per group required to detect a 0.12 μmol/L difference between the groups in change of serum retinol concentration was 55 and to detect a 5 g/L difference in change of haemoglobin concentration was 49, with a power of 0.90 and α of 0.05. To allow for 15% dropouts, we decided to recruit three groups each of 65 subjects. In the two villages, more than 95% of all women breastfeeding a child younger than 18 months (n = 730) were screened for anaemia (haemoglobin < 120 g/L) with the Hemocue device (HemoCue, Angelholm, Sweden). We selected women with anaemia (58% of total) because they are more likely to have low serum retinol concentrations [4]. They were asked to provide two samples of faeces. 294 (72%) women did so and became eligble for the study. For each of the ten administrative areas of the village assigned wafers (8000 inhabitants), eligble women were ranked by age of the breastfed child and assigned alternately to enriched or control wafers. Eligble women in the vegetable-assigned village (6000 inhabitants) were matched to the women in the wafer-assigned village for age of the breastfed child, with equal distribution over the eight administrative areas. The groups did not differ in parasitic infestation. When a woman could not participate, another eligble woman from the same area with a breastfed child of similar age was invited. Of the 256 women approached, 191 were enrolled in the study. The main reasons for non-participation were: increased haemoglobin at baseline blood collection (11%), logistic difficulties (7%), and refusal (6%). The women enrolled were breastfeeding children aged 3-17 months, and had baseline packed-cell volumes below < 0.38 and baseline haemoglobin below 130 g/L. (Because of difficulties with the baseline haemoglobin measurement, we were unable to comply strictly with our enrolment criteria for haemoglobin of < 120 g/L).

All participants gave written informed consent, and the study was approved by the medical ethics committee of the Ministry of Health, Indonesia, and the Indonesian Institute of Science.

Supplements

To avoid changes in food consumption due to knowledge of the other treatment, assignment to treatment groups was done by village. A study during the previous year (unpublished) had shown that within-village differences in food consumption and nutritional status of breastfeeding women were larger than between-village differences. Supplements were provided 5 days per week for 12 weeks.

Vegetable supplements were stir-fried according to local recipes, one for each day of the week. They consisted of 100-150 g vegetables per portion - cassava leaves (*Manihot utilissima*), water spinach (*Ipomoea aquatica*), spinach (*Amaranthus viridis*), or carrots (*Daucus carota*). In the other village, wafers were delivered personally each day by village volunteers. The enriched wafer contained β-carotene, vitamin C and folic acid and iron. The taste and appearance of the enriched and control wafers were identical but the foil wrappings were different (red or blue), which permitted double- masked distribution. The code of the wrapping was known only to the manufacturer (General Biscuits, Netherlands) until after data analysis.

Special care was taken to ensure consumption of the supplements and to avoid replacement of part of the usual diet by the supplement. Consumption of all supplements was observed and vegetable portions were given to subjects in the early morning when they would not replace vegetable dishes prepared by the participants. When participants were absent from the village, they were given replacement vegetable portions on weekend days or wafers to be consumed on the days spent away from home. Participants were carefully informed about the purpose of the study and the importance of not changing the usual diet. Possible changes in food consumption, especially of vegetables, were checked by assessing food consumption before the intervention and in the 7th week, by means of a 24 h recall questionnaire administered on 2 consecutive days to a subgroup (n = 108). 54 women from the vegetable group were compared with 54 women drawn equally from both wafer groups, because no difference in adaptation was expected between those receiving the two types of wafer.

Methods

Samples of blood and urine were collected from each participant at baseline (1 day before the intervention) and at follow-up (1 day after consumption of the last supplement). Breastmilk samples were collected 1 or 2 days before blood collection. Measurements of height at baseline and body weight with light clothing at baseline and follow-up were all made by the same person. For each subject, baseline and follow-up samples were analysed in the same run for each substance. Samples were coded to conceal treatment group.

Breastmilk was collected in a standard way. Between 0800 and 1100 h all milk from one breast, which had not been used to feed the child for at least 1 h, was collected by means of a breast pump, stored in dark-brown glass bottles, and transported to the laboratory on ice. Creamatocrit was measured in triplicate and averaged. Fat content was related to that measured by extraction, based on measurements by both methods made on 22 breastmilk samples. Breastmilk was stored at -20°C (2-6 weeks) then at -80°C until analysis of retinol. The samples were analysed in a room illuminated with yellow light. 500 μL samples were incubated overnight at room temperature with 500 μL ethanolic (50% by volume) potassium hydroxide (4 mol/L). 2 mL acetonitrile with 5% acetic acid was added. After seperation, 50 μL of the upper layer was drawn off and subjected to high performance liquid chromatography (HPLC). Vitamin A was detected spectrophotometrically at 325 nm and quantified by relating the peak area to a calibration curve constructed from standards analysed in the same run. Within-run and between-run coefficients of variation were 3.4% and 4.5%. Recovery in the method was 95-105%.

Blood - For the modified-relative-dose-reponse (MRDR) measurement, participants were asked not to eat vitamin-A-rich foods after the evening meal on the day before blood sampling. At 0730 h the next morning they were given 8.8 μmol 3,4-didehydroretinol acetate in 250 μL corn oil, followed by a snack high in fat and low in vitamin A. 5 h later subjects were physically examined and 6 mL blood was drawn from an antecubital vein, placed on ice, and protected from light. Haemoglobin concentrations were measured, at first with the Hemocue device, but we found it gave erratic results when the seal of the bottle of cuvettes had been broken for more than a few days (probably because of the warm and humid climate). During initial screening for anaemic women this problem did not arise

because all cuvettes in a bottle were used within 1 or 2 days. After a third of the subjects' haemoglobin concentrations had been measured at baseline with the Hemocue, those of other subjects were measured by the cyanmethaemoglobin method. During the third week, haemoglobin concentration was measured by this method in all subjects; it was found not to differ from the values measured at baseline (95% CI for difference -1.4 to 1.8 g/L], n = 117). White blood cells were counted and zinc protoporphyrin concentration was measured (Proto-Fluor-Z, Helena Laboratories, Beaumont, Texas, USA).

Serum - Remaining blood was centrifuged, and serum was frozen for HPLC analysis of retinol and dehydroretinol [9] (within-run coefficients of variation 5.1% for retinol, 10.4% for dehydroretinol, 9.5% for dehydroretinol/retinol ratio). Serum carotenoids were measured by HPLC [10] (within-run and between-run coefficients of variation 3.4% and 8.2% for β-carotene, 4.6% and 7.0% for α-carotene, 3.6% and 11.4% for β-cryptoxanthin, 5.7% and 6.6% for lutein, and 9.6% and 9.3% for zeaxanthin). Serum ferritin was measured by radio immuno assay (Ciba Corning, Medfield, Massachusetts, USA), serum transferrin receptor by ELISA [11], and serum albumin by a standard method.

Urine - Pregnancy tests were done on urine samples at baseline and follow-up and women found to be pregnant were excluded.

Faeces - Samples (562) were collected on ice from 294 women and stored at 4°C for no more than 2 d before examination for protozoa, cysts, and helminth eggs. To check the results, 17 samples with multiple infections were examined both in Bogor and at the Laboratory for Parasitology, Leiden University, Netherlands.

Supplements - Duplicate portions of vegetables (5 consecutive days, four occasions) and wafers (8 enriched, 8 control, two occasions), were analysed for fat, proten, fibre, and iron [12, 13] and carbohydrate was analysed by difference. Carotenoids were measured in vegetable portions [14] and β-carotene in wafers [15]. The α-carotene content of the wafers was estimated from information supplied by the manufacturer of the β-carotene used in the wafers. Vitamin C [16] and folic acid (microbiological assay with *Lactobacillus casei*, coefficient of variation 8.9%) were measured in vegetables and wafers. Results of the analyses are shown in **Table 1**.

Table 1. Composition of the supplements as analysed, averaged from the results of analysis of individual samples, expressed per one-day portion

	Vegetables	Enriched wafer	Control wafer
Carotenoids (mg)			
All-*trans*-ß-carotene	3.5	3.5	0.1
α-carotene	0.4	<0.1 ¶	-
Lycopene	ND	-	-
ß-cryptoxanthin	ND	-	-
Lutein and zeaxanthin	5.5	0	0
Micronutrients (mg)			
Iron	5.2	4.8	0.4
Vitamin C	11.4	21.5	0.2
Folic acid	0.13	0.10	0
Major nutrients (g)			
Fat	7.8	4.4	4.5
Protein	3.1	0.8	0.8
Carbohydrates	2.7	7.3	7.6
Dietary fiber	3.6	0.1	0.4
Energy (kJ)	395	304	315

¶ Estimated. Results averaged from analysis of individual samples, expressed per 1-day portion. ND = not detected. Some analyses were not done on the wafers since the nutrients were not added to the wafers.

Food intake - For the 24 h recall questionnaire, amounts of the wafer and vegetable supplement were not recorded. Where possible, duplicate portions of rice were weighed to the nearest gram. Carotenoid content was taken from a table of foods from developing countries [17], and content of other nutrients from local food-composition tables (references available from CEW). The Micronap programme (Northern Technical Data Inc, Winnipeg, Canada) was used for all calculations.

Statistics

To examine differences between groups, analysis of covariance was used for normally distributed variables and the Mann-Whitney test (two groups) or Kruskal-Wallis test (three groups) for non-normally distributed variables. Changes

from baseline to follow-up were calculated for each individual by subtraction, even for variables with skewed distributions, because the calculated changes were normally distributed. To compare baseline and follow-up values within treatment groups, a paired-t-test was used for all variables, except intake of foods rich in vitamin A (excluding supplements), for which Wilcoxon's signed-rank test was used because many subjects did not consume the foods of one or more subgroups. To compare calculated changes from baseline to follow-up among treatment groups, with control for other factors, analysis of covariance was used with dummies for treatment groups [18]. In addition, when the variation of calculated changes differed greatly between groups, analysis of covariance was used to compare two groups with similar variation.

RESULTS

Follow-up data were obtained was 175 (91.6%) women. 5 women had became pregnant, 3 had moved away from the study area, the breastfed child of 1 had died, 1 woman had excessive menstrual blood loss, and 6 women refused further participation. Apart from a small difference in height, the characteristics of the remaining subjects did not differ among the groups (**Table 2**).

Serum albumin concentrations ranged from 32 to 56 g/L, a little lower than the normal range (40-60 g/L). The range of leucocyte counts ($4.0\text{-}10.4 \times 10^9$/L) was close to the normal range ($3.2\text{-}9.8 \times 10^9$/L). Based on these findings and on the absence of signs of infection at clinical examination, no subject was excluded from data analyses.

Food consumption

During the intervention period, there was no change in food availability in the study area. Intake of macronutrients and iron (**Table 3**) did not change significantly during the intervention (supplements excluded) in the vegetable group or in the combined wafer group (there were no differences between the two wafer groups). Baseline carbohydrate intake, and thus the proportion of energy from various nutrients, differed between the vegetable and combined wafer groups, but this

Table 2. Characteristics of participants at baseline

	Vegetable group (n = 57)	Enriched-wafer group (n = 62)	Control-wafer group (n = 56)
Demographic and anthropometric data			
Age breastfed child (months)	8.9 (3.9)	8.9 (4.3)	8.9 (4.0)
Parity	5.0 (3.4)¶	5.0 (2.9)	4.6 (2.6)¶
Bodyweight (kg)	46.1 (4.9)	46.4 (5.5)	48.1 (6.9)
Height (m)	1.48 (0.05)*	1.49 (0.05)	1.51 (0.06)
Body mass index (kg/m^2)	21.1 (1.9)	20.9 (2.1)	21.2 (2.7)
Weight change (kg), baseline to follow-up	-0.5 (1.5)	-0.4 (1.3)	-0.3 (1.4)
Parasitic infestation (% with positive stool)§			
Ascaris	82	84	80
Trichuris	93	95	88
Giardia lamblia	2	8	7
Entamoeba histolytica	29	26	25
Hookworm	0	5	5

Data presented as mean (SD) or % of group. ¶ 1 woman was breastfeeding twins. * $p < 0.05$ *vs* control-wafer group (ANOVA). § Data missing for 1 woman in vegetable group.

difference is unlikely to affect vitamin A status. During the intervention, carbohydrate intake was similar in the two groups.

Because the very large day-to-day variation of vitamin A intake made it difficult to assess changes in this variable with a 24-h recall questionnaire, changes were examined also in terms of vegetable and fruit intake (**Table 4**). Intake during the intervention, excluding supplements, did not differ from that at baseline in either group. The combined wafer group consumed more non-leafy green vegetables at baseline and more fruits during the intervention. However, these differences are unlikely to have had much impact on vitamin A status. Total vitamin A intake did not differ between the groups.

Table 3. Daily intake of macronutrients and iron and change from baseline to 7th week of intervention in subgroup who answered 24 h recall questionnaire

	Vegetable group (n = 50)		Combined wafer group (n = 54)	
	Baseline mean (SD)	Change (95% CI)	Baseline mean (SD)	Change (95% CI)
Energy (MJ)	10.2 (2.7)	0.4 (-0.6 to 1.3)	10.7 (3.3)	0.5 (-1.4 to 0.5)
Protein				
Weight (g)	65 (20)	-4 (-11 to 3)	63 (20)	-3 (-8 to 2)
% of energy	10.9 (1.9)*	-0.9 (-1.6 to -0.3)*	10.2 (1.6)	0 (-0.6 to 0.5)
Fat				
Weight (g)	73 (20)	0 (-11 to 11)	63 (24)	6(-3 to 14)
% energy	27.0 (8.0)**	-1.3 (-3.9 to 1.3)*	22.7 (6.9)	2.6 (0.7 to 4.6)
Carbohydrates				
Weight (g)	376 (111)*	29 (-12 to 70)*	431 (155)	-31 (-74 to 12)
% energy	64.1 (7.4)**	2.3 (-0.4 to 4.9)*	68.5 (6.6)	-1.6 (-3.6 to 0.4)
Iron (mg)	13 (6)	-2 (-3 to 0)	13 (6)	0 (-2 to 2)

Supplements excluded. * $p<0.05$, ** $p<0.01$ for difference between vegetable and combined wafer group (ANOVA).

Vitamin A status

3% of women had deficient serum retinol concentrations (<0.35 μmol/L) and 33% had marginal values (0.35-0.70 μmol/L). Breastmilk concentrations were deficient (<0.35 μmol/L) in 7%, and marginal (0.35-0.70 μmol/L) in 39%. There were no significant differences between the groups at baseline (**Table 5**). During the intervention, serum retinol increased significantly (38%) in the enriched-wafer group; this change differed significantly from those in the other two groups (**Figure 1**). Similarly, the substantial increase in breastmilk retinol in the enriched wafer group (67%) differed significantly from the changes in the other two groups ($p<0.01$, ANOVA).

Serum β-carotene concentration increased by 390% in the enriched wafer group and by 17% in the vegetable group; there was no significant change in the control-wafer group. In terms of change in serum β-carotene concentration, the

Table 4. Daily intake of carotenoids, retinol and groups of vegetables and fruits by weight and vitamin A content at baseline and in 7th week of intervention, median (interquartile range)

	Vegetable group (n = 50)		Combined wafer group (n = 54)	
	Baseline	Intervention	Baseline	Intervention
Green leafy vegetables				
Weight (g)	44 (4-117)	38 (0-90)	50 (11-78)	33 (2-81)
Vitamin A content (RE)	137 (9-447)	117 (0-403)	136 (27-373)	111 (11-271)
Non-leafy red and yellow vegetables				
Weight (g)	24 (15-53)	29 (12-68)	26 (13-62)	37 (21-65)
Vitamin A content (RE)	9 (5-59)	17 (10-109)	18 (10-81)	21 (12-128)
Non-leafy green				
Weight (g)	3 (0-43)*	5 (0-34)	32 (2-87)	14 (2-41)§
Vitamin A content (RE)	2 (0-11)*	3 (0-9)	8 (1-24)	4 (1-10)§
Fruits				
Weight (g)	0 (0-65)	0 (0-65)¶	0 (0-38)	55 (0-86)§
Vitamin A content (RE)	0 (0-8)	0 (0-8)¶	0 (0-5)	8 (0-14)§
Total carotenoids (RE)	337 (124-605)	289 (37-597)	351 (132-558)	273 (137-575)
Total retinol (RE)	19 (8-53)	13 (5-43)	16 (6-33)	12 (5-29)

Supplements excluded. * Differed significantly ($p < 0.05$) from baseline value of wafer group. § Differed significantly ($p < 0.05$) from baseline within group. ¶ Differed significantly ($p < 0.05$) from follow-up value of wafer group.

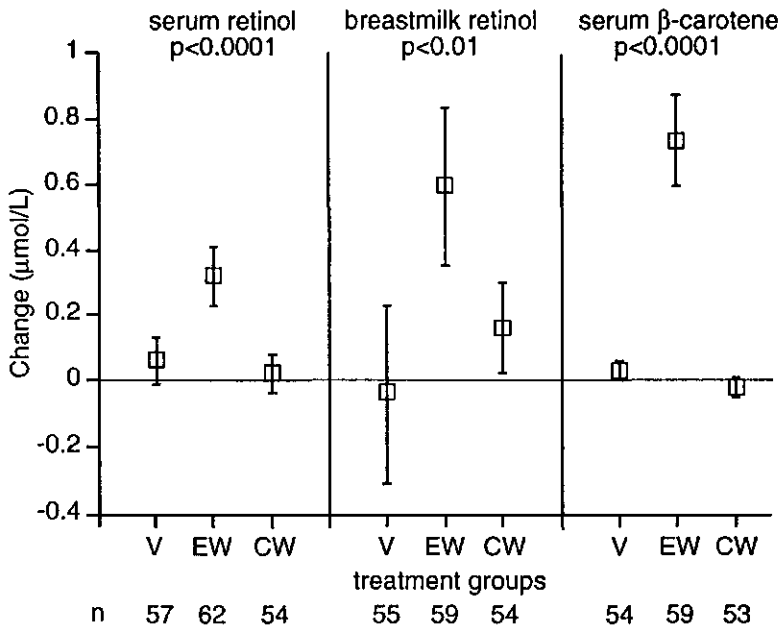

Figure 1. Changes in serum retinol and *β*-carotene and breastmilk retinol concentrations from baseline to 12 weeks in vegetable (V), enriched-wafer (EW), and control-wafer (CW) groups

Mean and 95% CI. p values for difference from other two groups (ANOVA), controlled for age of breastfed child, individual weight change, and for breastmilk, milk fat changes.

vegetable group and the control-wafer group differed significantly ($p < 0.01$) from each other only when analysis of covariance was done irrespective of the largevariation in response in the enriched wafer group.

Changes in the serum concentration of carotenoids other than *β*-carotene were very small (**Table 5**). Serum *β*-cryptoxanthin concentrations did not change significantly in any group. Serum *α*-carotene increased more in the enriched-wafer group than in the other two groups, whereas the changes in serum lutein and

Table 5. Vitamin A status at baseline and changes from baseline to 12 weeks

	Vegetable group (n = 53-57) ‖		Enriched-wafer group (n = 58-62) ‖		Control-wafer group (n = 53-54) ‖	
	Mean (SD) at baseline	Mean (95% CI) change	Mean (SD) at baseline	Mean (95% CI) change	Mean (SD) at baseline	Mean (95% CI) change
Serum retinol (μmol/L) §	0.89 (0.04)	0.06 (-0.01 to 0.14)	0.84 (0.04)	0.32 (0.23-0.40)*	0.81 (0.04)	0.02 (-0.04 to 0.09)
Breastmilk retinol (μmol/L) ¶	0.98 (0.92)	-0.04 (-0.31 to 0.23)	0.88 (0.59)	0.59 (0.35-0.84)*	0.84 (0.51)	0.16 (0.02-0.30)*
Serum β-carotene (μmol/L) §	0.19 (0.12)	0.03 (0-0.06)*	0.19 (0.12)	0.73 (0.59-0.88)*	0.17 (0.09)	-0.02 (-0.04 to 0.01)*
Serum α-carotene (μmol/L) ¶	0.07 (0.04)	0.01 (0-0.02)*	0.06 (0.03)	0.03 (0.02-0.04)*	0.06 (0.03)	0 (-0.01 to 0.02)
Serum β-cryptoxanthin (μmol/L)	0.08 (0.07)	0.02 (-0.01 to 0.04)	0.07 (0.06)	-0.01 (-0.02 to 0.01)	0.06 (0.04)	0 (-0.01 to 0.01)
Serum zeaxanthin (μmol/L) †	0.11 (0.05)	0.01 (0-0.02)	0.10 (0.05)	-0.01 (-0.03 to 0)*	0.11 (0.05)	-0.02 (-0.03 to 0)*
Serum lutein (μmol/L) †	0.48 (0.27)	0.05 (-0.01 to 0.11)	0.47 (0.24)	-0.12 (-0.18 to -0.06)*	0.43 (0.19)	-0.06 (-0.11 to -0.01)*
Median dehydroretinol/retinol ratio (IQR)	0.09 (0.05-0.15)	-0.01 (0.03 to 0)	0.07 (0.04-0.11)	-0.03 (-0.05 to -0.01)*	0.09 (0.04-0.13)	0.02 (-0.01 to 0.4)

‖ Dehydroretinol/retinol ratio, n = 47, 52, 49 for vegetable, enriched-wafer, control-wafer groups. * Significantly different from baseline ($p < 0.05$). §, ¶ For comparisons between groups, change in enriched wafer group was greater than changes in other groups (ANOVA, control for age of breastfed child, individual weight change, and (for breastmilk) milk fat changes; § $p < 0.001$, ¶ $p < 0.01$. † Vegetable group differed from other groups in change from baseline (ANOVA).

zeaxanthin in the vegetable group differed from those of the other groups ($p < 0.01$).

At baseline, 68% of the women had dehydroretinol/retinol ratios above 0.06, indicating low vitamin A status [9], but there were no differences in the ratio among the groups (**Table 5**). There was a significant ($p < 0.001$) decrease in the dehydroretinol/retinol ratio in the enriched-wafer group but not in the other two groups. The change in the enriched wafer group was therefore significantly different from that in the other two groups. The small significant difference ($p < 0.05$) between the vegetable group and the control-wafer group was related to a slightly higher ratio in the vegetable group at baseline (mean 0.12 vs 0.10). Analysis of the difference between the two groups corrected for baseline level showed no difference.

Iron status

At baseline, the only difference between the groups in iron status variables was in the transferrin receptor concentration, which was slightly higher in the vegetable group than in the control-wafer group (**Table 6**). The changes during the intervention did not differ among the groups. At follow-up, haemoglobin concentration was significantly higher and serum transferrin receptor concentration significantly lower in all three groups. The transferrin receptor level reflects iron requirements at tissue level. The increase in packed-cell volume and the decrease in zinc protoporphyrin concentrations reached statistical significance in two groups. A decrease in zinc protoporphyrin shows a reduction of iron shortage in red blood cells. The trend of increased serum ferritin concentration in all groups suggests increased iron stores. The changes in iron status could be explained by the fact that the women were recovering from iron loss resulting from pregnancy and delivery, and also by regression to the mean, because a low haemoglobin concentration was one of our selection criteria.

Among women with low (< 0.70 μmol/L) serum retinol concentrations at baseline, packed-cell volume increased significantly ($p < 0.05$) more in the 20 women who received the enriched wafer (0.02 [0.01-0.02]) than in 24 women who received the control-wafer (0 [-0.01 to 0.01]) or in 18 who received vegetable supplements (0 [0-0.01]).

Table 6. Iron status at baseline and changes to 12 weeks' follow-up

	Vegetable group (n = 55-57)		Enriched-wafer group (n = 61-62)		Control-wafer group (n = 55-56)	
	Baseline	Change (95% CI)	Baseline	Change (95% CI)	Baseline	Change (95% CI)
Packed-cell volume*	0.35 (0.02)	0.01 (0-0.01)§	0.34 (0.03)	0.01 (0.01-0.02)§	0.35 (0.02)	0 (0-0.01)
Haemoglobin (g/L)*	110 (10)	8 (6-10)§	108 (10)	9 (7-12)§	109 (10)	8 (5-10)§
Zinc protoporphyrin* (μmol/mol heme)	89 (46)	-7 (-13 to -1)¶	92 (45)	-8 (-14 to -1)¶	77 (23)	-1 (-4 to 2)
Serum ferritin (μg/L)†	9.0 (2.3-25.8)	3 (-1 to 7)	8.5 (2.8-20.0)	2 (-1 to 5)	10.0 (3.0-25.5)	2 (-1 to 7)
Serum transferrin receptor (mg/L)†	4.83 (3.59;5.95)	-0.56 (-0.9 to -0.21)§	4.45 (3.56-5.65)	-0.35 (-0.64 to -0.07)¶	4.08 (3.30-4.91)	-0.33 (-0.52 to 0.15)§

Baseline values are * mean (SD) or median (IQR). Haemoglobin values measured during week 3, rather than baseline. §,¶ Significant change from baseline: § $p < 0.01$, ¶ $p < 0.05$.

DISCUSSION

Our results indicate that β-carotene is very poorly absorbed from dark-green leafy vegetables, but that absorption from an enriched wafer is good. The vitamin A status of women who received the enriched wafer improved substantially but there was no improvement in the vegetable or control-wafer groups. The smaller increase in serum than in breastmilk retinol in the enriched wafer group is possibly due to more precise homeostatic control of retinol concentrations in serum than in breastmilk. In this study population with a wide age range of the breastfed children, the variation in serum retinol response was smaller than that in breastmilk retinol response; serum retinol is therefore a more sensitive indicator of change of vitamin A status. Expression of the breastmilk retinol concentration per gram of milk fat did not affect the within-individual variation in retinol content. A high proportion of the women in this study, 36%, had deficient or marginal serum retinol concentrations, perhaps partly because anaemia was a selection criterion [4] and because the women were at a late stage of lactation [5].

The increase in serum β-carotene concentration in the vegetable group was too small to have had much impact on nutritional status. Serum concentrations of provitamin A carotenoids (α-carotene and β-cryptoxanthin) did not change. The small increase in serum α-carotene in the enriched wafer group could be due to α-carotene in the β-carotene preparation used to enrich the wafer, or to β-carotene supplementation itself [19].

Vitamin A supplementation of anaemic subjects with low vitamin A status can increase packed-cell volume and haemoglobin concentrations [3, 4]. In this study, although vitamin A status improved in women who received the enriched wafer, iron status did not improve in any treatment group. For women with baseline serum retinol below 0.70 μmol/L, however, the increase in packed-cell volume was greater in the enriched wafer group than in the other groups. Increases in haemoglobin showed a similar, but not significant, trend. A possible explanation for the smaller difference in iron status changes between the treatment groups, than was observed in previous studies [4] is that the dose of vitamin A was smaller.

The enriched wafer contained 4.8 mg carbonyl iron and 21.5 mg vitamin C, which could be expected to improve iron status. The bioavailability of carbonyl iron is similar to that of ferrous sulphate [20], but it may be reduced by inclusion in

foods [21]. The vegetable supplement contained 5.2 mg iron and 11.4 mg vitamin C, but bioavailability of iron in vegetables is low [6]. The iron content of our supplements may have been too low to affect iron status.

We established before this study that nutritional status and food consumption of breastfeeding women in the two neighbouring villages were similar. Allocation to treatment had to be by village, because it was impossible to distribute vegetables and wafers in a double-masked way. Randomization of treatment across villages could lead to changes in food consumption by participants because they would be aware of the other method of supplementation. We had to be sure that the vegetable portions provided were eaten and that they did not replace vegetable dishes normally consumed.

Consumption was assured by close supervision of distribution and consumption of supplements. In addition, replacement was avoided by giving the vegetable portions to the participants in the morning. Participants were informed carefully about the purpose of the study and that they could benefit from the supplements only if they did not change their diet and ate every vegetable portion or wafer supplied. Their commitment to the study is shown by the low drop out rate. In addition, the 24-h recall questionnaire, which was used to examine differences in consumption large enough to affect vitamin A status, revealed no within-group differences in intake during the intervention. Weight loss in all three groups was similar and less than expected for unsupplemented breastfeeding women [22], which confirms that the supplements were eaten and that compliance was the same in all three groups. Subjects who gained weight and those who lost weight had almost identical changes in vitamin A status; thus differences between the treatment groups can be ascribed to the supplements. Furthermore, even if a woman did not eat one of her regular vegetable servings, the total amount of vegetables consumed that day would still be increased, because one portion of vegetable supplement contained the amount of vegetables usually consumed in two or three servings on one day.

A difference in compliance between participants would lead to a range of changes in serum β-carotene concentration. The large confidence interval of the increase of serum β-carotene in the enriched-wafer group reflects not so much a possible difference in compliance as a large between-individual variation in bioavailability of β-carotene [23]. The virtual absence of variation in serum β-carotene response in the vegetable group suggests that bioavailability was equally

poor for all subjects, overshadowing any possible difference in compliance. The small increase (in relation to the two wafer groups) in serum concentrations of lutein and zeaxanthin in the vegetable group also supports the evidence that the vegetable supplements were eaten, but that carotene bioavailability was poor.

The current recommendations for consumption of fruit and vegetables to meet vitamin A requirements are largely based on the Sheffield experiment by Hume and Krebs [24]. They found that bioavailability of β-carotene from vegetables and carrots was, on average, only a third of β-carotene in oil. This finding was taken into account when establishing the conversion factor for dietary β-carotene - 6 μg β-carotene equals 1 μg retinol or 1 retinol equivalent. The recommended daily allowance for breastfeeding women is 850 retinol equivalents [6]. In our study, vegetables portions contained 3.5 mg β-carotene (583 retinol equivalents) and α-carotene supplied an additional 33 retinol equivalents. The enriched wafer also contained 3.5 mg β-carotene, presumably with higher bioavailability. Our findings challenge the established factor for converting amounts of carotenoids in vegetables to vitamin A activity. Other studies have also suggested that the conversion factor overstates the bioavailability of β-carotene from vegetables and its conversion to vitamin A [23, 25, and Muhilal, Karyadi; unpublished].

The low bioavailability of β-carotene in dark-green leafy vegetables may be due to several factors. First, the recommended daily allowance was established in vitamin-A-deficient subjects, whereas only 3% of our study population were vitamin A deficient. In our subjects, however, the response in serum retinol was independent of baseline serum retinol, which suggests that there was no effect of vitamin A status on carotene absorption or bioconversion in this range of serum retinol concentrations. Second, physical inaccessibility of carotenoids in plant tissues may reduce their bioavailability. In green leaves, β-carotene molecules are organised in pigment-protein complexes located in cell chloroplasts, and in fruits, β-carotene is found in lipid droplets and chromoplasts. It may be difficult to free β-carotene in dark-green leafy vegetables from its matrix. Perhaps β-carotene in fruits is more bioavailable, as suggested by the seasonal variation in vitamin A status in areas where mangoes are eaten [26]. Third, carotenoids other than β-carotene in the vegetable supplement may have inhibited β-carotene absorption by competing for absorption [27]. However, the increases in serum lutein and zeaxanthin in the vegetable group were also small. Fourth, light cooking can increase bioavailability, but further cooking can produce isomers of all-*trans*-β-carotene such as 13-*cis*-β-

carotene or 9-*cis*-β-carotene with much lower provitamin A activity [28]. We did not measure *cis*-β-carotene isomers in the vegetable supplement, but the carotenoid profile showed that about 30-35% of β-carotene was *cis* isomers. Analyses of raw vegetables from the same area showed that about 15% of β-carotene was *cis* isomers. Although the all-*trans*-β-carotene content of vegetable supplement and the enriched wafer was the same, isomerisation could have led to competition for absorption between all-*trans* and *cis* isomers, at the expense of all-*trans*-β-carotene with higher provitamin A activity. This possibility, however, is unlikely, because the evidence of competition by other carotenoids is poor. Fifth, the amount of fat consumed with carotenoids has a strong effect on carotenoid absorption, but cannot explain our findings since the vegetable supplement contained more fat (7.8 g) than the wafer supplement (4.4 g). It is possible that β-carotene absorption is affected by the type of fat [29]. Sixth, there may have been differences between the groups in the extent of conversion of absorbed β-carotene to retinol. However, neither serum retinol nor serum β-carotene increased in the vegetable group. Other factors which might affect bioavailability, such as parasitic infestation, infection with bacteria, viruses or protozoa, and intestinal malabsorption, cannot explain the differences between the groups.

It is unlikely that a longer intervention would have resulted in an increase of vitamin A status. Jalal [8] provided subjects with 850 retinol equivalents daily from red sweet potato and vegetables and found an increase in serum retinol concentration after 21 days. It is possible that parasitic infestations and perhaps other factors such as competition for absorption between carotenoids exacerbate the difference in the matrix where the carotenoid is found between leafy vegetables and other carotenoid-rich foods such as red sweet potato, but we have no quantitative information to support this hypothesis.

Our findings do not support the long-standing assumption that vitamin A deficiency can be combatted by increasing the intake of dark-green leafy vegetables. Consumption of vegetables should never be discouraged because they supply other valuable dietary constituents. Our results need to be confirmed and more work needs to be done on factors influencing the bioavailability of carotenoids from different foods. Other food approaches to overcoming vitamin A deficiency, such as the use of foods naturally rich in retinol (eggs, whole fish, and liver) and fortified foods, should be developed further.

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CHAPTER 4

Reactions on the article

"Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables"

(*Lancet* 1995; **346**: 75-81)

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replace vitamin A in the diet. However, their biological equivalence needs to be established. Increasing the consumption of locally available carotenoid-rich foods is the most rational strategy against vitamin A deficiency. Bioavailability is determined by several factors, including the choice of vegetables and the way they are prepared, and programmes designed to improve vitamin A status must find ways of maximising the bioavailability of dietary carotenoids.

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Sir - Despite the opening statement by de Pee et al, their results are inconsistent with several studies in children. Epidemiological studies [5] and community-based clinical trials in children show that where vitamin A deficiency is confirmed biochemically (serum retinol <0.70 µmol/L) or clinically, provitamin A carotenoids from food [3, 6 - 8], including dark-green leafy vegetables [1, 9, 10], raise serum concentrations of retinol and ward off or even correct [11 - 12] clinical deficiency. Some studies can be criticised for lack of randomisation, lack of controls, or masking. However, one looks for consistency in results as a gauge for determining likely causality.

When initial levels are not low serum retinol does not usually respond to a food or other supplemental source of vitamin A [4, 13, 14]. De Pee et al do not tell us specifically how the subset of women who had low serum values (36%) or the 46% who had deficient or marginal breastmilk concentrations were distributed among treatment groups or how these women, who could be expected to respond, reacted to the interventions. An alternative explanation for lack of increased serum β-carotene in the vegetable-supplemented group could be very efficient bioconversion (the non-convertible carotenoids lutein and zeaxanthin increased in serum) and deposition in body stores. The methodology used was not sensitive enough to detect small changes in body stores of vitamin A.

The study by Jalal [3], cited by de Pee et al, is a good example of a carefully masked clinical trial among children who were vitamin-A-deficient and whose vitamin status improved with vegetable sources of β-carotene, most notably when they were dewormed and the fat content of their diet was concurrently increased. De Pee et al noted a uniform distribution of parasite species among the groups but did not report intensity of infections and did not deworm the women before interventions. Nor do they think that a difference in fat intake could account for the lowered absorption of carotenoids from the vegetable-based supplement. Yet there was only 7.8 g of fat in the stir-fried vegetable supplement, and this was fed seperately from any other meal component that could have provided additional fat. Presumably the fortified wafer, which contained 4.4 g fat, was given under the same conditions. Hence, very little fat was provided; this was probably sufficient when this small amount of β-carotene (3.5 mg) was given as a supplement in a wafer but possibly not when β-carotene was provided as part of a complex milieu in competition with other carotenoids and lipid-soluble substances released from the vegetable matrix. Jalal [3] found that fat in the amount of 15 g from supplemental foods fed daily with the vegetable-containing meals was important among vitamin-A-deficient Indonesian children fed carotene from vegetable sources, including spinach. Other studies in adults show that supplements of β-carotene require dietary fat for absorption and that supplement absorption is enhanced by administering them with meals [15]. Insufficient fat, and perhaps intense parasite infection, could be reasons for the lack of improvement in vitamin A status among the vegetable-supplemented group in the study by de Pee et al. If so, dark-green leafy vegetables should not be labelled as ineffective.

Much remains to be learned about the biochemistry of factors affecting bioavailability, bioconversion, and control over serum and breastmilk levels of retinol and carotenoids, and the sensitivity of indicators of change in vitamin A status. While pursuing studies to clarify these biochemical indices, every effort should be made to increase the accessibility to and consumption of food sources of carotenoids, including dark-green leafy vegetables. Experience shows

Several types of study on intake of provitamin A carotenoids from vegetables and fruits and vitamin A status do indeed point to an association but only intervention studies can test whether the relationship is causal or not. Of the 16 intervention studies reported and discussed in our review [16] (advanced copies have been widely distributed), 13 showed a positive effect on vitamin A status while 3 studies showed no effect. In our view 14 have weaknesses, such as no or small negative control groups, untreated controls, high and unexplained drop-out rates, no baseline data, or very few participants with very variable responses so that unequivocal conclusions cannot be drawn. A weak study design does not justify ignoring results of a study but the results need to be confirmed in well-designed studies.

2 studies, discussed by Underwood and Reddy, were designed well. Bulux and co-workers [4] did not find an improvement of serum β-carotene levels in children given carrots but did find an improvement in those fed purified ß-carotene despite the fact that the children were not vitamin A deficient. Jalal's study [3] reported an improvement of vitamin A status in children fed red sweet potato and dark-green leafy vegetables. Red sweet potato was the major source of provitamin A carotenoids (personal communication).

What about possible explanations for the lack of an effect of dark green leafy vegetables? A very efficient rate of bioconversion of ß-carotene to retinol in the vegetable group but not in the enriched-wafer group is unlikely. The groups were well matched with a similar proportion of women with marginal retinol levels in serum or breastmilk initially. The extensive absorption and/or bioconversion of β-carotene would be expected to result in increased retinol levels in serum and breastmilk and/or increased serum β-carotene levels in the vegetable and the enriched-wafer group.

The parasite load was high; in many stools we found >1000 *Ascaris* eggs per 25 mg. A high parasite load may make the freeing of β-carotene more difficult from a complex matrix (vegetables) than from a simpler one (wafer). Deworming medication is not often taken and we decided therefore not to deworm the women before the intervention.

The fat content of the vegetable supplement (7.8 g) was high. The study by Jayarajan, Reddy and co-workers [2], which is often cited, found that 5 g oil added to spinach and rice resulted in a larger increment in vitamin A status than the same meal without fat. In our case the fat accounted for 75 energy % of the vegetable

supplement, while in the normal diet fat accounted for 25%. When the vegetable supplement would have been taken as part of a complete meal, the relative fat content of the meal would have been smaller and interference of other components in the diet would have been greater.

We agree that often animal foods cannot play a major part in sustainable strategies against vitamin A deficiency. They are too expensive. This fact was the basis for our study, which was designed to measure the extent to which vegetables can improve vitamin A status. We also agree that our findings need to be examined carefully because of the policy implications.

Saskia de Pee, Clive E. West, Muhilal, Darwin Karyadi, Joseph G.A.J. Hautvast

OTHER ISSUES RAISED

Apart from the points raised by Reddy and Underwood, some other aspects of the paper have been discussed with colleagues, as reported below.

"The subjects with marginal serum retinol concentrations did not show an increase of serum retinol concentrations after consumption of dark-green leafy vegetables, because their vitamin A status was not low enough."

Whether serum retinol concentrations of people with deficient levels (<0.35 μmol/L) would increase after consumption of dark-green leafy vegetables in comparison with a control group, cannot be answered by our findings because only very few of our participants had such low serum retinol concentrations. Analysis of the subgroup of women with retinol concentrations in serum and/or breastmilk <0.70 μmol/L, however, showed no difference between the responses of the vegetable group and the control-wafer group (**Table 1**).

We see no reason to expect different results when studying participants with deficient serum retinol levels, because that would mean that only subjects with deficient serum retinol levels are able to absorb β-carotene from dark-green leafy vegetables in physiologically meaningful amounts. We cannot think of a physiological phenomena which would underly such a difference, for several

reasons. Subjects with a deficient vitamin A status as well as subjects with a marginal vitamin A status need vitamin A and they should thus both be able to free β-carotene from its matrix. Subjects with an adequate vitamin A status, and who are not consuming synthetic β-carotene supplements, have serum β-carotene concentrations which are 2-3 times higher than those found in our population [18], indicating that they absorb β-carotene from foods. Thus, it is very unlikely that the digestive system is influenced by vitamin A status which would determine whether β-carotene can be released from its matrix in dark-green leafy vegetables or not, because, according to that hypothesis, a subject with deficient serum retinol levels would be able to absorb β-carotene from dark-green leafy vegetables, but a subject with marginal or normal serum retinol levels would only be able to absorb β-carotene from carotene-rich foods other than dark-green leafy vegetables.

"If dark-green leafy vegetables were the main food source of vitamin A in the study area, and their consumption would not contribute to an increase in serum concentrations of retinol and β-carotene, how could it then be explained that not more than 36% of the women in the study had serum retinol levels < 0.70 μmol/L, and that serum β-carotene concentrations before the intervention were approximately 0.19 μmol/L?"

We hypothesize that the bioavailability of retinol from animal foods and provitamin A from foods with a matrix which is relatively easy to access and with a lower content of absorption inhibitors, is much better than the bioavailability of provitamin A from dark-green leafy vegetables and carrots. "Matrix" refers to the possible bonds between β-carotene and other molecules and to the way in which it is packed in organelles and cell walls. "Absorption modifiers" refers to components eaten in the same meal, as well as to components of the carotene-rich foods themselves. Examples of components which may inhibit carotene absorption are fibre, including pectin [19], cellulose [20] and chlorophyll [20], which may entrap carotenoids, and lycopene [20] which may compete for absorption (see below). Those components are particularly found in vegetables and to a lesser extent in non-leafy vegetables such as red sweet potato. It has also been found that in fruit, β-carotene is already surrounded by fat in the chromoplasts which facilitates its absorption [21], while the β-carotene molecules in leafy vegetables

have to be brought in contact with the fat first, before their absorption can be enhanced. Animal foods contain 10-30% of their vitamin A in the form of provitamin A [17] with good bioavailability.

According to our hypothesis, the limited consumption in the study area of foods with a better accessible matrix and fewer absorption inhibitors such as fruits, pumpkin and animal foods, explains why marginal serum retinol concentrations were not found in more women and why β-carotene was detected in serum. Some of the serum β-carotene could even have come from dark-green leafy vegetables and carrots, because we found a very small increase of serum β-carotene concentration (0.03 μmol/L) in the vegetable group, which was significantly different from the change in the control-wafer group (-0.03 μmol/L) (anova, controlled for age of breastfed child and individual weight change, and excluding the enriched-wafer group). This increase was however too small to result in an improvement of vitamin A status. We have tested our hypothesis of better bioavailability of (pro)vitamin A from animal sources and fruits as compared to dark-green leafy vegetables and carrots in a study with school children in the same area in Indonesia as where we conducted the study with breastfeeding women. Complete results were not yet known when this thesis was printed.

"Competition for absorption between carotenoids could reduce the bioavailability of β-carotene from dark-green leafy vegetables."

Competition for absorption between carotenoids could reduce β-carotene bioavailability [20, 22], but the amount of β-carotene absorbed from the dark-green leafy vegetables in our study was too small to be entirely explained by competition for absorption. If competition for absorption would be a very important factor, serum β-carotene levels of people who are not consuming synthetic β-carotene supplements should be much lower than generally found.

"The bioavailability of carotenoids may differ between populations."

The possibility that, as yet unidentified, factors involved in β-carotene bioavailability and bioconversion may differ between populations, and that

therefore the bioavailability of carotenoids from vegetables differs between populations cannot be ruled out. Such factors could be related to (micro)nutrient status, genetic characteristics (different enzymes), and diet. Possibly the easiest way to investigate this hypothesis is by studying the bioavailability of provitamin A from different types of food in different populations. For that reason, we are preparing an intervention study with breastfeeding women in Vietnam [23], with a design similar to the study with school children mentioned above.

CONCLUSION

In our opinion, the design of our study was appropriate to study the effectiveness of dark-green leafy vegetables and its results are reliable. The discrepancy with studies which found a positive effect can be due to weaknesses of design of those studies [16] and/or differences between study populations. Our major hypotheses to explain the lack of an effect of dark-green leafy vegetables on vitamin A status, are:

1. The bioavailability of carotene from dark-green leafy vegetables is much lower than from other carotene-rich foods, because the matrix in which carotenoids are embedded in dark-green leafy vegetables and absorption modifiers present in those vegetables, reduce bioavailability much more than the matrix and absorption modifiers of other carotene-rich foods such as fruits and non-leafy vegetables.

2. Parasite infections exacerbate the difficulty of freeing carotenoids from dark-green leafy vegetables.

3. Factors which are not yet identified and which differ between populations cause a different degree of bioavailability of carotenoids from dark-green leafy vegetables, but most likely not from synthetic β-carotene, between those populations.

Results of only one study should not immediately be generalized to other situations nor should policies be changed on the basis of one study. In order to develop

effective dietary approaches to combat vitamin A deficiency, more research should be done on the bioavailability of dietary carotenoids from different foods, in different populations, and under different circumstances.

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CHAPTER 5

Dietary carotenoids and their role in combating vitamin A deficiency: review of the literature

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ABSTRACT

Objective: To evaluate the evidence that carotene-rich fruits and vegetables can overcome vitamin A deficiency.

Design: Results of studies on the relationship between dietary carotenoids and vitamin A deficiency were evaluated critically.

Results: Increased intake of fruits and vegetables has been shown to be related to improved vitamin A status in many cross-sectional, case-control and community based studies, but this does not prove causality of the relationship. Many experimental studies indicating a positive effect of fruits and vegetables can be criticized because of poor experimental design while recent experimental studies have found no effect of vegetables on vitamin A status. Thus, it is too early to draw firm conclusions about the role of carotene-rich fruits and vegetables in overcoming vitamin A deficiency. Bioavailability of dietary carotenoids and their conversion to retinol are influenced by the following factors: Species of carotene; molecular Linkage; Amount of carotene in a meal; Matrix in which the carotenoid is incorporated; Absorption modifiers; Nutrient status of the host; Genetic factors; Host related factors; and Interactions ('SLAMANGHI'). Studies are required to quantify the impact of these factors, especially of the matrix, host related factors and absorption modifiers.

Conclusions: The effectiveness of carotene-rich foods in improving vitamin A status and ways of improving carotene bioavailability need further investigation.

INTRODUCTION

Vitamin A supplements and foods fortified with vitamin A can reduce child mortality by about 23% [1]. In addition, vitamin A supplements have been shown to reduce duration and severity of illness episodes [2], to increase haemoglobin concentrations when vitamin A deficiency co-exists with anaemia [3, 4], and to increase serum and breastmilk retinol levels when given to breastfeeding women shortly after delivery [5].

Vitamin A can be obtained from supplements, from fortified foods or from foods naturally rich in vitamin A. Food-based approaches deserve attention because they are more likely to be sustainable in the long term and will increase the intake of other nutrients simultaneously. They are also the most feasible way of providing an adequate dose of vitamin A to women of childbearing age who should restrict their intake to less than 10,000 IU/d, except during the first month after delivery [6].

Retinol-rich foods are most effective in improving vitamin A status. However, except for breastmilk, they are also the most expensive. Thus yellow and orange fruits and dark-green leafy vegetables are often promoted as a means to improve vitamin A status because such foods are rich in provitamin A carotenoids, especially β-carotene. However, the assumption that provitamin A from plant sources can effectively combat vitamin A deficiency has been challenged by results from recent studies [7-10]. Therefore, the aim of this paper is to evaluate the evidence that foods rich in provitamin A carotenoids can improve vitamin A status and to examine the factors which influence the bioavailability of dietary carotenoids.

EARLY STUDIES ON VITAMIN A REQUIREMENTS AND CAROTENE ABSORPTION

Between 1930 and 1950 a number of studies were performed in Europe to establish the requirements for vitamin A. This was done by repleting subjects fed a vitamin A deficient diet and by examining the absorption of carotene from various food sources.

Hume & Krebs [11] conducted a thorough study, the so-called 'Sheffield experiment', and concluded that 750 μg retinol or 1800 μg purified β-carotene are

required to maintain adequate vitamin A levels. These requirements were supported in the extensive review on vitamin A by Rodriguez & Irwin [12] and confirmed in a repletion study by Sauberlich and co-workers [13] who concluded that 1200 μg retinol or 2400 μg β-carotene are required to maintain serum retinol concentrations above 30 μg/dL. Current recommended daily allowances (RDA) for retinol range from 350 μg for infants to 850 μg for breastfeeding women. The RDA for vitamin A in terms of β-carotene intake depends on the amount consumed in each meal [14], on average it is 2100 μg for infants and 5100 μg for breastfeeding women.

The bioavailability of carotene from various foods has been studied by looking at their effectiveness in restoring dark adaptation or maintaining serum levels of retinol and carotene. Carotene from peas and spinach has been found to restore dark adaptation as effectively or even better than carotene in oil [15, 16], while carotene from carrots appear to be less effective than carotene in oil [16]. The method of preparation is important: cooked carrots were found to be as effective as blanched carrots [16], while grated raw carrots were found to increase serum carotene levels but cooked carrots not [17]. Leitner, Simpson & Gerber [18] found that it was possible to increase serum concentrations of retinol and carotene by feeding large amounts of carrots and spinach containing 17 mg carotene/d for 3 months.

Other studies have compared the bioavailability of β-carotene from various sources by studying apparent absorption. This is calculated by subtracting the amount of carotene in faeces from the amount consumed and dividing the difference by the amount consumed. Between 1935 and 1950 a number of such studies were undertaken in Europe, mainly in adults while between 1960 and 1980, there were a number of studies in Ruanda, India and Indonesia, mainly in children. Many of these studies have been reviewed by Hume and Krebs [11] and by Rodriguez & Irwin [12], and some of the studies included in **Table 1** also measured apparent absorption. In summary, apparent absorption of carotene dissolved in oil ranged from 30 to 99%; from cooked carrots, 1 to 60%; from raw carrots without fat, 1 to 20%; from raw carrots with fat, 25 to 50%; from vegetables with or without fat, 5 to 77%; and from papaya, 46 to 77%. James & Hollinger [19] reported that the apparent absorption of carotene from sweet potato was 46%. The apparent absorption of total carotene and β-carotene from different sources as measured by Nageswaro Rao & Narasinga Rao [20] (carrots, 36% and 81%; dark-green leafy vegetables, 58% and 76%; and papaya, 46% and 90%,

respectively) gives the impression that absorption of β-carotene may be greater than that of total carotene. All of the studies were carried out with a small number of subjects and absorption varied widely between individuals in the same study.

Absorption is underestimated when carotene extraction from the diet is incomplete, or when no correction is made for the amount of carotene excreted in faeces when the basal diet is being consumed. However, carotene absorption is more likely to be overestimated. This can happen when carotene extraction from faeces is incomplete. It can also happen if the assumption that carotenoid absorption is represented by the total amount of carotenoids not found in the faeces is incorrect, which is probably the case [21]. In two studies investigating the absorption of β-carotene and retinol using ^{14}C-labelled compounds [22, 23], 8 to 17% of ingested β-carotene and 7 to 41% of ingested retinol was recovered in the lymph. This suggests that a considerable proportion of β-carotene and retinol is metabolized in the gut and is not available for absorption. Thus studies on apparent absorption almost certainly overestimate real absorption.

We conclude, as did the Expert Groups of FAO/WHO [14, 24] and Rodriguez & Irwin in 1972 [12], that data on the absorption of dietary carotenoids are too few and too variable to predict their bioavailability accurately. One of the reasons for the variability is the large number of factors which influence bioavailability of dietary carotenoids as discussed below.

At the same time that the studies on requirements and absorption were being conducted in Europe, Bloch [25] reported from Denmark that cases of xerophthalmia appeared when consumers changed from butter to margarine. Practically no further cases of xerophthalmia were reported once margarine was fortified with vitamin A. Since then, margarine and fat spreads in Europe have been fortified with vitamin A and almost no further studies on vitamin A deficiency have been carried out in Europe because the condition has become so rare. Contrary to what may be expected, vegetarians in Europe also do not seem to be at high risk of developing vitamin A deficiency as illustrated by a study in France which found no difference in serum retinol concentrations between vegetarians and non-vegetarians [26]. This seems to be due to the relatively large amount of retinol in the diet of the vegetarians, 300-500 RE/d, in addition to the 1200-1400 RE from β-carotene. In developing countries however, where foods naturally rich in retinol are scarce and often beyond the reach of most people and where foods are rarely fortified with retinol, investigation of the effectiveness of dietary carotenoids to

reduce vitamin A deficiency continued.

CROSS-SECTIONAL, CASE-CONTROL AND COMMUNITY BASED INTERVENTION STUDIES IN VITAMIN A DEPLETED POPULATIONS

In developing countries very few cross-sectional studies have been carried out on the relationship between vitamin A status and vitamin A intake. In Senegal, a correlation was found between serum concentrations of retinol and carotene [27]. This may reflect a concurrent intake of carotene-rich and retinol-rich foods or it may be that carotene-rich foods increase serum concentrations of both carotene and retinol. Morris and co-workers [28] reported that no significant association was found between the increase in serum retinol and the consumption of mangoes and red palm oil, while the increase in serum retinol concentration was associated with the consumption of dried leaves.

There are a considerable number of case-control studies on the relationship between diet and vitamin A deficiency. One of the earliest was reported from Indonesia by Blankhart [29]. Children aged 2-5 y consumed very little retinol, while consumption of β-carotene supplied one-third of the recommended vitamin A intake in healthy children and one-fifth in malnourished children some of whom were night blind. Protein and energy consumption of the groups were comparable. Another case-control study in Indonesia found that controls consumed breastmilk, eggs, fish, dark-green leafy vegetables, carrots, and carotene containing fruits more frequently than did children with corneal xerophthalmia [30]. Pepping and co-workers [31] observed in Tanzania that controls consumed green leaves, whole milk and butter more frequently than did cases. A study in Aceh, Indonesia used logistic regression to examine the relationship of various factors with xerophthalmia. It was found that the consumption of dark-green leafy vegetables, yellow fruits and eggs was inversely related to the risk of xerophthalmia [32]. Breastfeeding was not included in the model and odds ratios were calculated from data on the group that never consumed a particular food and from the group that consumed the food at least once per month. When analyses are performed in such a way, it is not surprising that consumption of common foods is found to be inversely related to xerophthalmia. Within a large vitamin A supplementation trial in Sudan, food consumption was also investigated [33, 34]. Although the

supplement did not have an impact on incidence of xerophthalmia or mortality, dietary intake of vitamin A and of carotene were both strongly and inversely associated with risk of xerophthalmia and mortality. Retinol intake was inversely asociated with incidence of xerophthalmia but not with mortality. Relative risks were calculated by comparing children in the lowest quintile, who often consumed no retinol or carotene, with those in the highest quintile.

Other studies have suggested a stronger protective role of breastmilk and other sources of retinol than of carotene-rich foods. Stanton and co-workers [35] found that lower consumption of both milk and eggs was related to a greater risk of xerophthalmia, but did not find an increased risk for lower consumption of vegetables and fruits. Keith West and colleagues reported a protective role of breastfeeding against xerophthalmia in early childhood. Cases tended to consume papaya, mango, eggs and fresh small fish less frequently than did controls, while the frequency of consumption of fresh green leaves was comparable among cases and controls [36].

A number of criticisms can be directed towards case-control studies. They do not test causality. It is not possible to determine whether or not the difference between cases and controls is indeed the cause of disease in the cases and it could also be possible that the difference arose after the subjects became cases. In addition, it is difficult to mask interviewers completely with respect to the status of a subject because a child with xerophthalmia cannot hide his or her affected eyes. If the interviewer is aware of the hypothesis, this will make it more difficult to obtain objective answers about food consumption. Therefore, although case-control studies are very useful for generating hypotheses, experimental studies are necessary to test them.

Community-based interventions which promote cultivation and/or consumption of vitamin-A-rich foods should first of all lead to increased consumption of carotene-rich foods. A number of community based interventions, and one intervention based in a nutrition rehabilitation centre [37], have been evaluated for their impact on both the consumption of carotene-rich foods and on vitamin A status. In Tamil Nadu in India, papaya and drumstick leaves were provided together with nutrition education. It was reported that after one year, β-carotene content of the diets had improved and serum retinol levels in children had increased [38]. Other evaluations reported a reduction of the prevalence of night blindness or xerophthalmia after providing meals and nutrition education [37, 39];

introduction of a leaf concentrate [40]; nutrition education combined with home garden promotion [41]; or promotion of green leaves through mass media [42]. The abstracts of five of the studies, presented at the XVth (1993) or XVIth (1994) IVACG Meeting, do not provide enough detail to carefully evaluate the results. Evaluation of a large home gardening programme in Bangladesh found an increase in vegetable and energy consumption, an increase in household income, and a small reduction of the prevalence of night blindness [43, 44]. Evaluation of a large social marketing programme of ivy gourd in Thailand, also reported increased production and consumption, while no clear impact on vitamin A status was found [45]. It was concluded that the programmes, despite the lack of an effect on vitamin A status, were successful in many other aspects.

In the evaluation of the community based programmes, only that of the Bangladesh programme [43] compared the changes in the intervention community with changes in a control group. Thus it is difficult to draw conclusions from the other studies. We realize that it is not easy to define an appropriate control group, because it is difficult to determine the exact boundaries between communities which are influenced by a programme and those which are not. If control communities are chosen far away from the intervention communities, any other factor could also lead to a difference. Ideally, carefully controlled experiments should be done to select foods which can improve vitamin A status. These could then be followed by properly evaluated community interventions which look at changes of both intake and status.

INTERVENTION STUDIES WITH DIETARY CAROTENOIDS IN VITAMIN A DEPLETED POPULATIONS

The designs and results of intervention studies with carotene-rich foods in vitamin A depleted populations (**Table 1**) are discussed below.

Study sites and subjects

All studies were carried out in countries with regions with a high prevalence of vitamin A deficiency and they were all done with children, mostly of preschool age. Sometimes it was reported that the children were poorly nourished (Studies

5, 6 and 8), but it is not known to what extent nutritional status would have influenced the response to dietary carotenoids. If the intake of vegetables was increased without correcting deficiencies other than vitamin A, the full potential of the dietary carotenoids would not have been achieved. On the other hand, if nutritional status with respect to other nutrients was also improved, the bioavailability of retinol and bioavailability / bioconversion of dietary carotenoids would also be increased.

Inclusion of control groups

Studies should have both negative and positive control groups. A negative control group should be included in order to check whether vitamin A status of the subjects would have also changed in the absence of an intervention. A change in vitamin A status of a negative control group can be due to changes in the diet, which can occur in any study group, and/or to regression to the mean when subjects are selected on the basis of low or high vitamin A status [61]. A positive control group should receive the same amount of β-carotene or retinol as the treatment group in a form which will produce a maximum effect. The effect of the treatment group can then be compared to the effect of the positive control group.

While many studies did not include a negative control group, five studies included a negative control group in which subjects received some type of placebo (Studies 1, 8 and 13 - 15). The design of Study 13 was very elegant, because all treatment groups received similar foods which only differed in vitamin A content. The negative control group showed a smaller increase in vitamin A status than the groups given red sweet potato and dark-green leafy vegetables, but the increase of 3.3 μg/dL for subjects with initial levels <20 μg/dL was significant. This emphasizes the need for a negative control group. Study 15 used a similar design but vitamin A status was only measured after the intervention. Although it was stated that subjects were randomly allocated to the groups, no conclusion can be made about whether the difference between the groups was indeed due to the intervention or whether the difference existed prior to the intervention.

Three studies included a negative control group which was not given any treatment (Studies 3, 6 and 16). In two of those studies there was a large difference in intake of total energy and fat between the treatment groups and the

control group (Studies 3 and 6). This could have lead to erroneous conclusions because adding fat to vegetables can increase vitamin A status much more than vegetables alone (Studies 7 and 13). In the third study (Study 16) the energy given to the carrot groups was minimal and therefore no large difference was created with the negative control group.

Five studies (Studies 1, 4, 8, 11 and 14) included a positive control group which received retinol (Study 8), carotene (Study 1), β-carotene (Study 14), salt fortified with retinol (Study 4), or a megadose of vitamin A (Study 11).

In Study 9, subjects were their own control but they did not have stable baseline serum retinol levels because their routine vitamin supplements were withold just before the study. Subjects should also be randomly allocated to the treatment groups, which did not happen in all studies (Studies 3, 4 and 6).

Sample size and drop out

It is important that group size is adequate, as determined by a power calculation, and that the number of subjects per group is approximately equal. In the studies examined, the number of subjects per group varied greatly between 15 and 60 but in some studies, very few subjects were involved (Studies 1, 6, 8 and 11) or the number of subjects per group were very different (Study 3). The variation in response within the groups with very few subjects was often large (Study 1).

When relatively many of the subjects do not complete a study the results can be affected, especially when the drop out is related to the design or the outcome of the study. For example, when subjects who do not experience any improvement during the study decide to withdraw, only the results of subjects who experienced an effect would remain for analysis. As many reports did not give details about drop out or the number of subjects initially enrolled, this could mean that all subjects completed the study. This is possible when the study was conducted in an institution, such as an orphanage, or it could mean that the authors have only reported results of those subjects who completed the study. Two of the studies reported a relatively large drop out. Study 4 reported results on only 75% of subjects for serum levels of retinol and carotene, but on 100% of subjects for the haemoglobin measurement. This difference may have been due to

problems in obtaining enough serum or to problems with the analysis of retinol in serum. In either case, the drop out would not have influenced the validity of the results because it was not selective. For Study 8, no reason was given for the relatively high rate of drop out in some of the groups. Therefore, selective exclusion of subjects cannot be excluded.

Duration of intervention

Carotene-rich foods were consumed over a period lasting from two weeks to three months. It is important that studies are carried out for sufficient time for an effect to be expected. For example, with respect to changes in haemoglobin levels, no changes were seen when vegetables were fed for a short period of time (<14 d; Study 9), but increased haemoglobin levels were reported when vegetables were fed for more than 60 d (Studies 4, 5 and 8). Other studies have shown that vitamin A supplementation of anaemic subjects with a marginal vitamin A status can result in an increase of their haemoglobin concentration [3, 4].

Avoiding unwanted differences in diet among groups

Designing a dietary intervention study is very difficult because it is essential that only the dietary component of interest differs among the groups being studied. Because vitamin-A-rich foods cannot be exchanged readily for foods with virtually no vitamin A without subjects noticing any difference, generally food interventions cannot be carried out using a completely masked study design. With a non-masked study design, complete randomization of treatment could lead to undesirable changes in food consumption in the control group since participants will realize that the other group is receiving a different treatment. A change in food consumption could counterbalance any effect of the food supplement, because often the supplement is small in relation to the daily diet.

Because allocation to treatment group and daily diet during the study are so critical, studies should report how subjects were allocated to treatment groups and whether their daily diet remained unchanged. Most reports of studies do not give such information or only give information about daily diet but not about changes

Table 1. Interventions with carotene rich foods and the effect on vitamin A status in vitamin A depleted populations

Authors; study site [ref]	Design: description of subjects; treatment¶; fat content of supplement; duration of intervention	Results§	Conclusions and comments: drop out; control group; sample size†; control over daily diet during study period
Roels et al., 1958; Ruanda (Study 1) [46]	* Subjects: boys aged 9-16 y * Treatment: Group 1: 200 g raw grated carrots/d (19 mg carotene/d) (n=5) Group 2: 200 g raw grated carrots + 18 g olive oil/d (19 mg carotene/d) (n=4) Group 3: 28 mg carotene + 18 g olive oil/d (n=4) Group 4: 18 g olive oil (n=4) Group 5: placebo (n=4). Supplements were supplied in two meals * Fat content of supplement: see above * Duration of intervention: 31 d after 8 d on basal diet * Other information: 17 subjects had Bitot spots, 4 subjects did not show signs of xerophthalmia	* Change in serum retinol concentration: - Group 1, from 36 to 51 μg/dL - Group 2, from 32 to 53 μg/dL (ns) - Group 3, from 35 to 56 μg/dL - Group 4, from 37 to 39 μg/dL(ns) - Group 5, from 33 to 38 μg/dL(ns) * Change in serum carotene concentration: - Group 1, from 43 to 84 μg/dL - Group 2, from 48 to 335 μg/dL - Group 3, from 64 to 501 μg/dL - Group 4, from 52 to 47 μg/dL(ns) - Group 5, from 49 to 43 μg/dL(ns) Changes in serum concentration of retinol and carotene in Groups 2 and 3 were different from those in other groups ($p<0.07$ and $p<0.001$ resp). Range of change of serum retinol concentration within groups was large (8-46 μg/dL)	* After consumption of carrots or purified carotene, both with fat, serum concentration of retinol and carotene increased. After consumption of carrots without fat the increase in serum retinol concentration was not statistically significant * Drop out: one subject developed diarrhoea * Control groups: negative and positive control groups included * Sample size: n<10 and responses varied considerably within groups * Daily diet: well controlled

Pereira & Begum, 1968; India (Study 2) [47]	* Subjects: children aged 2-5 y, from an orphanage * Treatment: 30 g cooked green leafy vegetables (1.5-2.25 mg β-carotene/d) (n = 29) * Fat content of supplement: no information given * Duration of intervention: 3 mo * Other information: after vegetables an intramuscular injection of vitamin A acetate in oily solution (100,000 / 200,000 / 300,000 IU) or placebo was given	* Change in serum retinol concentration: - after vegetable consumption, from 22 to 31 μg/dL - after intramuscular injection of vitamin A, decrease in all groups, including the placebo group	* Increase in serum retinol concentration after consumption of vegetables * Drop out: none * Control groups: not included * Sample size: n = 20-30 * Daily diet: controlled * Other comment: lack of effect of intramuscular injection of vitamin A is difficult to explain
Lala & Reddy, 1970; India (Study 3) [48]	* Subjects: children aged 2-6 y * Treatment: Group 1: 40 g amaranth (1.2 mg β-carotene/d) and one chapati (n = 29) Group 2: no intervention (n = 6) * Fat content of supplement: none; daily diet contained 5-7 g fat * Duration of intervention: 15 d	* Change in serum retinol concentration: Group 1: - In subjects with initial concentration < 25 μg/dL (n = 17), it increased by 12.6 μg/dL. - In subjects with initial concentration > 25 μg/dL (n = 12), it increased by 6.2 μg/dL (ns) Group 2: no changes	* Low serum retinol concentration increased after consumption of vegetables * Drop out: three subjects, due to respiratory infections * Control groups: negative control group included but it was much smaller than the vegetable group and did not receive any treatment * Sample size: Group 1: n = 20-30; Group 2: n < 10 * Daily diet: little information given; different for Groups 1 and 2

Devadas et al., 1980; India (Study 8) [53]	* Subjects: children aged 3-5 y * Treatment: 1st month basic diet with virtually no vitamin A for all subjects, then 2 months: Group 1: basic diet (n = 5) Group 2: 142 g papaya (1.2 mg β-carotene/d) (n = 8) Group 3: 30 g amaranth (1.2 mg β-carotene/d) (n = 5) Group 4: vitamin A (300 RE/d) (n = 10) * Fat content of supplement: no information given; daily diet contained 5-7 g fat * Duration of intervention: 2 mo	* Change in serum retinol concentration: - Group 1, from 13 to 13 μg/dL(ns) - Group 2, from 13 to 29 μg/dL - Group 3, from 14 to 29 μg/dL - Group 4, from 13 to 35 μg/dL * Change in haemoglobin concentration: - Group 1, from 9.2 to 9.8 g/dL(ns) - Group 2, from 9.2 to 10.9 g/dL - Group 3, from 8.0 to 10.2 g/dL - Group 4, from 8.9 to 10.8 g/dL	* Serum retinol concentration increased as much after consumption of papaya as after consumption of amaranth and it increased most after vitamin A * Drop out: each group started with 10 subjects, no information about 30% drop out * Control groups: negative and positive control group included * Sample size: n < 10 * Daily diet: controlled

Charoen-kiatkul et al., 1986; Thailand (Study 9) [54]	* Subjects: children of preschool age who had been in an orphanage >6 mo, where they were given vitamin supplements * Treatment: Group 1: in wet season; 2 wk no intervention, 2 wk cooked ivygourd (1.1 mg β-carotene/d) (n = 15) Group 2: in cool, dry season; 2 wk cooked ivygourd (1.2 mg β-carotene/day), 2 wk vitamin supplement (450 RE/day) (n = 15) * Fat content of supplement: ivy gourd supplement contained 0.2-0.4 g fat per portion * Duration of intervention: 2 wk per treatment	* Change in serum concentration of retinol and β-carotene: - Group 1: 2 wk no intervention: serum concentration of retinol and β-carotene decreased from 39 to 25 μg/dL and from 44 to 27 μg/dL resp; 2 wk vegetables: serum concentration of retinol and β-carotene increased from 25 to 49 μg/dL and from 27 to 106 μg/dL resp - Group 2: 2 wk vegetables: initial serum retinol concentration of 35 μg/dL unchanged, serum β-carotene concentration increased from 36 to 87 μg/dL; 2 wk vitamin supplements: serum retinol concentration increased from 35 to 48 μg/dL and β-carotene concentration decreased from 87 to 57 μg/dL * Haematocrit remained the same throughout the study period in both groups	* The authors ascribe the decrease in serum retinol levels in Group 1 and no increase in Group 2 during first two weeks to discontinuation of vitamin supplements. Authors conclude that 50 g of vegetables can maintain vitamin A status * Drop out: none * Control groups: not included * Sample size: n = 10-15 * Daily diet: controlled * Other comment: increases of serum retinol concentrations occured very rapidly and to very high levels

Bulux et al., 1994; Guatemala (Study 14) [9]	* Subjects: children aged 7-12 y * Treatment: Group 1: placebo (capsule) (n = 17) Group 2: retinyl palmitate (1000 RE/d, orally by syringe) (n = 17) Group 3: 50 g carrots (6 mg β-carotene/d) with 10 g fat (n = 17) Group 4: β-carotene supplement (6 mg/d) (n = 16) * Fat content of supplement: see above * Duration of intervention: 20 d	* Serum retinol concentration remained the same in all four groups (34 μg/dL) * Serum β-carotene concentration increased almost 3 times in Group 4. It remained unchanged (13 μg/dL) in the other three groups	* The already normal serum retinol concentration did not increase after consumption of retinol, β-carotene or carrots. Consumption of purified β-carotene (6 mg) increased serum β-carotene concentration, but it was not increased after consumption of carrots * Drop out: 3% * Control groups: negative and positive control group included * Sample size: n = 16-20 * Daily diet: recorded
Wadhwa et al., 1994; India (Study 15) [59]	* Subjects: children aged 7-12 y from an orphanage * Treatment: Group 1: carrots (5d/wk), papaya (2d/wk), coriander-mint chutney (2d/wk) (2.3 mg β-carotene for 7-9 y old and 3.3 mg for 10-12 y old) (n = 60) Group 2: radishes (3 μg β-carotene/d) (n = 54) * Fat content of supplement: no information given; daily diet contained 17 g fat * Duration of intervention: 1 mo	* Serum retinol concentration after the intervention was: - Group 1, 25.1 μg/dL - Group 2, 15.5 μg/dL	* Higher serum retinol concentration after consumption of carrots, papaya, coriander, and mint, than after consumption of radishes * Drop out: 5.8% * Control groups: negative control group included * Sample size: n > 50 * Diet: recorded * Other comment: serum retinol concentration was not determined at baseline

Nasoetion et al., 1994; Indonesia (Study 16) [60]	* Subjects: children aged 10-13 y * Treatment: Group 1: carrot soup (1.8 mg β-carotene/d) (n = 37) Group 2: carrot juice (1.8 mg β-carotene/d) (n = 39) Group 3: no intervention (n = 37) * Fat content of supplements: none * Duration of intervention: 3 mo	* Change in serum retinol concentration: - in subjects with initial concentration < 30 μg/dL: - Group 1, from 24 to 28 μg/dL (n = 18) - Group 2, from 25 to 31 μg/dL (n = 21) - Group 3, from 23 to 27 μg/dL (n = 20) - in subjects with initial concentration > 30 μg/dL: - Group 1, from 37 to 35 μg/dL (n = 19) - Group 2, from 38 to 36 μg/dL (n = 18) - Group 3, from 37 to 34 μg/dL (n = 17) Changes in serum retinol concentration were not different between Groups 1, 2 and 3	* No increase of serum retinol concentration after consumption of carrot soup and carrot juice * Drop out: 5.8% * Control groups: negative control group included, but not given any treatment * Sample size: n = 30-50 * Daily diet: *no information given*

¶ Number of subjects reported is the number used in the data analysis. Data on the number of subjects enrolled in the study can be calculated from the drop out which is mentioned in column 4. Not all authors provided data about the number of subjects enrolled or that dropped out, or reason for drop out.

§ Most studies reported serum retinol concentration in μg/dL: therefore this unit of measurement has been used in the table. For some of the later studies, serum retinol concentration was reported in μmol/L and these values have been converted to μg/dL (1 μmol/L = 28.6 μg/dL).

† For evaluating the size of the study, sample size was classified into the following categories: n < 10, 10-15, 16-20, 20-30, 30-50, > 50.

‖ ns: not significant ($p > 0.05$). All changes reported without comment were statistically significant.

we have to conclude that the design of most of the studies was weak. Three of the studies (Studies 4, 14 and 16) did not support the hypothesis that carotene-rich vegetables and fruits can improve vitamin A status, while the other 13 studies (Studies 1, 2, 3, 5 - 13 and 15) supported the hypothesis. Only half of the studies (Studies 1, 3, 6, 8 and 13 - 16) fulfilled one of the most important criteria of an intervention study, inclusion of a negative control group. Six of those studies however suffered from other weaknesses in study design: the control group was very small (Study 3); the control group did not receive any treatment (Studies 6 and 16); no information was provided about the high rate of drop out (Study 8); baseline values were not available (Study 15); or the variation in response within treatment groups was very large and the number of subjects very small (Study 1). The two remaining studies that included a negative control group suffered less from weaknesses in design. The study by Bulux and co-workers (Study 14) was conducted with vitamin A replete subjects. Therefore no effect was found on vitamin A status, but an effect on serum carotene levels, which was found after feeding purified β-carotene, was not found after feeding carrots with fat. Jalal (Study 13) reported an increase of serum retinol, which was mainly attributed to the consumption of red sweet potato, rather than of vegetables. It may well be that the bioavailability of β-carotene from red sweet potato is better than that from vegetables because of the nature of the matrix (see below).

Three studies not included in **Table 1** reported that red palm oil, which is very rich in carotenoids, has a positive impact on vitamin A status. The studies suffered from weaknesses in design, but two of them [62, 63] included a negative control group in which serum retinol levels did not change and in the other study [64] changes were almost the same as in the positive control group that received retinol. An ecological study did report a negative correlation between consumption of red palm oil and prevalence of vitamin A deficiency [65]. This supports the suggestion that red palm oil improves vitamin A status. The improvements of vitamin A status of the subjects given a buriti sweet (Study 10) could indeed be due to the buriti sweet because the matrix in which the β-carotene is found is more favourable in the fruit than in leafy vegetables and its composition is comparable to red palm oil. However, the poor design of that study, does not allow firm conclusions.

In conclusion, there is no good reason to doubt the effectiveness of red palm oil in improving vitamin A status. It might be that yellow and red sweet potato can

improve vitamin A status, but the assumption that carotene-rich vegetables and fruits can improve vitamin A status is based on results of studies with poor designs. Well designed studies which argue against the assumption are however also scarce. Therefore, there is an urgent need for studies with good designs to test which carotene-rich vegetables and fruits can combat vitamin A deficiency.

RECENT STUDIES IN VITAMIN A REPLETE POPULATIONS

In recent years, interest in the bioavailability of β-carotene from fruit and vegetables in vitamin A replete populations has grown because the incidence of cancer and of coronary heart disease has been found to be correlated negatively with the consumption of fruit and vegetables [66, 67]. This correlation has been attributed in part to the anti-oxidant properties of β-carotene. Some investigators have conducted intervention studies to examine whether an increased intake of vegetables and/or fruits has an effect on serum β-carotene concentrations.

Feeding carrot juice containing 20 mg β-carotene, 10 mg α-carotene and 10 mg other carotenoids daily for 7 or 14 days was found to increase serum concentrations of α- and β-carotene [68]. In another study, the concentration of β-carotene in serum reached its maximum 5 hours after consumption of carrots containing 8 mg β-carotene [69]. Brown and co-workers [7] provided meals containing 40 energy% fat with foods naturally rich in β-carotene or containing synthetic β-carotene in a cross-over design. Group 1 received 30 mg synthetic β-carotene or 29 mg dietary β-carotene in carrots. Group 2 received 12 mg synthetic β-carotene or 6 mg dietary β-carotene in broccoli or tomato juice without β-carotene. Serum β-carotene concentrations were monitored for 11 days after the meal. In Group 1 the increase after the carrot meal was only 14% of that after synthetic β-carotene. In Group 2 serum β-carotene concentration increased only after synthetic β-carotene. Micozzi and co-workers [8] gave the same foods for a period of 6 weeks and observed similar effects. In another study, the maximum increase after feeding 6 mg dietary β-carotene in cooked carrots for 7 days was only 9% of the maximum increase after synthetic β-carotene [70]. No information was provided about the fat content of the meals. It can be concluded that the relative bioavailability of β-carotene from carrots compared with that of β-carotene in oil (9-18%) is less than the factor of one third which is generally used [14].

SUGGESTIONS FOR RESEARCH ON THE ROLE OF CAROTENE-RICH FOODS IN COMBATING VITAMIN A DEFICIENCY

From what has been discussed until now, it is clear that there is need for additional intervention studies to be carried out on the effectiveness of carotenoids from vegetables and fruits. In addition, factors that influence bioavailability should be investigated in order to optimize bioavailability. Operational research should investigate ways of increasing the consumption of effective foods and whether increased intake indeed results in improved status. Criteria for the design of food intervention studies can be identified as follows:

Which carotene-rich foods should be investigated?

First of all, bioavailability of carotenoids from dark-green leafy vegetables and carrots need to be studied because we cannot draw firm conclusions from the data now available. Red palm oil has been shown to improve vitamin A status, while almost no intervention studies have used fruits. Fruits are expected to contain β-carotene which is reasonably bioavailable because cross-sectional and case-control studies have suggested a positive relationship between the consumption of carotene-rich fruits, such as mango, and vitamin A status [91] and β-carotene located in the chromoplasts of fruits is expected to be more bioavailable than β-carotene located in the chloroplasts of dark-green leafy vegetables. Studies with roots and tubers, are very few and have mainly focussed on yellow and red sweet potato [19, 58]. Because of the cytological characteristics of β-carotene in roots and tubers, it is expected that carotenoid bioavailability would be comparable to that of β-carotene in fruits.

Some work has been done on developing processed foods. Red sweet potato has been used to make snacks in Indonesia [58] and flour for gruel and pancakes in Guatemala [92]. Solar drying devices have been developed to dry vegetables and mangoes in order to extend the period for which they are available [93]. In addition, vegetable concentrates have been made to reduce bulkiness [40, 94]. The buriti sweet [55] and chicken liver chips [95] are examples of processed foods which are not expected to have a large bioavailability problem. Processed and preserved foods should also be tested for their effectiveness in improving vitamin A status.

How to design a good food intervention study?

Intervention studies aimed at investigating the effectiveness of different foods or food groups in improving vitamin A status should be designed very carefully, taking into account the following aspects of design:

Hypothesis and outcome parameters

Studies should be designed to test a clearly defined hypothesis. The hypothesis should test an effect on a parameter of vitamin A status. Of the many parameters for measuring vitamin A status [96][2] most intervention studies test hypotheses based on measurements of serum retinol concentration. As an increase in serum concentration of carotenes is also expected when carotene containing foods are fed, we suggest that the concentration, especially of β-carotene, in serum should also be measured. An additional reason for such measurements is that they are more sensitive than changes in retinol concentration which is more closely regulated. Because an increase in vitamin A status can also improve suboptimal iron status [3, 4] measuring iron status parameters could also be considered. However, it should be remembered that attempts to test secondary hypotheses should in no way distract from testing the primary hypothesis.

Sample size and drop out

The hypothesis should state the difference in the change in vitamin A status parameter among the groups which is relevant. In addition, the minimum sample size required to test the hypothesis should be established prior to carrying out the study. When reporting results, information should be provided about the number of subjects dropping out of the study and the reasons for this drop out. This will allow a judgement to be made about whether selective drop out has confounded the results of the study.

Study population

Bioavailability of dietary carotenoids can be studied in vitamin A replete populations by measuring changes in serum carotenoid concentrations. However, studies on the effectiveness of foods in improving vitamin A status should be

[2] See also *Chapter 6* of this thesis.

conducted in subjects with a marginal or deficient vitamin A status because only then will serum retinol concentrations increase. In addition, other aspects of nutritional status and nutrient intake may differ between vitamin A deficient and vitamin A replete populations. It is also important to provide additional information such as on anthropometry and parasitic infestation which may affect nutritional status of the subjects.

Control groups

Because changes over time could be related to factors other than the food supplement such as seasonal change and infection, it is necessary to include a negative control group in the study. A positive control group should be included to monitor the effect which could be achieved when conditions are most favourable, for example when synthetic β-carotene in fat or retinol is fed.

Diet of treatment groups

The diet of the treatment groups should differ only with respect to vitamin A content because other differences will confound the effect. Differences can occur, for example, when one group is given a capsule, which would not change normal food consumption, and another group is given vegetables which could reduce the consumption of vegetables normally consumed. Although it is not possible to mask foods, large differences between groups can be avoided by giving foods of the same type, for example by giving one group dark-green leafy vegetables and the other group cabbage. Even better would be to feed such vegetables in a composite food such as a lumpia.

The ideal way to ensure that the consumption of other foods remains unchanged when the intake of a particular food is increased, is to provide subjects with all their food for the entire study period. However, this cannot be realized in most settings. A good alternative in order to avoid unwanted changes in the daily diet is to explain to participants the importance of not changing their normal diet and to introduce a similar dietary change in all groups. The latter reduces the risk that changes in food consumption will differ among groups. When a similar change in food consumption occurs in all groups, a negative control group will correct for such change. An even better solution would be, if supplying all the food is not possible, to supply a large part of the diet as this would markedly reduce compensation from consuming other food. In order to check whether indeed no

unwanted dietary changes occurred during the study period within and between groups, as much information as possible should be obtained about food consumption of participants before and during the intervention.

Allocation of treatment

Ideally, subjects should be randomly allocated to treatment groups. However when the treatment is not masked, this can lead to problems as subjects will be aware of the different treatments in the study. As mentioned before, when a large part of the diet is fed under close supervision, the possibility for substantial compensation may be very small. This would especially be the case when potions of particular vegetables or fruits are significantly larger than normal daily intake. If the control over the diet is smaller, groups should better not be in contact with one another. In that case however, care has to be taken to ensure that the groups do not differ in any parameter which may invalidate conclusions from the study. It could for example be decided to allocate treatment by village or school after making sure that the treatment groups indeed do not differ from each other in important aspects.

Composition of food supplements

In general, the carotenoid content of foods as reported in food composition tables provides an overestimate, often two-fold, of the content measured by modern techniques such as high-pressure-liquid-chromatography (HPLC) [24]. Therefore, the carotenoid content of the prepared supplements should be analysed, preferably by HPLC as this technique can also provide data on the content of a range of carotenoids including isomers which differ in their provitamin A activity. In addition, the fat content of the supplement should be determined, and the preparation method and the matrix of the supplements described.

The design aspects described above apply to studies which test whether there is any effect of particular foods or food groups on vitamin A status. Because large dietary interventions are usually not only aimed at improving vitamin A status, but more general at reducing morbidity and mortality, these endpoints should be included when evaluating large community interventions.

DISCUSSION:
COMBATING VITAMIN A DEFICIENCY WITH A FOOD-BASED APPROACH?

Many uncertainties still remain about the capacity of carotene-rich fruits and vegetables to improve vitamin A status and about the bioavailability of dietary carotenoids in general. These uncertainties need to be swept away if we wish to reach the goal agreed to by all national governments of eliminating vitamin A deficiency by the year 2000 [97]. Meanwhile, we should continue to use strategies proven to be effective: vitamin A supplementation, food fortification, and consumption of retinol-rich foods and red palm oil. Home garden programmes should be continued because they have a much broader role then just providing vegetables for household consumption. Even if vegetables do contribute little to improvement of vitamin A status, they still provide other nutrients and add variety to the diet. When more is known, preparation methods could be developed which would improve carotene bioavailability and thus possibly the effectiveness of vegetables. Where home garden programmes include poultry, small animal husbandry or fish, as in the VAC programme in Vietnam [98], the consumption of such foods, especially eggs, livers and whole fish (with the liver) should be encouraged.

In order to develop locally feasible and acceptable food-based approaches to combat vitamin A deficiency, each region, province or country should identify acceptable and affordable dietary sources of retinol and carotenes, with high bioavailability, including processed and fortified foods. The effectiveness of these foods should be evaluated by intervention studies. Operational research should be done to investigate ways of achieving sufficient consumption of an array of effective foods and to measure the impact on vitamin A status, morbidity and mortality in the population.

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CHAPTER 6

Performance of biochemical indicators of vitamin A status in breastfeeding and non-breastfeeding Indonesian women

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ABSTRACT

Indicators of vitamin A status were evaluated in 265 breastfeeding and 49 non-breastfeeding Indonesian women. The concentration of retinol in breastmilk (mainly retinyl esters) was 30% higher and breastmilk fat content 20% higher, for women with a breastfed infant >6 mo old compared to women with an infant of 3-6 mo old. The retinol content of milk fat remained constant throughout lactation, but it is better to relate breastmilk retinol to volume, because that was more sensitive to changes in vitamin A status. Sensitivity and specificity of the concentration of breastmilk retinol and of serum retinol binding protein for detecting serum retinol concentrations <0.70 μmol/L was <75%. The modified relative dose response (MRDR) method suffers from a relatively large variation in the dehydroretinol/retinol ratio, because of the vulnerability of the dehydroretinol concentration to laboratory errors, to variation in the dose and to variation in absorption. In addition, within categories of the dehydroretinol/retinol ratio, serum retinol concentration was lower in breastfeeding women than in non-breastfeeding women, raising the question as to whether different cut-off values should be used for the ratio and/or for serum retinol concentration. Serum retinol concentration, which was just above marginal (0.85 μmol/L), had the smallest within-person variation and was also the most sensitive indicator for detecting a difference in change in vitamin A status, requiring 19 subjects per group. Serum retinol binding protein concentration, breastmilk retinol content expressed per volume or per gram milk fat and the modified relative doses response method required groups of 35, 36, 139 and 53 subjects respectively.

INTRODUCTION

The most commonly used biochemical method for determining vitamin A status of individuals and populations is the concentration of retinol in serum. However, serum retinol concentration is not regarded as sensitive to changes over a wide range of vitamin A status, it is lowered temporarily during infection, and it requires a blood sample. Therefore, other methods have been proposed to supplement or replace the measurement of serum retinol for determining vitamin A status.

The aim of this paper was to evaluate the following indicators of vitamin A status in breastfeeding women by comparing them to serum retinol concentration: breastmilk retinol concentration, serum retinol-binding protein concentration, and the modified relative dose response (MRDR) method. In addition, the performance of the modified relative dose response method in breastfeeding women was compared to the performance in non-breastfeeding women. Vitamin A status should ideally be measured using methods that provide a direct estimate of vitamin A stores, such as isotope dilution. In this paper, serum retinol was used as the parameter with which all other parameters were compared. This is acceptable, because a clinical examination was used to exclude women with signs of infection from the measurements and their average serum retinol concentration of 0.85 μmol/L was sufficiently low to enable it to increase in response to increase in status.

Retinol concentration in breastmilk[1] has been proposed as an indicator of vitamin A status in breastfeeding women, because it appears to be sensitive to changes in intake and because breastmilk is relatively easy to collect compared to serum [1]. Retinol concentration varies during a feed and throughout the day because the content of milk fat, in which retinol is dissolved, varies [2]. Milk retinol concentration is therefore more vulnerable to sampling errors than the retinol content of milk fat. However, it is difficult to predict an infant's vitamin A intake from the retinol content of milk fat. Up until now, breastmilk retinol content has been expressed in terms of concentration. Concentrations >1.75 μmol/L are regarded as sufficient to meet an infant's requirements for vitamin A, while

[1] Please note that retinol in breastmilk exists mainly as retinyl esters and that it does not include provitamin A carotenoids.

Methods

In the intervention study, the concentration of serum retinol, breastmilk retinol, breastmilk fat, serum retinol binding protein, serum β-carotene, the dehydroretinol/retinol ratio and serum albumin were measured, while from the cross-sectional study data on serum retinol, the dehydroretinol/retinol ratio and serum albumin were available. In both studies, pregnancy tests were performed in order to exclude pregnant women from the study. Biochemical analyses were carried out in both studies in the same way. For the intervention study, baseline and follow-up samples of serum and breastmilk from the same woman were analysed in the same run for each respective indicator. The samples were coded to mask the treatment group of the subjects.

Serum retinol and the modified relative dose response. At 7.30 a.m. on the day of blood collection, women received an oral dose of 3,4-didehydroretinol acetate (8.8 μmol) dissolved in 250 μL corn oil. This was followed by a high-fat, low-vitamin A snack such as fried banana. Participants were instructed not to eat vitamin A-rich foods from the time of the previous evening meal until blood was taken. Five hours after ingesting the dehydroretinol, women were examined clinically by a clinician or a nurse, and blood (10 mL in the cross-sectional study and 6 mL in the intervention study) was drawn from an antecubital vein and placed on ice protected from light. The blood was centrifuged and the serum collected stored at -20°C until analysis of retinol, dehydroretinol and albumin. Serum retinol and dehydroretinol were analysed at the Nutrition Research and Development Centre (NRDC) in Bogor by HPLC (column, Bondapak C18, Waters, Milford, MA, USA; detector, Shimadzu SPD-6AV, Tokyo, Japan) with methanol/water (90/10 v/v) as mobile phase [7]. For analysis of the samples from the intervention study, within-run coefficient of variation was 5.1% for retinol, 10.4% for dehydroretinol and 9.5% for the dehydroretinol/retinol ratio.

Breastmilk retinol and breastmilk fat. Breastmilk was collected in a standardized way. Between 8 and 11 a.m. all milk from one breast, which had not been used to feed the child during at least the previous hour, was collected using a breast pump (White River Concepts, San Clemente, CA, USA). The breastmilk was stored in dark brown glass bottles and transported to the field laboratory on ice. Creamatocrit, analogous to hematocrit for the cream fraction of milk [8], was measured in triplet and averaged. The fat content was related to the fat content

measured by extraction [9], based on measurements made on 22 breastmilk samples measured by both methods. Breastmilk was stored at -20°C for 0.5-1.5 mo, and subsequently at -80°C, until analysis of retinol. Analyses were performed at Nutricia Research, Zoetermeer, the Netherlands in a room illuminated with a yellow light, using the following method. An aliquot of a thawed and well mixed sample (500 μL) was incubated overnight at room temperature with 500 μL ethanolic (50% v/v) potassium hydroxide (4 M). Then, 2 mL acetonitrile (containing 5% v/v acetic acid) was added and mixed thoroughly. After the two phases had separated, 50 μL of the upper layer was injected onto an HPLC column (Merck, RP-18) which was eluted with a mobile phase of methanol/water (95/5 v/v). Vitamin A was detected spectrophotometrically at 325 nm and quantified by relating the peak area to a calibration curve constructed using standards analysed in the same run. Within- and between-run coefficients of variation were 3.4% and 4.5%, respectively. Recovery of the method was 95-105%.

Serum retinol binding protein. Serum retinol binding protein was analysed by immunonephelomery at Hoechst laboratories, Amsterdam, using a Behring Nephelometer and reagents supplied by Behringwerke AG, Germany [10]. The retinol binding protein concentrations were converted to μmol/L based on a molecular weight of 21,000 daltons.

Serum β-carotene. Serum carotenoids [11] were analysed at the Department of Human Nutrition, Wageningen Agricultural University. Within and between run coefficients of variation for β-carotene were 3.4% and 8.2% respectively.

Serum albumin. Serum albumin was analysed at the NRDC using the bromocresol green method [12].

Statistics

Differences between groups were tested using analysis of variance, while differences within subjects were tested with a paired t-test. Changes between the start and the end of the intervention study were calculated for each individual by subtraction. Within-person variation was estimated for each biochemical indicator by subtracting the change in the treatment group after the intervention from the individual changes. This estimate is expected to be larger than the real within-person variation because we could not correct for the variation of treatment effect.

Table 2. Serum concentrations of retinol and RBP and breastmilk retinol content of breastfeeding women, by age category of the breastfed child‖ (data from baseline of intervention study)

Age of child		Serum retinol	Serum RBP	Breastmilk composition		
				retinol	fat	retinol / fat
mo	*n*	*μmol/L*	*μmol/L*	*μmol/L*	*g/L*	*nmol/g fat*
3- 6	64	0.85 ± 0.32[ab]	1.56 ± 0.70¶	0.74 ± 0.41[a],§	29.1 ± 12.2[a]	28 ± 19
7- 9	48	0.90 ± 0.30[a]	1.73 ± 0.80¶	1.03 ± 0.72[b]	35.7 ± 15.0[b]	29 ± 14
10-12	21	0.72 ± 0.28[b]	1.48 ± 0.74¶	0.88 ± 0.67[ab]	34.5 ± 18.4[ab]	28 ± 19
13-18	35	0.86 ± 0.34[ab]	1.48 ± 0.51¶	1.05 ± 0.98[b]	35.8 ± 18.0[b]	28 ± 18

‖ $\bar{x}$ ± SD

[a,b] Values in the same column with different superscript are significantly different from each other, $p<0.05$ (analysis of variance)

¶ Significantly different from serum retinol and from breastmilk retinol, $p<0.05$ (paired t-test)

§ Significantly different from serum retinol, $p<0.05$ (paired t-test)

for women with a breastfed infant older than 6 mo of age as compared to those with a breastfed infant between 3-6 mo (**Table 2**). The fat content of the milk was also higher for women with an older breastfed infant (20%), while the retinol content of milk fat remained constant throughout lactation. In mothers of breastfed infants ≤6 mo the concentration of retinol in milk was slightly lower than in serum. In mothers with infants aged >6 mo, the retinol concentration in milk tended to be higher than in serum. The correlations between retinol concentrations in serum and breastmilk were poor, they ranged from 0.17 to 0.53 within the different categories for age of the breastfed child.

Sensitivity and specificity for detecting serum retinol concentrations <0.70 μmol/L were calculated for breastmilk retinol content expressed in relation to milk volume and to milk fat (**Table 3**). An iterative procedure was used to select cut-off values for which both sensitivity and specificity were optimal. Sensitivity was slightly higher when milk retinol content was expressed in relation to volume.

Table 3. Comparison of the sensitivity and specificity for detecting low serum retinol concentrations based on measurements of breastmilk retinol content related to milk volume and to milk fat (data from baseline of intervention study)

	Serum retinol concentration		
	<0.70 μmol/L	≥0.70 μmol/L	
Breastmilk retinol concentration (μmol/L)			
<0.68	47	36	Sensitivity: 47/ 63 = 74.6 %
≥0.68	16	76	Specificity: 76/112 = 67.9 %
	63	112	
Retinol content of milk fat (nmol/g fat)			
<22	42	36	Sensitivity: 42/ 63 = 66.7 %
≥22	21	76	Specificity: 76/112 = 67.9 %
	63	112	

Figure 1 shows the changes in fat content of milk, retinol content of milk fat, and milk retinol concentration in the intervention study. Women in the enriched-wafer group showed an increase in milk retinol concentration which was significantly greater than in the vegetable group and the control-wafer group, and could be attributed to an increase in both the fat content of the milk and the retinol content of milk fat.

Retinol binding protein

Table 2 shows that serum retinol binding protein concentration was the same for women with a breastfed child of different ages and that it was significantly higher than the retinol concentration in serum and breastmilk. A cut-off level, determined using an interative procedure, for serum retinol binding protein concentration of 1.29 μmol/L (or 27.1 mg/L) gave the highest levels of sensitivity and specificity to detect serum retinol levels <0.70 μmol/L, 72.1% and 70.5% respectively (**Table 4**).

Table 4. Sensitivity and specificity of serum retinol binding protein (RBP) concentration for detecting low serum retinol concentrations in breastfeeding women (data from baseline of intervention study)

	Serum retinol concentration		
	<0.70 μmol/L	≥0.70 μmol/L	
Serum RBP concentration (μmol/L)			
<1.29	44	33	Sensitivity: 44/ 61 = 72.1 %
≥1.29	17	79	Specificity: 79/112 = 70.5 %
	61	112	

Modified relative dose response method

When breastfeeding and non-breastfeeding women were categorized on the basis of dehydroretinol/retinol ratio, breastfeeding women had 20-25% lower serum retinol concentrations than non-breastfeeding women (**Figure 2**). The distribution of breastfeeding women with retinol concentrations <0.70 μmol/L in serum and breastmilk is shown in **Table 5**. Of breastfeeding women with either a serum or breastmilk retinol concentration <0.70 μmol/L, 26% had dehydroretinol/retinol ratios <0.06. When the cut-off for serum retinol concentration was reduced by 20% to 0.56 μmol/L, to reflect the difference between breastfeeding and non-breastfeeding women as shown in **Figure 2**, 22% (10 of 46) of the breastfeeding women had dehydroretinol/retinol ratios <0.06 (data not shown). The low ratios for women with retinol concentrations <0.70 μmol/L in serum and/or breastmilk were not significantly related to lower serum concentrations of albumin and/or

Table 5. Breastfeeding women with retinol concentration in serum and/or breastmilk <0.70 μmol/L by dehydroretinol/retinol ratio and their serum concentrations of albumin and retinol binding protein (RBP) (baseline and follow-up data from intervention study treated as independent data).

	dehydroretinol/retinol ratio		
	<0.03	≥0.03 - <0.06	≥0.06
Serum retinol <0.70 μmol/L, % (*n*)	12.0 (11)	14.1 (13)	73.9 (68)
Serum albumin (g/L)	4.20 ± 0.38¶[ab]	4.07 ± 0.21[a]	4.30 ± 0.33[b]
Serum RBP (μmol/L)	1.22 ± 0.34	1.09 ± 0.28	1.19 ± 0.42
Breastmilk retinol <0.70 μmol/L, % (n)	9.6 (12)	16.0 (20)	74.4 (93)
Serum albumin (g/L)	4.21 ± 0.38	4.18 ± 0.24	4.28 ± 0.33
Serum RBP (μmol/L)	1.50 ± 0.60[ab]	1.82 ± 0.94[a]	1.32 ± 0.43[b]

¶ $\bar{x}$ ± SD

[a,b] Values in the same row with different superscript are significantly different from each other ($p<0.05$)

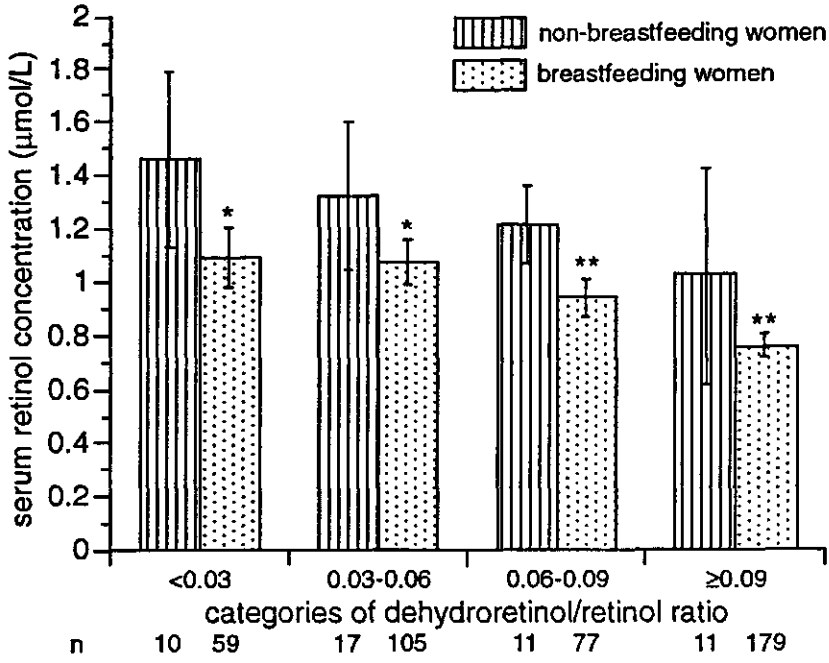

Figure 2. Serum retinol concentrations for different categories of dehydroretinol/retinol ratio for breastfeeding and non-breastfeeding women (data from cross-sectional study and intervention study, baseline and follow-up data treated as independent data).

Mean and 95% CI. *,** Serum retinol concentration is significantly lower in breastfeeding women as compared to non-breastfeeding women, * $p<0.05$, ** $p<0.01$

retinol binding protein.

Sample size required to detect a change in vitamin A status

Table 6 shows, for the different indicators of vitamin A status, the within-person coefficient of variation, the difference in change between the enriched-wafer and the control-wafer group of the intervention study, and the sample size required to detect this difference as being statistically significant. All indicators could potentially improve due to the intervention. The proportion of women who

Table 6. Within-person variation, coefficient of variation, and required sample sizes to detect differences in change of indicators as significant (data from intervention study)

Parameter	n	Baseline level	Within-person variation	Within-person coefficient of variation (%)	Observed difference¶	Sample size required to detect difference as significant
Serum retinol (μmol/L)	173	0.85 ± 0.32§	0.20	24.1	0.29 ± 0.41§	19
Serum RBP (μmol/L)	167	1.59 ± 0.70	0.49	31.2	0.44 ± 0.79	35
Breastmilk retinol (μmol/L)	168	0.90 ± 0.69	0.60	66.6	0.56 ± 1.03	36
Milk fat retinol (nmol/g)	168	28 ± 17	18	62.6	11 ± 39	139
DR/R ratio	148	0.097 ± 0.089	0.047	48.1	0.043 ± 0.096	53
Serum β-carotene (μmol/L)	159	0.19 ± 0.11	0.24	126.7	0.71 ± 0.24	5

¶ Difference between change in enriched-wafer group ($n = 46$) and control-wafer group ($n = 44$) of intervention study
§ $\bar{x}$ ± SD

after delivery those values may not have been very accurate.

Other differences between the studies are that they studied a relatively homogenous group, women were enrolled 2 weeks after delivery and followed for 2.5 mo, whereas we started with women with a breastfed child of 3-15 mo old and followed them for 3 mo. The treatment in the two studies was also different, a megadose of vitamin A in their study, 3.5 mg β-carotene daily for 12 weeks in our study. Although baseline values were higher in their study compared to our study, the increases in serum retinol concentration and retinol content of milk fat were the same in both studies, while breastmilk retinol concentration increased twice as much in their study as compared to our study. From the formula used to calculate sample sizes it follows that the more heterogenous the group in its response relative to the difference in response to be detected, the larger the sample size required. Two questions can be raised: 1. is breastmilk retinol more sensitive to detect a difference in change than serum retinol when the breastfeeding women are all at the same and early stage of lactation?; and 2. does a large dose of vitamin A increase retinol concentration in breastmilk so much more than in serum, that milk retinol can be more sensitive to change than serum retinol?

We found that expressing breastmilk retinol content per volume was better than expressing it per gram milk fat, because it was more sensitive to change, because both the retinol content of milk fat and milk fat content increased. Also, milk fat content was lower for women with a breastfed infant of 3-6 mo old as compared for women with an older breastfed child. However, Stoltzfus and colleagues however, reported that the fat content of breastmilk did not change during lactation and, as discussed above, found that milk retinol content expressed per gram fat was more sensitive to detect a difference between their two groups than milk retinol expressed per volume [17]. Rice and colleagues also concluded that breastmilk retinol content is better expressed per gram fat than per volume, because the expression per volume is sensitive to sampling errors [18]. Our data suggest that retinol content of milk fat may not only be less sensitive to sampling errors, but also relatively insensitive to changes in vitamin A status. The apparent controversy about the usefulness of expressing breastmilk retinol content per gram fat should be solved by examining more data. Such an examination should take differences in stages of lactation of the women into account and should use the calculations appropriate for the purpose of the comparison, should it for example evaluate treatment effects or compare two groups in different places or different

years? When breastmilk retinol content is expressed per volume, the vitamin A intake of the infant can be calculated using an estimate of the volume drank. For breastmilk retinol content to be expressed per volume, sampling procedures should be well standardized. Also, a choice between indicators of vitamin A status, such as breastmilk retinol or serum retinol, depends not only on their sensitivity to changes of vitamin A status, but also on other factors such as the feasibility of collecting the samples required.

Serum retinol binding protein concentration was the same for women with breastfed children of different ages. The cut-off level for serum retinol binding protein concentration at which sensitivity and specificity to detect a low serum retinol concentration was highest, was 1.29 μmol/L (27 mg/L). This is almost the same as a previously suggested cut-off level for low serum retinol binding protein concentration of 1.24 μmol/L (26 mg/L) [19]. The sensitivity and specificity of serum retinol binding protein to detect low serum retinol concentrations, of 72 and 70% respectively, could be improved by taking more than one blood sample per subject. If it is decided, in order to reduce the burden for the subjects, to collect blood by finger prick for the measurement of retinol binding protein, instead of by venous puncture for the measurement of retinol, the within-person variation of both indicators should first be compared. On basis of that comparison it could be decided whether more than one sample should be taken per subject. Actually, some investigators have managed to collect enough blood from the finger for the determination of serum retinol concentration [20, 21], but that requires a special technique.

Breastfeeding women had a significantly lower serum retinol concentration than non-breastfeeding women, irrespective of their dehydroretinol/retinol ratio. That is, for the same serum retinol concentration breastfeeding women had a lower dehydroretinol/retinol ratio than non-breastfeeding women. Because the determination of the ratio requires the measurement of serum retinol concentration, the modified relative dose response method could be most valuable in identifying groups with normal serum retinol concentrations but low stores. We were not able to compare the performance of the modified relative dose response method to a "gold standard" of vitamin A stores, but we were able to determine the sensitivity to detect retinol concentrations <0.70 μmol/L in both serum and breastmilk of breastfeeding women. The sensitivity appeared to be 74% for both fluids. Lowering the cut-off for serum retinol to 0.56 μmol/L did not improve the

sensitivity.

Data reported by Tanumihardjo and co-workers showed similar levels of sensitivity to detect serum retinol concentrations <0.70 μmol/L [4, 22], whereas very poor sensitivity has been reported when the method was used in moderately malnourished children. Only 71% of the children with serum retinol <0.35 μmol/L and 33% of the children with concentrations of 0.35-0.70 μmol/L had dehydroretinol/retinol ratios ≥ 0.06 [23]. Based on a comparison with the relative dose response (RDR) method, the investigators hypothesized that dehydroretinol could not effectively compete with retinol for binding to retinol binding protein (apo-RBP) in malnourished subjects. However, the availability of apo-RBP does not seem to have limited the dehydroretinol/retinol ratio in the women of our study, because we found no relationship between serum concentrations of retinol binding protein or albumin and the dehydroretinol/retinol ratio in the women with low serum retinol concentrations. The poor sensitivity seems to have been due to the relatively large within-person variation, which was twice as large as for serum retinol. Another study conducted over a 7 mo period in healthy subjects found a three times larger variation for the dehydroretinol/retinol ratio (CV, 27%) as compared to serum retinol concentration (CV, 9%) [24]. In fact, the ratio is very sensitive to small aberrations in the dehydroretinol concentration, especially at low serum retinol levels. When for example serum retinol concentration is 0.80 μmol/L, a dehydroretinol concentration of 0.04 μmol/L gives a dehydroretinol/retinol ratio of 0.050, while a concentration of 0.05 μmol/L gives a ratio of 0.063. Thus, random errors in laboratory measurements, as well as variation in administering the oral dose of dehydroretinol (dissolved in 250 μl corn oil), within-subject variation in absorption of the dose, and variation in the timing of taking the blood sample, all contribute to a relatively large within-person variation.

This relatively large within-person variation combined with the relatively small change to be detected, made the modified relative dose response method relatively insensitive to detect the difference in change between groups due to the food intervention. The recent conclusion by Tanumihardjo and co-workers [22] that the modified relative dose response method has more statistical power than serum retinol, cannot be confirmed by our results. With the same sample size as for serum retinol, 19, the difference in the change of dehydroretinol/retinol ratio between our treatement groups which could have been detected with statistical significance was ≥ 0.071. Especially in food-based interventions where the change

in vitamin A status may not be as large as compared to when a megadose is given, serum retinol will be more sensitive than the dehydroretinol/retinol ratio, as long as the levels at the start could still increase, as they did in our study population with starting concentrations of 0.85 μmol/L.

A difference in serum retinol concentration between breastfeeding and non-breastfeeding women with the same dehydroretinol/retinol ratio has not been reported previously. Possible reasons for this finding are a difference between the two groups in the specificity of retinol binding protein to bind dehydroretinol, in physiological serum retinol concentrations, or in the rate of mobilization of apo-RBP. The latter phenomena has also been suggested by Bulux and co-workers, who found that the maximal response of the relative dose response method in older adults occured 6-7 h after dosing, which is later than the 5 h after dosing recommended for measurements in young adults and children [25]. Tanumihardjo and co-workers [7] reported an increase of the dehydroretinol/retinol ratio from 3 through 6 hours after dosing in breastfeeding as well as non-breastfeeding women. Whether both groups of women have reached the same proportion of their maximal response at 5 h is not known. The performance of the method and the appropriateness of the suggested cut-off values should thus be evaluated for physiologically different groups.

Serum β-carotene concentration was the parameter with the largest within-person coefficient of variation, which may be due to its responsiveness to intake. Paradoxically, it also required the smallest sample size to detect a difference in change between groups, because of the large increase in response to the intervention. Although serum β-carotene does not necessarily reflect vitamin A status, these data show that where a change in vitamin A status is expected because of an intervention with β-carotene, albeit from isolated β-carotene or from foods, it is worthwhile to include the measurement of serum β-carotene.

In conclusion, serum retinol was the best indicator of vitamin A status in our population because of its relatively low within-person variation and its responsiveness to apparent change in vitamin A nutriture. This level of responsiveness was found when average baseline concentrations were 0.85 μmol/L. More data are required about breastmilk retinol composition and its changes at different stages of lactation, before firm conclusions can be drawn about the use of breastmilk retinol as an indicator of vitamin A status.

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CHAPTER 7

General discussion

CONTROLLING VITAMIN A DEFICIENCY

After a general introduction on strategies for controlling vitamin A deficiency with particular emphasis on food-based approaches, this chapter discusses the main findings presented in this thesis. The results of the intervention study are discussed first, because they also have a bearing upon the assessment of vitamin A intake, followed by a discussion of the assessment of vitamin A intake and vitamin A status. At the end of the chapter, the main conclusions are presented together with implications for policy development and recommendations for future research.

Different strategies can be used to control vitamin A deficiency. Clinical signs of vitamin A deficiency and conditions which rapidly reduce vitamin A status, such as measles infection, should be treated with megadoses of vitamin A [1]. Megadoses can also be used for prophylaxis but in situations where clinical signs of vitamin A deficiency are virtually absent, such as has recently become the case in Indonesia, food-based approaches are being assigned a major role in the prevention of vitamin A deficiency [1]. A variety of foods can be used for this approach: foods naturally rich in retinol, foods naturally rich in provitamin A carotenoids, or foods fortified with retinol and/or β-carotene.

Effectiveness of food-based approaches

In ancient Egypt and Greece, as early as 1500 BC, liver was already given to people who suffered from night blindness [2]. It is now known that night blindness is the first clinical sign of vitamin A deficiency and that liver is very rich in retinol. The effectiveness of another retinol-rich food, butter, was evident when xerophthalmia cases started to occur in the 1920s in Denmark after butter was being exported to Great Britain. When margarine, which replaced butter, was fortified with retinol, xerophthalmia cases were no longer found [3]. Fat spreads in most countries of Europe are still fortified with retinol, while food fortification is also practiced in some developing countries. Guatemala and other Central American countries have for example been very successful in reducing vitamin A deficiency through the fortification of sugar with vitamin A [4]. Research in

Indonesia has shown that fortification of monosodium glutamate with retinol can be highly effective in improving vitamin A status [5]. However, this has not yet been introduced, mainly because the costs of fortifying this particular food vehicle are relatively high. Thus, retinol-rich foods, whether naturally rich or because of fortification, can improve vitamin A status. For carotene-rich foods the picture is less clear. The effectiveness of synthetic β-carotene [6, 7] (*Chapter 3*) and carotene-rich red palm oil [8-10] have been shown, but for carotene-rich fruits and vegetables there is little evidence for an important role in the prevention of vitamin A deficiency (*Chapter 5*).

In Western populations, vitamin A deficiency has been eliminated, except in those people who suffer from lipid malabsorption which interferes with the absorption of fat soluble vitamins such as vitamin A. While this absence of vitamin A deficiency is mainly due to fortification of foods with retinol, there is growing interest in fruits and vegetables because of a negative association between their consumption and the incidence of cancer [11] and coronary heart disease [12]. Whether this negative assocation is related to the content of carotenoids and/or other anti-oxidants is the topic of many studies [13].

The bioavailability of carotenoids is thus of interest because of their provitamin A activity, as well as their possible role in the prevention of degenerative diseases.

DARK-GREEN LEAFY VEGETABLES FOR IMPROVING VITAMIN A STATUS?

Findings

No change in vitamin A or in iron status were found after providing dark-green leafy vegetables and carrots or a control-wafer to breastfeeding women for 12 weeks, while vitamin A status improved greatly after consumption of the same amount of β-carotene fed in an enriched-wafer (*Chapter 3*). This result does not confirm the generally held assumption that dark-green leafy vegetables can improve vitamin A status and has produced many reactions (*Chapter 4*). Most of the reactions did not question the design and the results of the study, but focussed on questions such as how the lack of effect could be explained and whether the

results could be generalized to other situations.

We have evaluated thoroughly the 16 other intervention studies with dietary carotenoids conducted in countries with a vitamin A deficiency problem, 13 of which found a positive effect on vitamin A status and 3 found no effect (*Chapter 5*). Of these studies, 14 had a weak study design. One of the two studies that were designed well was done in Guatemala with carrots and found no effect on serum concentrations of retinol and β-carotene [14], while the other was done in Indonesia using a combination of red sweet potato and dark-green leafy vegetables which resulted in an increase of serum retinol concentrations [15]. Not only our study and the study in Guatemala, but also studies in Western populations have found a much lower bioavailability of β-carotene from vegetables than generally assumed [6, 7, 16].

Hypotheses to explain the limited role of dark-green leafy vegetables

Hypotheses to explain the lack of improvement in vitamin A status after feeding dark-green leafy vegetables are mainly related to bioavailability, while differences between individuals and populations of as yet unidentified factors could further modify the differences in carotene bioavailability between foods.

Bioavailability

We have grouped the factors which influence bioavailability of carotenoids, into a mnemonic, "**SLAMANGHI**" (*Chapter 5*):

Species of carotenoids;

Linkages at molecular level;

Amount of carotenoids in a meal;

Matrix in which the carotenoid is incorporated;

Absorption modifiers;

Nutrient status of the host;

Genetic factors;

Host related factors;

Interactions between all those factors.

The most important factors to explain the poor bioavailability of carotenoids from dark-green leafy vegetables, seem to be the **matrix**, the **absorption modifiers**, and the **host related factors**, especially parasitic infestations. The matrix of dark-green leafy vegetables is characterized by many membranes and a lack of fat inside the chloroplast, the organel of the plant cell which contains the carotenoids [17]. Dark-green leafy vegetables also contain a relatively large amount of absorption inhibitors such as fibre [18, 19] and other carotenoids which could compete for absorption [20]. When parasitic infestations or other factors that interfere with the digestive system, are added to the effect of the difficult matrix and absorption inhibitors of vegetables, the bioavailability of carotenoids from dark-green leafy vegetables may become very poor.

The matrix and absorption inhibitors present in dark-green leafy vegetables can also reduce carotene bioavailability in Western populations. Most of the studies in Western populations have used carrots instead of dark-green leafy vegetables [6, 7, 16], but the effect of the matrix of dark-green leafy vegetables may be similar to that of the matrix of carrots, because in carrots, β-carotene exists in a crystalline form which may be difficult to dissolve [17]. The position of β-carotene in carrots is different from that in tubers, such as red sweet potato, and fruits, where it is usually found in the chromoplast, surrounded by fat which enhances bioavailability [17].

Possible differences between individuals and populations

The fact that almost all studies evaluated in *Chapter 5* can be criticized for their design does not simply rule out the possibility that in the places and under the circumstances where those studies were conducted dark-green leafy vegetables can improve vitamin A status. Limited bioavailability of carotenoids due to a difficult matrix and absorption inhibitors would also apply to those studies, although these characteristics may differ between different varieties of the same vegetable, but it is possible that additional factors which reduce bioavailability were less prevalent. Such additional factors could be related to the kind and the prevalence of infections which affect the gastro-intestinal tract and/or to as yet unidentified factors which influence carotene bioavailability.

All studies included in our review were done with children and for some studies they were dewormed prior to the intervention. Even if they were not

dewormed, their parasite load may have been lower than the load in the women we studied (*Chapter 5*). It could be hypothesized that if, for example, *Ascaris lumbricoides*, a helminth which lives in the small intestine [21], interferes with the digestion and absorption of nutrients from dark-green leafy vegetables, a higher worm load could theoretically cause a larger reduction of bioavailability.

Examples of factors which may reduce carotene bioavailability but which have been studied inadequately would include differences in the activity of enzymes essential for the release and absorption of carotenoids from foods. Such differences in activity could be due to the deficiency of a specific nutrient in the diet, or to differences in the genetic coding for such an enzyme. Studies in Western populations have for example documented a large range of responsiveness between individuals [6, 22, 23], and the women in our study who received the enriched-wafer also showed a large range of responsiveness (*Chapter 3*). This large range may be related to genetic and/or dietary factors, identified in the mnemonic "SLAMANGHI" as **nutrient status of the host** and **genetic factors**. Whether these factors could explain differences between populations remains to be elucidated.

Thus, we hypothesize that the major causes of the lack of an effect of dark-green leafy vegetables and carrots on vitamin A status are the matrix and the absorption inhibitors of the vegetables, possibly aggrevated by parasitic infestation and genetic and/or dietary factors which reduce carotene bioavailability through as yet unknown mechanisms.

ASSESSMENT OF VITAMIN A INTAKE

Findings

The food frequency questionnaire for assessing vitamin A intake confirmed that, based on what has been generally assumed, plant sources, especially dark-green leafy vegetables, are by far the most important source of vitamin A in developing countries [24]. They accounted for 80-85% of the dietary vitamin A intake of breastfeeding women in West Java (*Chapter 2*).

The estimate of total vitamin A intake depends very much on the method

used to analyse the carotene content of foods. When we used very recent data on carotene content of foods, vitamin A intake was 25% lower as compared to when we used data from Indonesian food composition tables (*Chapter 2*). The vegetable consumption estimated with the food frequency questionnaire was approximately twice as high as the estimate from a 24-h recall questionnaire, and the reproducibility of vegetable consumption was much lower than of consumption of fish and meat.

Improving a questionnaire on vitamin A intake

An overestimate of intake has also been found by other investigators in West- and Central-Java [25-27] and is mainly due to the abundance of vegetables, which makes the list of vegetables included in the questionnaire very long. The overestimate could be reduced by correcting intake of individual vegetables on the basis of the consumption of foods by food group. If the choice of foods to be included in the questionnaire could be based on food consumption data already available, the length of the list of foods could be limited and it would also be possible to include prepared foods and to establish portion sizes. Such modifications would also improve reproducibility of the questionnaire.

Factors to convert carotene content into vitamin A activity

Because the bioavailability of carotenoids seems to depend on the food in which they are found, it would be preferable to express vitamin A intake in terms of the amount of the active compounds (retinol, β-carotene and other provitamin A carotenoids) and to group the compounds according to their source, such as dark-green leafy vegetables, non-leafy vegetables and fruits. For the expression of vitamin A intake, the intake of individual compounds in groups of foods should be multiplied by different bioavailability factors. However, there is insufficient scientific basis at the present time to develop such new factors. This was also recognized when the current recommended conversion factors for dietary carotenoids were set [24, 28].

RELATIONSHIP BETWEEN VITAMIN A INTAKE AND STATUS

Findings

The correlation between individual vitamin A intake and status was poor. This can be attributed only in part to the relatively poor reproducibility of the questionnaire and its overestimate of intake. Other investigators have also reported poor correlations, from developing countries [29] as well as, although to a smaller extent, from Western countries [30-33]. The main causes of poor correlation are limited quality of food composition data (*Chapter 2*), influence of other factors such as infection on status, and limited knowledge about bioavailability and bioconversion of carotenoids (*Chapters 3, 4 and 5*).

Estimating risk of vitamin A deficiency on the basis of vitamin A intake?

Because the relationship between vitamin A intake and status was found to be poor, and as yet only partly understood, a vitamin A intake questionnaire should be used only, if at all, to assess the risk of vitamin A deficiency at the community level. For such a purpose, Helen Keller International has suggested the development of a very simple questionnaire, which only collects information on consumption at the level of food groups, uses a limited reference period, does not require food composition data, and does not use portion sizes [34]. It was developed to assess the risk that at least 15% of the children in a population have serum retinol concentrations <0.70 μmol/L, but the currently recommended cut-off values still have to be evaluated by studying communities other than those in which the questionnaire was developed. The concept of only collecting information on consumption of foods grouped into food groups is interesting because it may capture the difference in bioavailability of carotenoids from foods with a different matrix and it does not require information on vitamin A content of foods.

If the vitamin A intake of a population can be classified as either high or low, the risk of misclassifying that population as at risk or not at risk of vitamin A deficiency may be small. However, if intake is neither high nor low, the risk of misclassification on the basis of intake will be larger, which makes it necessary to measure vitamin A status using biochemical indicators. On the other hand, in some

situations it may not even be necessary to develop a questionnaire, because a quick assessment of available sources of vitamin A, by visiting markets and talking to key informants, may already lead to the conclusion that food sources are too scarce to prevent vitamin A deficiency.

When to use a vitamin A intake questionnaire

Vitamin A intake questionnaires should thus not be used to estimate an individual's risk of poor vitamin A status. They should only be used to estimate the risk of a population, but the number of situations for which a reliable questionnaire can be developed are limited. In case a questionnaire is developed, its senstivity and specificity should be evaluated carefully.

Elaborate vitamin A intake questionnaires, such as the one developed by us, should only be used to answer questions such as what the most important food sources of vitamin A are, whether vitamin A intake of the population has changed, or whether different populations have different food consumption patterns. Questionnaires developed for this purpose should also be evaluated carefully before they are used.

BIOCHEMICAL INDICATORS OF VITAMIN A STATUS

Findings

The evaluation of biochemical indicators of vitamin A status (*Chapter 6*), showed that in our study population of breastfeeding Indonesian women who did not suffer from infection and had average serum retinol concentrations of 0.85 μmol/L, serum retinol concentration was more sensitive for detecting a moderate change in vitamin A status than the modified relative dose response (MRDR) method, breastmilk retinol concentration and serum retinol binding protein concentration. It was found that it is not necessary to measure the fat content of breastmilk, unless the fat content itself is of interest, because the retinol content of milk fat remained almost constant when vitamin A status changed. Breast-

feeding women had lower serum retinol concentrations for specific categories of the dehydroretinol/retinol ratio (MRDR method) than non-breastfeeding women and the dehydroretinol/retinol ratio is relatively vulnerable to small errors in the administration, absorption and determination of dehydroretinol. Serum β-carotene concentration was the most responsive parameter of the food intervention study.

Evaluating indicators of vitamin A status

There is an urgent need for a new "gold standard" of vitamin A status which can be used to evaluate the performance of various indicators of vitamin A status, because use of the current "gold standard", liver retinol stores, is usually impractical. Such a new "gold standard" which is currently being developed, is based on the labelling of retinol with stable isotopes and the measurement of its dilution after administration to humans. Until now, indicators are usually evaluated on the basis of a comparison with other indicators which themselves are not ideal indicators of vitamin A status. In our study, indicators were compared with serum retinol, which was justified because subjects with clinical signs of infection or elevated white blood cell counts were excluded from data analysis (*Chapter 6*).

Indicators recommended for the measurement of vitamin A status

For populations with serum retinol concentrations below 0.85 μmol/L, serum retinol concentration seems the best biochemical indicator of vitamin A status because within-person variation was small and it proved to be the most sensitive indicator to detect a moderate change of vitamin A status. When collection of venous blood samples is difficult, breastmilk samples could be collected for the analysis of retinol content, or serum retinol binding protein concentration could be analysed in blood obtained by finger prick. Analyses of serum retinol binding protein generally requires less serum than does the analysis of serum retinol. Some investigators have been able to measure serum retinol concentration in blood obtained from the finger [35, 36], but the technique used for this type of blood collection has not been adopted widely and does not allow the measurement of additional indicators, except haemoglobin concentration and haematocrit.

Breastmilk retinol content can best be related to milk volume. It is also necessary to specify the age of the breastfed children when reporting breastmilk retinol content. For both breastmilk retinol concentration and serum retinol binding protein concentration it would be best to collect more than one sample of milk or blood for the assessment of individual vitamin A status. For dietary interventions we also recommend the measurement of serum β-carotene concentration because it is the most responsive indicator.

CONCLUSIONS

Dark-geen leafy vegetables and carrots

In the present study, dark-green leafy vegetables and carrots did not improve vitamin A status of breastfeeding women in Indonesia (*Chapter 3*), which suggested that the bioavailability of β-carotene from vegetables is lower than generally assumed. This has also been suggested by other investigators (*Chapters 4 and 5*) and the most important hypotheses are that it is due to:

- the complex matrix of dark-green leafy vegetables;
- the crystalline structure of carotene in carrots is difficult to dissolve;
- relatively large amounts of absorption inhibitors in those vegetables, such as fibre and other carotenoids which may compete for absorption;
- parasitic infestations which aggrevate the limited bioavailability of carotenoids from those vegetables;
- as yet unknown factors which could be of genetic and/or dietary origin, which also reduce bioavailability of carotenoids from those vegetables.

Assessment of vitamin A intake

The assessment of vitamin A intake depends very much upon the quality of the food composition data (*Chapter 2*) and the development of a questionnaire with good reproducibility and validity requires a great effort. The relationship between intake and status depends on many factors, including:

scientists, food industry, plant breeders, soil scientists), whether recommended foods are affordable and acceptable to the population (a question for social scientists and economists [38]), and whether those foods are really consumed (a question for social scientists and nutritionists).

It is encouraging to note an increase in the dialogue between people from many different disciplines working in programmes to reduce malnutrition, in science and in industry. Recent examples of meetings where such interdisciplinary dialogues were held, are the Meeting of the Programme Against Micronutrient Malnutrition (PAMM) in Papendal, the Netherlands, in June 1994, the carotenoid meeting in Washington in April 1995, and the meeting on food-based appraoches in Salt Lake City in November 1995.

Assessment of vitamin A status

Different indicators can be used to assess the extent of a problem of vitamin A deficiency in a population. Biochemical indicators provide the most precise information, while vitamin A intake questionnaires should only be used at community level and after careful testing. However, in some cases, checking for clinical signs of vitamin A deficiency and observing food availability may already be sufficient to conclude that a vitamin A deficiency problem exists.

RECOMMENDATIONS FOR FUTURE RESEARCH

Research with immediate relevance to the development of food-based approaches for the control of vitamin A deficiency should focuss on:

- monitoring the effectiveness of ongoing food-based programmes to control vitamin A deficiency;
- quantification of the role of different carotene- and retinol-rich foods, and also of fat, in improving vitamin A status;
- the influence of different parasitic infestations, and of varying intensity, on the effectiveness of different foods in improving vitamin A status;
- analysis of the composition of food in order to obtain reliable data on the content of carotenoids and of absorption modifiers in different foods.

Future research on carotene bioavailability should focuss on:

- development of methods for measuring carotene bioavailability;
- identification of the characteristics of the food matrix which limit the bioavailability of carotenoids;
- identification of components of foods which influence carotene absorption;
- determining within-individual, between-individual, and between-population differences in carotene bioavailability and identification of underlying factors.

The answers to those questions should guide the development of methods to increase carotene bioavailability.

Research with respect to vitamin A status and vitamin A intake should focuss on:

- development of a "gold standard" for vitamin A status, possibly using stable isotopes;
- longitudinal studies on changes in the composition of breastmilk: this will enable better interpretation of data on breastmilk retinol content;
- development and validation of a simple vitamin A intake questionnaire for estimating the risk of vitamin A deficiency at community level.

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Summary

The research described in this thesis focussed on the role of foods in controlling vitamin A deficiency in breastfeeding women in Indonesia and on ways of assessing vitamin A intake and vitamin A status.

Micronutrient deficiencies, such as vitamin A deficiency, have a devastating impact on development and are, together with protein energy malnutrition, a major cause of death in developing countries. Especially young children and pregnant and breastfeeding women are affected. To reduce micronutrient deficiencies, the following goals for the year 2000 have been set at the World Summit for Children (New York, 1990), and were affirmed at the Ending Hidden Hunger Conference (Montreal, 1991) and the International Conference on Nutrition (Rome, 1992):

- vitamin A deficiency should be virtually eliminated;
- iodine deficiency should be virtually eliminated;
- iron deficiency anaemia in women of reproductive age should be reduced by one-third of 1990 levels.

Different strategies can be used to achieve these goals. Indirect measures are used to reduce losses and/or needs for micronutrients, while direct measures should increase their intake and/or bioavailability. In case of emergencies and/or severe deficiencies, intake should be increased with a pharmaceutical approach, while for the control over the longer term, food-based appraoches are being developed. Food-based approaches can use fortified foods as well as foods naturally rich in micronutrients. The simultaneous intake of other nutrients can be an advantage of foods naturally rich in micronutrients, as well as a disadvantage, in the case that other components reduce bioavailability of micronutrients.

In Indonesia, the prevalence of xerophthalmia, the clinical sign of vitamin A deficiency, has decreased from 1.3% in 1978 to 0.3% in 1992. This is a great achievement, but the need for vitamin A deficiency control programmes still remains. In 1992, serum retinol concentration, a biochemical indicator of vitamin A status, was still low (<0.70 μmol/L) in 51% of children. Therefore, vitamin A capsule distribution for prophylaxis was continued in 1995, while the development and testing of food-based approaches continued so they could replace the capsule distribution programme in the near future.

Vitamin A occurs in two forms in foods: as retinol which exists only in

animal foods, such as liver, eggs and milk, and as carotenoids with provitamin A activity, especially β-carotene, which are mainly found in plant foods, such as dark-green leafy vegetables and orange and yellow fruits. We developed a questionnaire to assess vitamin A intake and confirmed that, based on what has been generally assumed about carotene bioavailability, plant sources, especially dark-green leafy vegetables, are the major source of vitamin A in developing countries. They accounted for 80-85% of vitamin A intake of breastfeeding women in West-Java.

However, feeding dark-green leafy vegetables to breastfeeding women did not improve their vitamin A status, while feeding the same amount of β-carotene in an enriched wafer resulted in a large improvement. This contradicts the general assumption that dark-green leafy vegetables play an important role in the control of vitamin A deficiency. Most of the previously reported studies, many of which suffered from weaknesses of design, found an increase of vitamin A status after feeding dark-green leafy vegetables, but some investigators, from developing as well as Western countries, have also reported smaller effects of carotene-rich vegetables, including carrots, than expected. The major hypotheses to explain the poor bioavailability of carotenoids from those vegetables are that it is due to:

- the complex matrix of green leafy vegetables;
- the crystalline structure of carotene in carrots is difficult to dissolve;
- relatively large amounts of absorption inhibitors in those vegetables, such as fibre and other carotenoids which may compete for absorption;
- parasitic infestations which aggrevate the limited bioavailability of carotenoids from those vegetables;
- as yet unknown factors which could be of genetic and/or dietary origin, which also reduce bioavailability of carotenoids from those vegetables.

Because dark-green leafy vegetables and carrots contribute other essential nutrients to the diet, the fact that their contribution to the control of vitamin A deficiency may be limited, should not lead to the conclusion that consumption and/or cultivation of these vegetables should no longer be promoted. However, food-based approaches should be based on a variety of foods rich in vitamin A, and it seems necessary to reconsider the contribution of various foods to vitamin A intake.

It would be preferable to express vitamin A intake in terms of amount of the active compounds (retinol, β-carotene and other provitamin A carotenoids) and to group the compounds according to their source based on differences in matrix

and/or content of absorption inhibitors. Thus for the expression of vitamin A intake bioavailability factors should be developed for each compound grouped by source. Questionnaires for the assessment of vitamin A intake should not be used to estimate risk of poor vitamin A status at the individual level because correlations found between intake and status are very poor. The relationship between intake and status is affected not only by the bioavailability of carotenoids, but also by factors which affect vitamin A status such as infection. All vitamin A intake questionnaires, regardless of their purpose, should be carefully evaluated for reproducibility and validity. Some suggestions for optimizing questionnaires, based on the experience with our questionnaire, have been given in *Chapter 2*.

Vitamin A status, of individuals as well as of populations, are best assessed with biochemical indicators. The "gold standard" for measuring vitamin A status, liver retinol stores, is not practical. Therefore, many indicators have only been evaluated by comparing them with serum retinol concentration, which is regarded as a reliable indicator of vitamin A status, but has some disadvantages; it is temporarily lowered during infection, can only be used for a limited range of status, and requires a blood sample. We have evaluated various biochemical indicators of vitamin A status by comparing them to serum retinol concentration in a population of breastfeeding women with average serum retinol concentration of 0.85 μmol/L. Serum retinol appeared to have a smaller within-subject variation and to be more sensitive to moderate differences in changes of vitamin A status between groups than breastmilk retinol concentration, the modified relative dose response (MRDR) method and serum retinol binding protein concentration. Breastmilk retinol content can best be expressed per volume and the age of the breastfed child should be specified. Individual concentrations of breastmilk retinol as well as of serum retinol binding protein, can best be based on more than one sample. The fact that the modified relative dose response (MRDR) method was less sensitive than serum retinol would appear to be due mainly to the poor reproducibility of the dehydroretinol concentration due to variation in administering, absorbing and analysing dehydroretinol. In addition, breastfeeding and non-breastfeeding women had different serum retinol concentrations within categories of the dehydroretinol/retinol ratio. Serum β-carotene concentration was very responsive to the food intervention. Its measurement should therefore be included in the evaluation of food-based interventions for improving vitamin A status.

Samenvatting

In dit proefschrift is onderzoek beschreven naar de rol van voedsel bij het terugdringen van vitamine A tekort bij borstvoedende vrouwen in Indonesië en naar methodes voor de bepaling van vitamine A inname en vitamine A status.

Lichamelijke tekorten aan eiwit, energie en/of micronutriënten kunnen een ernstige achterstand in lichamelijke en psychische ontwikkeling veroorzaken en zijn nog steeds de belangrijkste doodsoorzaken in veel ontwikkelingslanden. Vooral jonge kinderen en zwangere en borstvoedende vrouwen zijn er gevoelig voor. Het volgende voorbeeld illustreert de omvangrijke gevolgen van een tekort aan micronutriënten: Een denkbeeldig land met 50 miljoen inwoners en een tekort aan de micronutriënten vitamine A, ijzer en jodium zoals dat nu in Zuid-Azië voorkomt, betreurt hierdoor per jaar:

- 20.000 doden;
- 11.000 kinderen die ernstig misvormd worden geboren ("cretins") en/of blind worden voordat ze de schoolgaande leeftijd hebben bereikt;
- 1.300.000 mensjaren die verloren gaan aan handicaps en/of apatie;
- 360.000 studentjaren die verloren gaan.

Om micronutriënten tekorten terug te dringen zijn er op de "World Summit for Children" in 1990 in New York, drie doelen opgesteld voor het jaar 2000. Deze doelen zijn ook aangenomen op de "Ending Hidden Hunger Conference" in 1991 in Montreal en op de "International Conference on Nutrition" in 1992 in Rome. De doelen luiden als volgt:

- vitamine A tekort komt niet meer voor;
- jodium tekort komt niet meer voor;
- bloedarmoede door ijzertekort bij vrouwen in de vruchtbare leeftijd is verminderd tot 2/3 van de mate waarin het in 1990 voorkwam.

Er zijn verschillende manieren om deze doelen te bereiken. Het verbruik van micronutriënten door het lichaam kan verminderd worden, bijvoorbeeld door infecties te voorkomen. Dit is een indirecte manier om een micronutriënten tekort terug te dringen; de behoefte wordt verlaagd. Een directe manier om het probleem aan te pakken is het geven van de micronutriënten waar tekort aan is. De micronutriënten kunnen in de vorm van een capsule of een pil gegeven worden wanneer het tekort heel ernstig is. Wanneer het tekort minder ernstig is of om een tekort te

voorkomen, kunnen voedingsmiddelen gegeven worden die rijk zijn aan micronutriënten. Voedingsmiddelen kunnen op twee manieren rijk zijn aan micronutriënten, 1. doordat ze er van nature veel van bevatten, of 2. doordat de micronutriënten er tijdens de produktie aan zijn toegevoegd (b.v. vitamines A en D in margarine). Voedsel dat van nature rijk is aan micronutriënten heeft als voordeel dat het meestal ook rijk is aan andere voedingsstoffen. Een hoog gehalte van bepaalde micronutriënten in een voedingsmiddel wil echter nog niet zeggen dat deze ook in grote hoeveelheden door het lichaam worden opgenomen. Ook de biologische beschikbaarheid van de micronutriënten (de mate waarin een voedingsstof wordt opgenomen) moet goed zijn. Zo kan een voedingsmiddel dat rijk is aan bepaalde micronutriënten ook stoffen bevatten die de opname van de micronutriënten hinderen, zoals vezel, waardoor de biologische beschikbaarheid van de micronutriënten uiteindelijk laag is. De biologische beschikbaarheid van micronutriënten is ook afhankelijk van de combinatie van voedingsmiddelen die gegeten wordt. De kennis over deze zogenaamde biologische beschikbaarheid is nog beperkt.

Het onderzoek dat we in Indonesië hebben gedaan was gericht op het terugdringen van vitamine A tekort met behulp van voedsel. Wereldwijd lopen 250 miljoen kinderen, vooral in ontwikkelingslanden, een risico op vitamine A tekort. Een vergevorderd tekort aan vitamine A is te herkennen aan xerophthalmie, een beschadiging aan het oppervlak van de oogbol die tot blindheid kan leiden. Een minder vergevorderd tekort aan vitamine A leidt tot een verminderd functioneren van het afweersysteem. De algemene conclusie uit acht grote onderzoeken uitgevoerd in verschillende ontwikkelingslanden waar vitamine A tekort voorkomt is dat het geven van hoge doses vitamine A aan alle jonge kinderen, de kindersterfte met 23% kan verminderen. In Indonesië kwam xerophthalmie in 1978 voor bij 1.3% van de kinderen en in 1992 nog bij 0.3%. Dit is een belangrijke vermindering, maar de serum retinol gehaltes, een maat voor vitamine A status, waren in 1992 nog van matig niveau bij 51% van de kinderen. Zij liepen daardoor dus nog steeds een verhoogd risico op ziekte en sterfte. Daarom is in Indonesië ook in 1995 met het verspreiden van vitamine A capsules doorgegaan, terwijl methodes op basis van voedsel ontwikkeld en getest werden. Een voedselaanpak moet op afzienbare termijn het uitdelen van capsules vervangen omdat het probleem van vitamine A tekort niet zo ernstig meer is dat het alleen met capsules opgelost kan

worden, het uitdelen van capsules niet oneindig door kan gaan, en dat niet iedereen die vitamine A nodig heeft een capsule kan of mag krijgen. Bij zwangere vrouwen is een hoge dosis vitamine A bijvoorbeeld giftig voor het ongeboren kind en daarom krijgt niet één vrouw in de vruchtbare leeftijd een hoge dosis vitamine A, want ze zou zwanger kunnen zijn.

Vitamine A komt in voedsel voor als retinol en als provitamine A carotenen. Slechts een deel van de in het voedsel voorkomende carotenen zijn provitamine A carotenen. β-caroteen is het belangrijkste provitamine A caroteen. Retinol is makkelijk door het lichaam op te nemen en direct in werkzame vorm beschikbaar, terwijl provitamine A carotenen moeilijker opneembaar zijn en na opname door het lichaam omgezet moeten worden in het werkzame retinol. Retinol is vetoplosbaar en komt uitsluitend voor in dierlijke produkten zoals lever, melk en eieren, terwijl provitamine A carotenen vooral voorkomen in plantaardige produkten zoals donkergroene bladgroenten en oranje en geel fruit. Met de vragenlijst die we ontwikkeld hebben om vitamine A inname te schatten, hebben we bevestigd dat plantaardige produkten, met name donkergroene bladgroenten, de belangrijkst bron zijn van vitamine A in ontwikkelingslanden. Ongeveer 80-85% van de vitamine A inname van borstvoedende vrouwen in West Java was van plantaardige oorsprong. We zijn hierbij uitgegaan van de algemeen geldende aannames over biologische beschikbaarheid van carotenen.

Het gedurende 12 weken dagelijks verstrekken van porties klaargemaakte donkergroene bladgroenten aan borstvoedende vrouwen leidde echter niet tot een verbetering van hun vitamine A status. Een wafel waaraan eenzelfde hoeveelheid β-caroteen was toegevoegd veroorzaakte daarintegen een belangrijke verbetering in vitamine A status. Deze bevinding is in strijd met de algemeen geldende aanname dat donkergroene bladgroenten een bijdrage kunnen leveren aan het terugdringen van een vitamine A tekort. Deze aanname is gebaseerd op eerder onderzoek dat veelal onder moeilijke omstandigheden was uitgevoerd met weinig financiële middelen en geringe kennis over hoe een onderzoek het beste opgezet kan worden. Maar enkele andere onderzoekers, zowel uit ontwikkelingslanden als uit Westerse landen, hebben ook slechts kleine, of zelfs geen, effecten van donkergroene bladgroenten en wortels op de vitamine A status gevonden. Dit duidt erop dat de biologische beschikbaarheid van carotenen uit donkergroene bladgroenten en wortels veel lager is dan tot nu toe werd aangenomen. Belangrijke

hypotheses voor de oorzaak van deze matige biologische beschikbaarheid zijn:

- een complexe structuur van groene bladgroenten;
- een moeilijk oplosbare kristalstructuur van carotenen in wortels;
- relatief grote hoeveelheden stoffen in groenten die caroteen opname remmen zoals vezel en andere carotenen die mogelijk meestrijden voor opname;
- parasitaire infecties die de biologische beschikbaarheid van de carotenen uit deze groenten verder verlagen;
- nog niet geïdentificeerde factoren van genetische en/of voedings aard die de biologische beschikbaarheid mogelijk nog verder verminderen.

Het feit dat de bijdrage van donkergroene bladgroenten en wortels aan het terugdringen van vitamine A tekort beperkt is kan geen reden zijn om hun consumptie en produktie niet langer te stimuleren, want ze voegen andere belangrijke dingen aan de voeding toe, zoals vezel en vitamine C. Daarnaast zorgen groenten voor variatie in de voeding en betekent de kleinschalige produktie ervan vaak extra inkomen voor het huishouden. Maar een voedselaanpak voor het terugdringen van vitamine A tekort zal gebaseerd moeten zijn op verschillende vitamine A rijke voedingsmiddelen en de bijdrage van verschillende voedingsmiddelen aan de vitamine A inname moet opnieuw vastgesteld worden.

Het zou het beste zijn om vitamine A inname (dat wat we consumeren en dus beschikbaar is voor opname) uit te drukken in de verschillende vormen (retinol, β-caroteen en andere provitamine A carotenen) en deze te groeperen naar hun bron waarbij rekening gehouden wordt met de verschillen wat betreft structuur en gehaltes aan stoffen die de opname van carotenen remmen. De totale vitamine A inname kan dan berekend worden met behulp van aparte factoren voor de biologische beschikbaarheid van de verschillende vormen en bronnen van vitamine A. Vragenlijsten voor het bepalen van vitamine A inname kunnen niet gebruikt worden om op individueel niveau de kans op een slechte vitamine A status te schatten om twee belangrijke redenen: 1. de biologische beschikbaarheid van carotenen is slecht bekend en erg variabel, en 2. vitamine A status is niet alleen afhankelijk van inname maar ook van andere factoren zoals infectie. Op basis van de ervaring met onze eigen vragenlijst hebben we in hoofdstuk 2 enkele aanbevelingen gedaan voor het gebruik en de verbetering van vragenlijsten voor vitamine A inname.

De vitamine A status van individuen en groepen kan het best bepaald

worden aan de hand van biochemische indicatoren. De 'gouden maat' van vitamine A status is de voorraad in de lever, maar die is erg moeilijk te bepalen. Het alternatief is de retinol concentratie in serum. Deze geldt als een betrouwbare indicator van vitamine A status, maar heeft enkele nadelen; 1. het is tijdelijk verlaagd in geval van infectie, 2. de bruikbaarheid is beperkt tot een bepaald bereik van status, en 3. het vereist een bloedmonster. Wij hebben in een groep borstvoedende vrouwen verschillende indicatoren van vitamine A status vergeleken met serum retinol, t.w. retinol in moedermelk, de aangepaste relatieve dosis-response methode ("modified relative dose response method or MRDR") en retinol bindend eiwit in serum. Serum retinol bleek in vergelijking met deze andere indicatoren een kleinere binnen-persoonsvariatie te hebben (d.w.z. dat de waardes gemeten bij één persoon op verschillende dagen niet zoveel verschilden) en een kleiner aantal deelnemers aan een experiment nodig te hebben om een verschil tussen behandelingen vast te stellen. We concluderen daarom dat vitamine A status en veranderingen daarin het best bepaald kunnen worden door het meten van serum retinol concentratie. Serum β-caroteen is geen maat voor vitamine A status, maar omdat β-caroteen een voorloper is van vitamine A en omdat de serum concentratie ervan heel snel verandert, raden wij aan om ook serum β-caroteen te meten wanneer een voedingsinterventie wordt uitgevoerd om vitamine A status te verbeteren.

Op basis van ons onderzoek bevelen we vervolgonderzoek aan naar:

- de mate waarin verschillende voedingsmiddelen vitamine A status verhogen;
- de invloed van verschillende parasitaire infecties op de relatie tussen voeding en voedingsstatus;
- factoren die de biologische beschikbaarheid van carotenen beïnvloeden;
- ontwikkeling van een eenvoudige en betrouwbare indicator voor vitamine A status; en
- ontwikkeling van een simpele vragenlijst om het risico dat een bepaalde groep mensen loopt op vitamine A tekort te schatten.

Belangrijkste conclusie

Donkergroene bladgroenten en wortels lijken een veel kleinere bijdrage te leveren aan het terugdringen van een vitamine A tekort dan tot nu toe werd aangenomen. De belangrijkste oorzaak hiervan is waarschijnlijk een slechte biologische beschikbaarheid van de provitamine A carotenen uit deze groenten.

Ringkasan

Topik penelitian yang diuraikan pada tesis ini adalah mengenai peranan makanan dalam mengatasi kekurangan vitamin A di kalangan wanita menyusui di Indonesia dan beberapa cara dalam hal mengukur masukan vitamin A dan status vitamin A.

Defisiensi mikronutrient seperti kekurangan vitamin A, mempunyai dampak dalam pertumbuhan fisik, dan bila disertai dengan kekurangan energi dan protein, merupakan penyebab kematian utama di negara-negara berkembang. Hal ini terjadi terutama pada anak-anak, wanita hamil dan wanita menyusui. Sebagai gambaran adalah sebagai berikut: sebuah negara yang berpenduduk 50 juta dengan prevalensi defisiensi vitamin A, zat besi dan yodium seperti di negara Asia Tenggara, maka akibat yang dapat terjadi per tahunnya adalah sebagai berikut:

- kematian 20.000 orang;
- 11.000 bayi yang lahir mengalami kelainan (kretinisme) dan/atau buta pada usia prasekolah;
- 1.300.000 orang/tahun mengalami cacat dan/atau keterbelakangan mental fisik;
- kehilangan 360.000 anak usia sekolah.

Untuk mengatasi defisiensi mikronutrient ini menjelang tahun 2000 seperti yang sudah diutarakan oleh "World Summit for Children" (1990) di New York, dan ditegaskan pada Kongres "Ending Hidden Hunger" (1991) di Montreal dan "International Conference on Nutrition" (1992) di Roma dinyatakan beberapa tujuan berikut:

- mengatasi kekurangan vitamin A;
- mengatasi kekurangan yodium
- menurunkan jumlah kasus kekurangan zat besi di kalangan wanita produktif sampai 2/3 kalinya di tahun 1990an.

Beberapa strategi dapat dilaksanakan untuk mencapai beberapa tujuan di atas. Cara tak langsung untuk mengatasi kekurangan mikronutrient ini yaitu dengan menurunkan pengeluaran dan/atau kebutuhan mikronutrient seperti pada penyakit infeksi, dan cara langsung untuk mengatasi masalah ini yaitu dengan memberikan mikronutrient pada saat kekurangan. Pada keadaan kekurangan berat, mikronutrient diberikan dalam bentuk kapsul atau pil. Tapi pada kasus ringan atau untuk

mencegah terjadinya kekurangan mikronutrient, maka intervensi yang diberikan dapat berupa pemberian makanan yang banyak mengandung beberapa mikronutrient. Pemberian makanan yang kaya akan mikronutrient ini dapat dilakukan dalam 2 bentuk yaitu 1) makanan yang secara alami kaya akan mikronutrient dan 2) menambahkan mikronutrient tertentu pada makanan yang diproduksi (contoh: pada margarin). Keuntungan dari makanan yang secara alami kaya akan mikronutrient ini adalah juga mengandung beberapa zat gizi lain yang berguna bagi tubuh. Tetapi, makanan yang kaya akan beberapa mikronutrient ini belum tentu dapat digunakan seluruhnya oleh tubuh. Dalam hal ini ketersediaan secara biologis makanan harus baik, sebagai contoh makanan yang banyak mengandung serat menyebabkan rendahnya ketersediaan secara biologis beberapa mikronutrient lain meskipun makanan tersebut kaya akan beberapa mikronutrient. Ketersediaaan secara biologis bagi tubuh juga tergantung dari kombinasi dari makanan yang dikonsumsi. Hingga kini, belum banyak yang diketahui tentang ketersediaan secara biologis ini.

Di Indonesia saat ini telah dilakukan studi tentang pemberian makanan untuk mengatasi kekurangan vitamin A. Seperti diketahui bahwa 250 juta anak di seluruh dunia, terutama di negara-negara berkembang beresiko tinggi akan kekurangan vitamin A. Kekurangan vitamin A yang berat dapat menyebabkan terjadinya xerophthalmia yaitu kerusakan pada bola mata yang dapat berakhir dengan kebutaan. Pada kasus kekurangan vitamin A yang ringan dapat menyebabkan gangguan pada sistim daya tahan tubuh. Beberapa penelitian yang telah dilakukan (8 penelitian) di negara-negara berkembang yang masih mengalami kasus kekurangan vitamin A melaporkan bahwa pemberian vitamin A dosis tinggi dapat menurunkan 23% kematian anak. Di Indonesia, prevalensi xerophthalmia menurun dari 1.3% pada tahun 1978 menjadi 0.3% pada tahun 1992. Hal ini merupakan perkembangan yang sangat baik, tapi pengukuran kadar serum retinol, sebagai parameter biokimia dari status vitamin A pada tahun 1992 menunjukkan bahwa 51% anak masih memiliki status vitamin A yang rendah. Hal ini menunjukkan bahwa resiko kematian dan penyakit akibat kekurangan vitamin A masih tinggi, sehingga distribusi vitamin A kapsul untuk pencegahan tetap dilakukan sampai tahun 1995, dimana pada waktu itu pula telah dikembangkan dan dicoba menggunakan makanan yang diharapkan dapat menggantkan program pemberian kapsul di tahun-tahun mendatang. Pemberian vitamin A dalam bentuk makanan sudah

dapat menggantikan pemberian vitamin A dalam bentuk kapsul sebab saat ini kekurangan vitamin A sudah dalam tingkat ringan dan alasan lain yaitu kita tidak dapat memberikan kapsul pada seseorang untuk selamanya dan tidak semua orang dapat dan boleh minum kapsul vitamin A. Wanita hamil tidak boleh mengkonsumsi vitamin A dosis tinggi karena sifat toksisnya yang dapat berbahaya bagi janin yang dikandungnya sehingga wanita usia reproduktif juga tidak boleh diberi vitamin A kapsul karena kemungkinan akan kehamilan pada saat itu. Vitamin A yang terkandung dalam makanan terdapat dalam 2 bentuk yaitu: sebagai retinol dan provitamin A karoten. Tidak semua jenis karoten adalah provitamin A. Beta karoten merupakan salah satu provitamin A yang penting. Retinol menurut strukturnya dapat digunakan secara langsung oleh tubuh, sedangkan absorpsi provitamin A karoten tidak semudah retinol dimana di dalam tubuh perlu diubah lebih dahulu ke dalam bentuk retinol. Retinol larut dalam lemak, dan hanya terdapat pada makanan yang berasal dari hewan seperti: hati, susu dan telur, sedangkan provitamin A karoten pada umumnya terdapat pada makanan yang berasal dari tumbuh-tumbuhan seperti: sayur-sayuran hijau dan buah-buahan oranye dan kuning. Dengan menggunakan kuesioner yang terstruktur, kami mendapat asumsi bahwa sumber makanan yang berasal dari tumbuh-tumbuhan terutama sayur-sayuran hijau merupakan sumber utama dari vitamin A di negara-negara berkembang. Sekitar 80-85% masukan vitamin A dari wanita menyusui di Jawa Barat berasal dari sumber makanan nabati. Hal ini juga berdasarkan dari asumsi umum bahwa ketersediaan secara biologis karoten yang baik di dalam tubuh.

Tetapi, pemberian sayur-sayuran hijau kepada wanita menyusui selama 12 minggu ternyata tidak meningkatkan status vitamin A, dimana pemberian beta-karoten dalam jumlah yang sama dalam bentuk wafer ternyata memberikan hasil yang lebih tinggi. Hal ini bertentangan dengan asumsi umum bahwa sayur-sayuran hijau dianggap berperanan dalam mengatasi kekurangan vitamin A. Asumsi ini dihasilkan dari beberapa studi sebelumnya yang dilakukan pada situasi yang sulit, kekurangan biaya dan kurangnya pengetahuan dalam melakukan suatu studi yang baik. Tetapi beberapa peneliti dari negara-negara berkembang seperti juga dari negara-negara Barat, melaporkan bahwa karoten di dalam sayur-sayuran termasuk wortel, hanya memberikan efek yang sedikit bahkan tidak memberikan efek sama sekali terhadap status vitamin A. Ketersediaan secara biologis dari karoten di dalam sayur-sayuran hijau dan wortel bahkan lebih rendah dari asumsi sebelumnya. Beberapa hipotesa yang penting yang dapat menjelaskan hal ini adalah sebagai berikut:

- matrix yang kompleks dari sayur-sayuran berdaun hijau;
- struktur kristal dari karoten pada wortel yang sukar larut;
- *secara relatif cukup banyak faktor lain di dalam sayur-sayuran yang* menghambat penyerapan zat-zat ini seperti serat dan beberapa karoten lain sebagai kompetitif;
- infestasi parasit yang dapat menurunkan kemampuan karoten dari sayur-sayuran untuk mencukupi ketersediaan secara biologis bagi tubuh;
- faktor-faktor lain yang belum diketahui, seperti faktor genetik dan/atau faktor dari jenis makanan asli itu sendiri yang dapat menurunkan kemampuan karoten dari sayur-sayuran tersebut untuk mencukupi ketersediaan secara biologis bagi tubuh.

Kenyataan bahwa kontribusi sayuran hijau tua dan wortel dalam mencegah kekurangan vitamin A adalah terbatas, tidak dapat langsung disimpulkan bahwa konsumsi dan pengolahan sayur-sayuran ini tidak lagi digalakkan, karena mereka merupakan salah satu sumber serat kasar dan vitamin C dalam makanan. Sayuran menambah variasi konsumsi makanan dan produksi sayuran pekarangan dapat meningkatkan pendapatan keluarga. Pendekatan pangan harus didasarkan pada suatu variasi makanan kaya vitamin A, dan tampaknya penting untuk dipertimbangkan kembali kontribusi berbagai makanan pada konsumsi vitamin A.

Jumlah konsumsi vitamin A lebih dianjurkan untuk dinyatakan dalam bentuk banyaknya senyawa aktif (retinol, ß-karoten, dan provitamin A karotenoid lainnya) dan mengelompokkan senyawa-senyawa tersebut menurut sumbernya berdasarkan perbedaan dalam struktur dan/atau kandungan dari faktor-faktor penghambat. Oleh karena itu, untuk mengekspresikan konsumsi vitamin A, faktor ketersediaan biologisnya harus dipertimbangkan untuk setiap kelompok senyawa menurut sumber dan strukturnya. Kuesioner untuk menentukan jumlah konsumsi vitamin A tidak dapat digunakan untuk memperkirakan resiko status vitamin A yang rendah pada individu karena status vitamin A tidak hanya dipengaruhi oleh ketersediaan biologis dari karotenoid, tetapi juga oleh faktor-faktor lain yang mempengaruhi status vitamin A seperti infeksi. Semua kuesioner konsumsi vitamin A, apapun tujuannya, harus benar-benar dievaluasi validitas dan kemampuan pengulangannya. Beberapa usulan untuk optimasi kuesioner berdasarkan pengalaman kuesioner kami dapat dilihat pada Bab 2.

Status vitamin A pada individu dan juga populasi, paling baik diukur dengan

indikator biokimia. Standard baku untuk mengukur status vitamin A, kandungan retinol hati, adalah tidak praktis dan sulit dilakukan. Karenanya, banyak parameter hanya dievaluasi dengan membandingkannya dengan konsentrasi serum retinol, yang merupakan suatu indikator status vitamin A yang dapat dipercaya, tetapi memiliki beberapa kelemahan; yaitu kadang-kadang menurun pada saat infeksi, hanya dapat digunakan untuk kisaran status terbatas, dan memerlukan sampel darah. Kami telah mengevaluasi beberapa indikator biokimia untuk pengukuran status vitamin A dengan membandingkan dengan konsentrasi serum retinol pada populasi ibu-ibu menyusui. Serum retinol tampak memiliki variasi lebih kecil dalam individu (pengukuran pada seorang subyek pada hari yang berlainan dengan hasil pengukuran tidak terlalu berbeda) dan lebih sensitif pada perubahan moderat status vitamin A daripada konsentrasi retinol air susu ibu (ASI), metoda "modified relative dose response" dan konsentrasi serum "retinol binding protein". Konsentrasi retinol pada ASI individual seperti halnya serum "retinol binding protein", paling baik berdasarkan pada pengukuran lebih dari satu sampel. Kami menyimpulkan bahwa status vitamin A sebaiknya dinyatakan dalam konsentrasi serum retinol. Serum ß-karoten tidak mengukur secara langsung status vitamin A, tetapi serum ß-karoten dapat dikonversikan ke vitamin A. Oleh karena itu, kami menyarankan pengukuran serum ß-karoten sebaiknya termasuk dalam evaluasi intervensi pangan untuk meningkatkan status vitamin A.

Berdasarkan hasil penelitian ini, kami menyarankan beberapa hal untuk diteliti lebih lanjut:

- sejauh mana berbagai jenis makanan dapat meningkatkan status vitamin A;
- pengaruh infeksi parasit terhadap hubungan antara konsumsi makanan dan status *gizi*;
- faktor-faktor yang mempengaruhi ketersediaan biologis karotenoid-pengembangan indikator status vitamin A yang mudah dan dapat dipercaya;
- pengembangan kuesioner yang sederhana untuk mengukur resiko kekurangan vitamin A pada sekelompok individu (populasi)

Kesimpulan utama

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About the author

Saskia de Pee was born on 21 August 1967 in Heemskerk, the Netherlands. In 1985 she completed secondary school ("Bonhoeffer College", Castricum) and started the studies in Biomedical Sciences at Leiden University. As part of those studies she conducted research projects in Human Nutrition (Dutch Institute for Applied Research "TNO-Voeding", Zeist, Sept - Dec 1988 and Department of Human Nutrition, Wageningen Agricultural University, May - Nov 1990), Epidemiology (Department of Epidemiology, University Hospital Leiden, May - July 1989 and UNICEF Malawi, Oct 1989 - Feb 1990) and Lung Physiology (Department of Pulmonology, University Hospital Leiden, Jan - April 1989). In addition, she completed courses in Medical Anthropology, University of Amsterdam; Problems in Developing Countries, Leiden University; and Human Nutrition, Wageningen Agricultural University. In 1991 she received the MSc-degree. The Netherlands Foundation for the Advancement of Tropical Research (WOTRO-NWO) then appointed her as a PhD-fellow to conduct research on food-based approaches for controlling vitamin A deficiency in West-Java. The work was done within the framework of collaboration between the Department of Human Nutrition, Wageningen Agricultural University and the Nutrition Research and Development Centre, Bogor, Indonesia. She joined the Netherlands Postgraduate Programme in Human Nutrition and the education programme of the Graduate School VLAG (advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences). In July 1994 she attended the Annual New England Epidemiology Summer Program at Tufts University, Boston, USA. From September 1995 to March 1996 she was appointed as a research associate to investigate the effectiveness of various foods for improving vitamin A status of Indonesian school children. In December 1995 she was involved in the development of a module on micronutrient malnutrition for an MSc-course in Nutrition for African Universities (NATURA-NECTAR programme of the European Community). It is expected that she will have fulfilled the requirements of the Netherlands Epidemiology Society for Epidemiologist B before the end of 1997.

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