Vitamin A intake, status and improvement using the dietary approach

Studies of vulnerable groups in three Asian countries

BY

VIVEKA PERSSON
ABSTRACT


Studies were performed on methodological issues on vitamin A intake, status and improvement in three Asian countries, to improve the dietary approach recommended by FAO/WHO to alleviate vitamin A deficiency in low-income countries.

The reliability of the practical 24-hour dietary recall method to assess individual intake of vitamin A during pregnancy was investigated in Central Java, Indonesia. The usual mean intake of vitamin A can be reliably measured, but data on attenuation of simple regression coefficients suggest that it is difficult to establish associations between vitamin A intake and some health outcome. The majority of women was below the recommended daily intake of vitamin A in all three trimesters and strategies to improve vitamin A intake in all women are thus needed.

The applicability of the simplified “Helen Keller International Food Frequency Method” to assess community risk of vitamin A deficiency in South Asia, even though it excludes breastmilk and animal milk, was tested in rural Bangladesh and rural India. Breast milk was found to be an important source of vitamin A even in the second and third years of life in rural areas of Bangladesh. Similarly, animal milk is likely to be an important source of vitamin A among preschoolers in certain areas of India. The method should be revalidated to make it a useful tool even in settings where breastmilk and animal milk are common in the diets of preschool children.

Whether it is possible to improve vitamin A status with dark green leafy vegetables in children free of Ascaris lumbricoides was investigated in northern Bangladesh. A substantial increase in serum β-carotene was seen after supplementary feeding of these vegetables for 6 weeks. The impact on serum retinol concentrations was less substantial.

Key words: Vitamin A deficiency, vitamin A intake, plant sources, preformed vitamin A, variability, reliability, 24-h recall, helminthiasis, retinol, β-carotene, iron, bioavailability, children, pregnancy.

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This study is dedicated to the millions of people still suffering from vitamin A deficiency and its consequences, and the people working on alleviating this public health problem.
PAPERS INCLUDED IN THE THESIS

The thesis is based on the following papers, which will be referred to by their numerals:


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CONTENTS

ABBREVIATIONS .................................................................................. 7

1 INTRODUCTION .............................................................................. 8

2 BACKGROUND .................................................................................. 8
  2.1 Functions of vitamin A ............................................................. 8
  2.2 Risk groups .................................................................................. 8
  2.3 Vitamin A intake .......................................................................... 9
    2.3.1 Food sources of vitamin A ..................................................... 9
    2.3.2 Bioavailability and bioefficacy of carotenoids ......................... 10
    2.3.3 Vitamin A requirements ......................................................... 11
    2.3.4 Assessing dietary intake of vitamin A ....................................... 11
    2.3.5 Methodological concerns in assessing dietary intake ................. 12
  2.4 Vitamin A status .......................................................................... 15
    2.4.1 Factors influencing vitamin A status ....................................... 15
    2.4.2 Association between iron and vitamin A ................................. 19
    2.4.3 Assessment of vitamin A status .............................................. 20
  2.5 Improving vitamin A status in communities .................................. 26
    2.5.1 Background ........................................................................... 26
    2.5.2 Cross-sectional and case-control studies ................................... 27
    2.5.3 Community-based interventions and social marketing ............... 28
    campaigns
    2.5.4 Experimental studies ............................................................... 29
    2.5.5 The basis for the current conversion factor of 6 µg β-carotene ...... 30
to 1 µg retinol
    2.5.6 Research questioning the current conversion factor .................. 31
  2.6 Health and vitamin A deficiency in Indonesia, Bangladesh and India .... 33
    2.6.1 Indonesia .............................................................................. 33
    2.6.2 Bangladesh .......................................................................... 36
    2.6.3 India ....................................................................................... 39

3 AIM OF THE STUDIES ...................................................................... 41
  3.1 Overall aim .................................................................................. 41
  3.2 Specific objectives ........................................................................ 41

4 SUBJECTS AND METHODS ............................................................. 42
  4.1 Variability in nutrient intake and vitamin A intake during pregnancy ... 42
  4.2 Vitamin A intake where sustained breastfeeding is common .......... 44
  4.3 Vitamin A intake where animal milk is a part of children’s diet ........ 44
  4.4 Relationship between vitamin A, iron and helminths and the effect .... 45
    of DGLVs on vitamin A status in primary school children

5 RESULTS .......................................................................................... 49
  5.1 Vitamin A intake .......................................................................... 49
    5.1.1 Methodological aspects of dietary assessment ......................... 49
    5.1.2 Vitamin A intake during pregnancy ........................................ 51
5.2 Vitamin A status ................................................................. 54
  5.2.1 Helen Keller International Food Frequency Method .......... 54
  5.2.2 Relationships between vitamin A, iron and helminths in children 55
5.3 Vitamin A improvement .................................................. 56
  5.3.1 The effect of DGLVs on vitamin A status in primary school children 56

6. DISCUSSION ........................................................................ 59
  6.1 Vitamin A intake .............................................................. 59
    6.1.1 Methodological aspects of dietary assessment during pregnancy in a developing country 59
    6.1.2 Vitamin A intake during pregnancy .............................. 61
    6.1.3 Implications and recommendations from the study in Indonesia 63
  6.2 Vitamin A status .............................................................. 64
    6.2.1 Helen Keller International Food Frequency Method .......... 64
    6.2.2 Relationships between vitamin A, iron and helminths in children 65
    6.2.3 Recommendations .................................................... 67
  6.3 Vitamin A improvement .................................................. 68
    6.3.1 The effect of DGLVs on vitamin A status in primary school children 68
    6.3.2 Relation to other studies ............................................ 69
    6.3.2 Lessons learned and recommendations ........................ 70

7. CONCLUSIONS ................................................................. 74

8. ACKNOWLEDGEMENTS .................................................... 76

9. REFERENCES ...................................................................... 78

Papers 1-6
ABBREVIATIONS

CHN-RL  Community health and nutrition laboratories
CRP     C-reactive protein
CV      Coefficient of variation
DGLV    Dark green leafy vegetables
EPG     Eggs per gram
FAO     Food and Agriculture Organization
FFQ     Food frequency questionnaire
FFM     Food frequency method
GNP     Gross national product per capita
HKI     Helen Keller International
HKI FFM Helen Keller International food frequency method
HPLC    High pressure liquid chromatography
IU      International units
NGO     Non-governmental organisation
PEM     Protein energy malnutrition
RDI     Recommended daily intakes
RE      Retinol equivalent
UNICEF  United Nations Children’s Fund
VAD     Vitamin A deficiency
WHO     World Health Organization
XN      Night blindness
XIB     Bitot’s spots
YOFV    Yellow orange fruits and vegetables
1. INTRODUCTION

Malnutrition is a great problem in the world and includes both under- and over-nutrition. The major under-nutrition problems are protein-energy malnutrition (PEM) and deficiencies of the micronutrients vitamin A, iron, iodine. This thesis deals with one of these micronutrients; vitamin A. Similar to iodine deficiency disorders, it was declared at the World Summit for children (1990) and at the International Conference on Nutrition (FAO/WHO, 1992) that all efforts should be made to eliminate vitamin A deficiency (VAD) by the year 2000. However, still it is estimated that approximately 250 million children in the world suffer from VAD, with approximately 50% living in South Asia (WHO/UNICEF, 1996). Research in three South Asian countries is included in the present thesis; Bangladesh, India and Indonesia. The thesis focuses on methodological issues regarding vitamin A intake, status and improvement that are important to clarify when dietary strategies are used to alleviate the problem of VAD, an approach recommended in the Plan of Action (FAO/WHO, 1993). The two major risk groups for VAD are included, namely children and pregnant women and the focus is both on the individual and the community levels.

2. BACKGROUND

2.1 Functions of vitamin A
Vitamin A is needed by the human body for many physiologically important functions, the most obvious deficiency symptom being blindness, preceded by night blindness (XN) and Bitot's spots (X1B). These and other ocular manifestations are termed xerophthalmia or "dry eye". Xerophthalmia affects 2.8-3 million children under five years of age. Vitamin A is also of great importance for growth and development of bone tissue, normal function of skin and mucous membranes, normal reproductive health and in the immune defence (Ross, 1992). The non-ocular manifestations are largely hidden from view and do not provide a ready basis for specific clinical diagnosis. However, subclinical deficiency affects an estimated 251 million children under five years of age. The results of a meta-analysis of studies performed on vitamin A supplementation and young child mortality concluded that improving a low to marginal vitamin A status will reduce the risk of death due to infectious diseases by 23% (Beaton et al., 1993).

2.2 Risk groups
Almost all suffering from VAD comes from the poorer socio-economic strata in low-income countries. Among these, pre-school children who are not breastfed any more are at greatest risk of VAD. The reasons behind this are that their nutritional demands are high, their consumption of vitamin A rich foods and the dietary fat required for absorption can be limited, and infections can deplete their body reserves of vitamin A. However, it could be assumed that in vitamin
A endemic areas also older groups of children would benefit from an adequate vitamin A status, through its role in immunity (Ross, 1992).

Other risk groups are pregnant and lactating women. Until recently, the main focus regarding low maternal vitamin A has been for its effect on foetal development and child health, e.g. pre-term birth, reduced intra-uterine growth and development and decreased birth weight (Shah and Rajalakshmi, 1984; Shah and Rajalakshmi, 1987). Also, it has been shown that vitamin A intake and serum vitamin A concentrations during pregnancy influence the composition of breast milk (Ortega et al., 1997). In Nepal, six-month mortality was higher among infants of women who had night blindness during pregnancy (Christian et al., 2001).

However, recent studies suggest that an improved vitamin A status during pregnancy also benefits the women. Addition of vitamin A to iron supplementation of pregnant women in Indonesia improved haemoglobin concentration (Muslimatum et al., 2001). Maternal mortality in Nepal was significantly reduced after supplementing pregnant women with weekly doses of vitamin A (West et al., 1999). In addition, a poor vitamin A status during pregnancy was found to be associated with greater risks of mild anaemia (Dreyfuss et al., 2000), severe anaemia and a lower body mass index as well as symptoms of urinary/reproductive tract infections, diarrhoea, pre-eclampsia and nausea (Christian et al., 1998). Also, maternal vitamin A or β-carotene supplementation resulted in a reduction in the postpartum prevalence of loose stools and night blindness, and vitamin A supplementation resulted in a reduction in reported number of days of illness symptoms during pregnancy (Christian et al., 2000).

Possible pathways that may explain the impact of vitamin A on pregnancy related infections are improved wound healing, increased resistance to infection, and if infection occurs, vitamin A’s effect as an immune enhancer. In addition, β-carotene can act as an antioxidant (Faisel and Pittrof, 2000).

### 2.3 Vitamin A intake

#### 2.3.1 Food sources of vitamin A

Dietary sources of vitamin A are of two categories: vitamin A or retinol, also known as preformed vitamin A; and provitamin A, which refers to those carotenoid precursors that can be bioconverted to retinol. Preformed vitamin A is found naturally in certain foods of animal origin: liver, fish liver oil, egg yolk, whole milk and products with milk fat, and breast milk. Provitamin A is formed by and found primarily in plant foods such as dark green leafy vegetables (DGLV) and yellow orange fruits and in vegetables (YOFV), except citrus fruits. In poorer countries, carotenoids are the major sources of vitamin A in the diet.
Vitamin A content of various food sources is shown in **Table 1**. Except for milk (Renner, 1989), the values are taken from the Swedish (SLV, 1996) and the Indian (Gopalan *et al.*, 1989) food composition tables.

To provide a basis for describing the vitamin A activities of carotenoids and retinol on a common basis, the joint Food and Agriculture Organization/World Health Organization (1967) expert group introduced the concept of the retinol equivalent (RE) (see also section 2.5.5).

The following relationships among dietary sources of vitamin A were established:

\[
\begin{align*}
1 \, \mu\text{g retinol} & = 1.0 \, \mu\text{g RE} \\
1 \, \mu\text{g } \beta\text{-carotene} & = 0.167 \, \mu\text{g RE} \\
1 \, \mu\text{g other provitamin A carotenoids} & = 0.084 \, \text{RE}
\end{align*}
\]

Thus, the vitamin A activity of β-carotene was estimated to be 1/6 of preformed vitamin A.

**Table 1. Approximate vitamin A content of various foods**

<table>
<thead>
<tr>
<th>Food item</th>
<th>vitamin A content/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal sources</strong></td>
<td></td>
</tr>
<tr>
<td>eggs with yolk</td>
<td>200</td>
</tr>
<tr>
<td>milk</td>
<td>10-90</td>
</tr>
<tr>
<td>liver</td>
<td>19,000</td>
</tr>
<tr>
<td>fortified margarine</td>
<td>900</td>
</tr>
<tr>
<td>butter</td>
<td>620</td>
</tr>
<tr>
<td><strong>Vegetable sources</strong></td>
<td></td>
</tr>
<tr>
<td>DGLV</td>
<td>2,700-19,000</td>
</tr>
<tr>
<td>papaya</td>
<td>800</td>
</tr>
<tr>
<td>mango</td>
<td>2000</td>
</tr>
<tr>
<td>yellow sweet potato</td>
<td>1800</td>
</tr>
<tr>
<td>sweet pumpkin</td>
<td>750</td>
</tr>
<tr>
<td>red palm oil</td>
<td>30,000-70,000</td>
</tr>
<tr>
<td>carrot</td>
<td>6000</td>
</tr>
</tbody>
</table>

1 in µg retinol
2 in µg β-carotene

### 2.3.2 Bioavailability and bioefficacy of carotenoids

As can be seen above, the vitamin A activity of carotenoids vary, with β-carotene having the highest. One reason for this variation is differences in the bioavailability of carotenoids. Bioavailability is now defined as the fraction of an ingested nutrient available for utilisation in normal physiological functions and storage, while bioconversion is the fraction of a bioavailable nutrient (an
absorbed provitamin A carotenoid) converted to the active form of the nutrient (retinol) (West and Eilander, 2001). Earlier, bioavailability included both absorption and conversion. A third term, which now is coming into use (van Lieshout et al., 2001) is bioefficacy, defined as the amount of ingested provitamin A required to yield 1 µg retinol to the body.

2.3.3 Vitamin A requirements
Between 1930 and 1950 a number of studies were conducted in Europe to establish the requirements for vitamin A. This was mainly done by repleting subjects fed a vitamin A deficient diet and by examining the absorption of carotene from various food sources. Hume & Krebs (1949), performed the “Sheffield experiment”, and concluded that 750 µg retinol or 1800 µg purified β-carotene were required to maintain adequate vitamin A levels. Similar requirements were found in a repletion study by Sauberlich and co-workers (1974), who concluded that 1200 µg retinol or 2400 µg β-carotene were required to maintain serum retinol levels above 30 µg/dl. They were also supported in the extensive review by Rodriguez & Irwin (1972).

The current recommended dietary intake (RDI) of vitamin A (FAO, 1988) in RE is shown in table 2, and includes both basal and safe requirements. The “basal requirement” is the amount needed to prevent clinical VAD and people meeting this requirement are capable of normal growth and reproduction. However, they have very low or non-existent reserves. Therefore, infection or short-term dietary inadequacies may make them susceptible to VAD. In comparison, “safe level of intake” is the level of intake which, when sustained, will maintain both health and appropriate reserves in almost all healthy people. For example, the safe requirements for children 1-6 years old and for pregnant women are 400 RE and 600 RE, respectively. The RDI for vitamin A from β-carotene depends on the amount consumed in each meal (FAO, 1988) but on average it is 2400 µg for infants and 4800 µg for pregnant women.

2.3.4 Assessing dietary intake of vitamin A
There are several methods which assess current/recent diet. Advantages and disadvantages of all food consumption survey methods should be carefully considered, and the limitations of the method must be accepted when planning studies and interpreting the results. In the weighed diet record method, all foods consumed, as well as plate wastes, are weighed on a scale. The records are generally written by the participants, which may be difficult and is time consuming. Food frequency questionnaires (FFQ), frequently used in cohort studies, are designed to assess usual eating habits and comprise a list of foods about the nutrients (e.g. vitamin A) or foods of interest. Generally they ask both for the frequency and amount consumed, for example in the last seven days. Thus, it include important days such as holidays and market days when diet may change. However, it often overestimates intakes of vitamin A (Russell-
Briefel et al., 1985; Bakari et al., 1997). Nowadays, the 24-hour recall method has come into frequent use (Thompson and Byers, 1994).

Table 2. Recommended dietary intake of vitamin A in RE

<table>
<thead>
<tr>
<th>Group</th>
<th>FAO/WHO (1988)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>Infants</td>
<td></td>
</tr>
<tr>
<td>0-0.5 y</td>
<td>180</td>
</tr>
<tr>
<td>0.5-1 y</td>
<td>180</td>
</tr>
<tr>
<td>Children</td>
<td></td>
</tr>
<tr>
<td>1-6 y</td>
<td>200</td>
</tr>
<tr>
<td>6-10 y</td>
<td>250</td>
</tr>
<tr>
<td>10-12 y</td>
<td>300</td>
</tr>
<tr>
<td>12-15 y</td>
<td>350</td>
</tr>
<tr>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>15-18</td>
<td>330</td>
</tr>
<tr>
<td>18 +</td>
<td>270</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>370</td>
</tr>
<tr>
<td>Lactation</td>
<td>450</td>
</tr>
</tbody>
</table>

24-hour recall method:
Dietary intake is often measured quantitatively by a 24-hour recall. Typically, the interviewer asks the respondent to estimate the quantities of all foods eaten during the previous 24 hours. A trained nutrition worker is required, as well as a reliable food composition table. Computers are playing an increasingly important role in dietary analysis by facilitating the development and organisation of large nutrient computation data banks and computation algorithms for converting the foods into nutrients. Attention needs to be given to important dietary days such as religious days and holidays. Individuals have difficulties in estimating portion size and weights of foods, both when reporting about foods previously consumed and examining displayed foods (Thompson and Byers, 1994). However, various types of food models can help the respondent to describe the amounts eaten more accurately (Simko et al., 1984).

In comparison with the more exact direct weighing record method, the 24-hour recall method is simpler and cheaper, making it more suitable for larger studies. In addition, the 24-hour recall method is considered suitable for measuring change over time (Block, 1982) and does not require literate respondents, making it suitable in developing countries. However, the 24-hour recall method tends to under-estimate dietary intake somewhat more than other methods (Block, 1982), including vitamin A (Russell-Briefel et al., 1985).

2.3.5 Methodological concerns in assessing dietary intake
Dietary data about the level of nutrient intake and sources of the nutrient of interest is used in relation to a broad range of goals within health and nutrition
Vitamin A intake, status and improvement using the dietary approach

programs, including assessment, monitoring and epidemiologic research (Wright et al., 1994). More precisely, the goals can include:

- assessing group level dietary intake
- assessing an individual’s mean intake with a given precision.
- ranking individuals into groups of intake, e.g. quartiles or quintiles. This classification is a commonly used technique in cross-sectional studies for analysing relationships between variables
- establishing links between dietary intake and some health outcome

Depending on the goal of the study, different sample size and different number of replicate measurements may be suitable. To make informed decisions about these, one needs to specify the degree of accuracy desired, and make assumptions about the intra-individual variation in nutrient intake, as well as the ratio of the intra-individual variation to the inter-individual variation.

- Intra-individual variation $\sigma^2_w$, is the variation of the individual about her true mean. A basic assumption is that variation within each individual represents truly random variation about her true mean and is not due to changes in her habitual dietary pattern.
- Inter-individual variation $\sigma^2_B$, is the variation among the true means of individuals within a population.

If intra-individual variation is large, and few replicate measures are taken or the sample size is small, the observed value $X$ (mean), may be quite different from the true mean $\mu$. The probability of misclassification may therefore be quite large. Thus, the statistical precision of intakes can be jeopardised and measures of diet-health outcome associations, such as correlations, regressions, odds ratios and relative risk, may be attenuated (Liu et al., 1978; Sempos et al., 1985; Walker and Blettner, 1985; Freudenheim et al., 1989; ). In contrast, taking too many replications or too large a sample wastes resources and disturbs respondents without serving any purpose.

Further, the equation to be used to calculate the number of replicate measurements needed depends on the objectives of the study. For example, if it is to assess an individual’s mean intake with a given precision, the following equation should be used (Willett, 1990):

$$n = (Z_\alpha CV_w/D)^2$$

where $n =$ number of replicate days required, and $Z_\alpha =$ the normal deviate for the percentage of times a confidence interval should cover the “true” mean intake of an individual and the length of the interval as percentage of the mean is 2D. $CV_w =$ coefficient of variation = $s_w$/mean intake (of that nutrient), where $s_w =$ square root of the estimated intra-individual variance.
If the objective is to rank groups of people, the same equation can also be used to determine whether or not a specified number of replicate diet recalls is sufficient to separate for example the first from the fifth quintile,

\[ Z_a = \left( \frac{\sqrt{n}}{D/CV_w} \right) \]

However, when the aim is to measure the association between two variables, i.e. to establish links between dietary intake and some health outcome, it is necessary to consider an error term, which indicates the influence that intra-individual variation has on the estimate. Then, the value being estimated is not the true correlation, \( r \), but \( r \) multiplied by an error term (Liu et al., 1978), i.e.

\[ r_{xy} \times \sqrt{\left[ \frac{1}{1 + \frac{\sigma^2_w}{\sigma^2_B}} \right]} \]

where \( n \) = number of replicates required, \( r_{xy} \) = the actual correlation between two variables, \( \sigma^2_w \) = within-subject variance, and \( \sigma^2_B \) = between-subject variance. Thus, if the ratio \( \frac{\sigma^2_w}{\sigma^2_B} \) is large, the correlation coefficient is strongly attenuated. The error term decreases as the number of replicate measurements per individual increases. The same equation can also be used to calculate the influence of \( \sigma^2_w \) on the regression coefficient, by replacing the correlation coefficient with the regression coefficient.

In this situation, the following equation should be used to calculate the number of replicates needed (Nelson et al., 1989):

\[ n = \left[ \frac{r^2}{1-r^2} \right] \times \left( \frac{\sigma^2_w}{\sigma^2_B} \right) \]

where \( r \) = the unobservable correlation between the observed and true mean intake of individuals over the period of observation.

In sum, it is very important to consider both the objectives of a study and method of analysis before deciding on the number of replicate days needed, since different assumptions underlie the equations for calculating the number of replications in the two cases described. In the first case, number of replicate days needed is a direct function of how large \( \sigma^2_w \) is in relation to the mean intake of the nutrient of interest, thus ignoring between-subject variance. In the second case the number of days required is a direct function of the ratio \( \frac{\sigma^2_w}{\sigma^2_B} \).

Even though a great body of research in developing countries aims at establishing links between diet and health, the majority of published research on variability in dietary intake that are used in calculating sample size and number of replicates needed, come from Western countries. It cannot be concluded, that data on variability from Western countries are generalisable to the situation in developing countries.
In addition, very few studies have looked at variability in dietary intake during pregnancy, despite the fact that much research both in Western and developing countries aims at ensuring optimal nutritional status and health of the pregnant women for her own sake as well as that of the newborn. Of course, one factor of great importance in achieving this is an adequate dietary intake during pregnancy. In calculating sample size and number of replicate measures needed, it may be incorrect to use data on variability based on non-pregnant subjects, since dietary intakes may change and vary significantly during pregnancy. Factors which can affect dietary patterns during pregnancy include nutritional recommendations on requirements (FAO/WHO/UNI Expert consultation, 1985; FAO, 1988), activity (Banerjee et al., 1971), appetite (Coons, 1933), and self-selected diet (Dickens and Trethowan, 1971). Any dietary intake measurement is specific to the stage of pregnancy.

Among the six studies identified which have assessed the precision of dietary intake methods during pregnancy, only one reported patterns of variability in women from a developing country (Launer et al., 1991). Three used the weighed diet record method (Thomson, 1958; Nelson et al., 1989; Launer et al., 1991), two used the 24-hour recall method (Osofsky, 1975; Rush and Kristal, 1982) and one used a seven-day food record (Cellier and Hankin, 1963).

The majority of the six studies on the variability in dietary intake during pregnancy report intra/inter variance component ratios. However, in most cases these were presented only for the macronutrients. Sample sizes were in many studies small, thus challenging the generalisability of the results. In addition, the number of replicate days used was also small in many studies and one (Rush and Kristal, 1982) did not specify time points for the recalls.

In conclusion, there is a need for thorough population-based studies of variability in dietary intake, measured with simple and practical methods that are suitable for use in developing countries.

2.4 Vitamin A status

2.4.1 Factors influencing vitamin A status

Associated risk factors:
Risk factors associated with vitamin A status include malnutrition, measles, respiratory infection, and diarrhoeal disease. It appears that VAD or marginal vitamin A status is often worsened by infectious disease and reciprocally, that poor vitamin A status is likely to prolong or exacerbate the course of illness (Scrimshaw et al., 1968). One explanation could be that severe infections in children lead to an increased urinary loss of retinol (Stephensen et al., 1994; Alvarez et al., 1995; Mitra et al., 1998). Therefore, among vitamin A deficient populations, attention should always be given to the vitamin A status of
children with measles, respiratory disease, diarrhoea, or severe protein-energy malnutrition. Improvement in community vitamin A status reduces the subsequent risk of measles mortality (Rahmathulla et al., 1990) and overall child mortality (Beaton et al., 1993). Children with preexisting mild VAD may be more prone to severe respiratory infection and diarrhoeal disease than children who are not vitamin A deficient (Sommer et al., 1984).

_Breastfeeding practises:_
Colostrum contains high levels of vitamin A as well as other vitamins and maternal antibodies. Breast milk is an important source of vitamin A for young children - short duration of breastfeeding and abrupt weaning may contribute to VAD. Xerophthalmia has been found more commonly among non-breastfed children (Tarwotjo et al., 1982; Bloem et al., 1995), even at older ages (Cohen, 1983; Mahalabanis, 1991).

_Food taboos:_
Food taboos can affect consumption of vitamin A-rich food items by young children and pregnant or lactating women. In parts of India, papaya and mangoes are avoided during pregnancy because they are classified as "hot" and thought to have the capacity to induce abortion (Johns et al., 1992; Persson, 1995). In Bangladesh, it was believed that young children could not digest DGLV (Rahman et al., 1993), though, a well-designed communication program in an entire district was apparently able to overcome this, leading to a doubling of DGLV consumption compared to a control district (Greiner and Mitra, 1995).

_Seasonal fluctuations of vitamin A-rich foods:_
In Bangladesh, the intake of vitamin A has been found to be inadequate in all seasons but is lowest in August and highest in October-November (Hassan et al., 1985). In Gambia, vitamin A intake among pregnant and lactating women ranged from 120 RE in December up to 900 RE/day in June. Plasma carotenoids showed synchronous fluctuation, whereas the seasonal variation in plasma retinol was less pronounced, with a small peak in May and June (Bates et al., 1994).

_Insufficient energy and/or protein derived from the diet:_
Insufficient energy and/or protein derived from the diet could lead to lowered levels of retinol-binding-protein in the blood, thereby impairing the transport of vitamin A in the body (Sommer, 1994). One of the biggest nutritional problems in India is PEM. However, studies have shown that the primary dietary problem underlying PEM in India is not a deficiency of protein, but rather a deficiency in calories (Gopalan, 1992).
Source of vitamin A: Control of VAD depends to a large degree on an adequate supply of vitamin A. However, the supply of vitamin A from plant sources is not only determined by the actual carotenoid content of a food, but also the bioavailability/bioefficacy of the carotenoids, especially β-carotene to retinol. Thus, the matrix in which β-carotene is embedded in a food is important. In DGLV, β-carotene molecules are organised in pigment-protein complexes. In other vegetables and fruits, where β-carotene does not play a role in photosynthesis, it is often found in lipid droplets, but can also be bound to protein. Releasing β-carotene from a pigment-protein complex is more difficult than freeing it from a lipid droplet. It has been shown that β-carotene fed in fat or a simple matrix is more bioavailable than β-carotene from vegetables (Brown et al., 1989; de Pee et al., 1995; de Pee et al., 1998; van het Hof et al., 1999; Huang et al., 2000). Cooking and reduction of particle size by grinding or homogenisation can reduce matrix effects (Hussein and El-Tohamy, 1990; Rock et al., 1998; Castenmiller et al., 1999). The differential impact of the disruption of the food matrix on bioavailability of various carotenoids is still uncertain. For example, van het Hof and co-workers (1999) showed that the plasma response of lutein is increased upon consumption of chopped spinach compared to the response with whole-leaf spinach, while the plasma response of β-carotene is not significantly affected. However, Castenmiller and co-workers (1999) found the opposite, i.e. disruption of the matrix of spinach by enzymatic treatment enhanced plasma response of β-carotene but not that of lutein.

In addition, plant sources generally contain more fibre, including pectin (Rock and Swendsen, 1992) and cellulose (AVRDC, 1987) which can reduce the bioavailability of provitamin A. Finally, it has been suggested that the carotenoid lutein, a non-provitamin A carotenoid with a very high concentration in DGLV inhibits both the absorption (van den Berg, 1998) and the conversion (van Vliet et al., 1996) of β-carotene to vitamin A. The steps of carotenoid absorption and dietary factors that affect carotenoid absorption are shown in figure 1.

The impact of fat on the absorption vitamin A: Fat is the dietary vehicle for transport of both vitamin A and carotenoids. Fat facilitates the absorption of β-carotene (El-Gorab et al., 1975; Jayaran et al., 1980; Jalal et al., 1998;) by increasing the bile-flow which in turn facilitates the transport of β-carotene into the mucosal cells (El-Gorab et al., 1975). Thus, one important issue is to determine the amount of fat needed for an optimal absorption. Raising the level of fat in a low fat diet by one gram per kg body weight (aged three to 13) per day improved the absorption of carotenoids (Roels et al., 1963). However, a study on healthy volunteers in the Netherlands showed that the optimal uptake of β-carotene requires a limited amount of fat,
3g per portion (Roodenburg et al., 2000). The type of fat in the meal ingested with β-carotene may also influence the degree of absorption; beef tallow resulted in a greater absorption when compared with sunflower oil (Hu et al., 2000) and long-chain triglycerides were better than medium-chain triglycerides, which primarily are absorbed via the portal vein (Borel et al., 1998).

**Figure 1. Steps of carotenoid absorption and dietary factors that affect absorption (with Permission from Journal of Nutrition)**
The impact of intestinal helminths on the bioavailability of Vitamin A:
Intestinal helminths may influence uptake and bioconversion of provitamin A. *Ascaris lumbricoides*, one of the most common intestinal helminths in the world, is reported to interfere with the absorption of vitamin A by most researchers (Sivakumar and Reddy, 1975; Mahalanabis *et al*., 1976; Mahalanabis *et al*., 1979; Marinho *et al*., 1991; Curtale *et al*., 1994; Kidala *et al*., 2000), but not all (Ahmed *et al*., 1993).

The impact of vitamin A status on the bioavailability of provitamin A:
There is no evidence that absorption of provitamin A is affected by either carotene or vitamin A status, because absorption occurs through passive diffusion. However, the conversion of provitamin A to retinol is influenced by serum retinol levels. A low vitamin A status appears to increase \(\beta\)-carotene cleavage (Villard and Bates, 1986; van Vliet *et al*., 1996). In addition, intra-individual variability in the conversion of \(\beta\)-carotene to retinol may contribute to the variable response to consumption of \(\beta\)-carotene (Lin *et al*., 2000; van Lieshout *et al*., 2001).

The impact of cooking practices on vitamin A activity:
Excessive or prolonged heating reduces provitamin A activity (Simpson and Chichester, 1981; Erdman, 1988). Foods should be kept out of the sun, as sunlight accelerates oxidation, which destroys the provitamin A activity (Simpson and Chichester, 1981; Erdman, 1988). In a study in Bangladesh, three traditional methods of cooking vegetables were compared. Losses of \(\beta\)-carotene ranged from 2.3% to 43% (Rahman *et al*., 1990).

### 2.4.2 Association between iron and vitamin A status
Vitamin A deficiency and iron deficiency anaemia, two of the major nutritional deficiencies in low-income countries, often coexist. This may be due to inadequate dietary intake of both vitamin A and iron. However, it may also be caused by a relative deficiency of one or the other. In an early study, adult subjects maintained on vitamin A deficient diets developed anaemia despite adequate iron intake (Hodges *et al*., 1978). Furthermore, vitamin A deficient subjects have been found to be unresponsive to dietary supplementation with iron (Meija and Arroyave, 1982). Clinical and community-based studies on humans have also documented an association between indicators of vitamin A and iron. For example, there is a clear evidence of association between serum concentrations of vitamin A and the level of haemoglobin in children (Mejia *et al*., 1977; Mohanram *et al*., 1977; Wolde-Gebriel *et al*., 1993; Ahmed *et al*., 1996) and in pregnant women (Panth *et al*., 1990; Suharno *et al*., 1992; Suharno *et al*., 1993; Muslimatum *et al*., 2001).
The mechanisms behind these relationships are still unknown. However, there may be a direct interaction between vitamin A nutritional status and the ability to effectively utilise both dietary and endogenous stored iron for haemoglobin formation (Meija and Arroyave, 1982; Meija and Chew, 1988; Bloem et al., 1989; Roodenburg et al., 1994; Bloem, 1995). Therefore, the possibility that iron deficiency anaemia might in part be a consequence of poor vitamin A status would have widespread implications for the public health interventions presently adopted for its prevention.

2.4.3 Assessment of vitamin A status
The choice of method to use to assess vitamin A status depends very much on the purpose of the study. For example, formulation of an effective intervention program for VAD begins with characterisation of the problem. The first concern is whether VAD exists and is likely to constitute a public health problem. A preliminary assessment can help to determine whether or not more intensive investigation is warranted. Searching for active or healed cases of xerophthalmia is, in many ways, a specific and efficient mean of preliminary assessment. Preliminary case-finding should also include interviews with individuals likely to be aware of the problem: eye specialists, clinicians, nutritionists and community health workers, staff of hospitals, feeding centres. However, to evaluate the impact of dietary interventions or social marketing campaigns on vitamin A status, other indicators are needed, such as clinical or biochemical ones. Lastly, if the purpose of the vitamin A assessment is to study the impact of experimental feeding trials, biochemical indicators must be used.

Clinical assessment:
Criteria for assessing the public health significance of xerophthalmia and VAD are presented in table 3. They are based on the prevalence among children less than 6 years old in the community. XN or impaired dark adaption is best assessed in a survey by careful, detailed history taken from a parent, or guardian. This recall may be quite sensitive in areas where a specific term describing the characteristics of the behaviour of the affected children is part of the vocabulary. Often local terms exist for “night eyes” or “chicken eyes” in many parts of Asia, Africa and Latin America. For example, in Bangladesh it is called raat-kaana (night blindness). The constraints to clinical assessment are that it is expensive and that a huge sample size is needed.

Biochemical assessment:
In countries where VAD is only a subclinical problem, the concentration of vitamin A (retinol) in serum can be measured by for example high pressure liquid chromatography (HPLC). The cut-off value of <0.70 µmol/L (20 µgram/dl) indicates a low vitamin A status (WHO/UNICEF, 1994). The
prevalence levels used to designate how serious VAD is as a public health problem are shown in Table 4.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Minimum prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night blindness (XN)</td>
<td>1.0</td>
</tr>
<tr>
<td>Bitot’s spot (X1B)</td>
<td>0.5</td>
</tr>
<tr>
<td>Corneal xerosis and/or ulceration/keratomalacia (X2 + X3a + X3b)</td>
<td>0.01</td>
</tr>
<tr>
<td>Xeophthalmia-related corneal scars (XS)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Source: Sommer, 1994

One constraint to the use of serum retinol is that it is vulnerable to infection (Salazar-Lindo et al., 1993; Friis et al., 1996) and common inflammatory conditions (Stephensen and Gildengorin, 2000). The concentration of retinol decreases transiently during the acute phase response and this can result in misclassification of vitamin A status (Paracha et al., 2000; Stephensen and Gildengorin, 2000). The acute phase response is the body’s immediate reaction to infection and inflammation. Changes in the synthesis of acute phase proteins are to maintain body homoeostasis and avert tissue damages. Biochemical markers of iron status are also altered by infection (Lipschitz et al., 1974; Hulthén et al., 1998), which reduces the sensitivity of for example serum ferritin as a marker for iron deficiency. Thus, acute phase proteins, e.g. C-reactive protein (CRP) or ß-acid glycoprotein (AGP) should be used when retinol is unlikely to be an accurate indicator of vitamin A status (Filteau et al., 1993) and ferritin of iron status (Punnonen et al., 1997; Mast et al., 1998). Subjects with elevated acute phase proteins are often excluded in experimental studies.

A second constraint is that the concentration of retinol in serum/plasma is fairly stable over a wide range of concentrations of vitamin A in liver. Thus, other methods must be used to determine vitamin A stores. These include the relative dose response test and modified relative dose response tests. The latter involves providing a person a dose of a derivate of retinol (dehydroretinol) and comparing its serum concentration 5 hours after dosing with the serum retinol concentration. The concentration of retinol in breast milk is also considered to be a good measure of the vitamin A status of the mother. In addition, the use of vitamin A isotopically labelled with deuterium has recently come to use. Then, total-body vitamin A stores can be measured, using isotope-dilution techniques (Tang et al., 1999; Ribaya-Mercado et al., 2000). Other constraints to the biochemical methods are financial, logistical and technical.
Table 4. Prevalence in children ≥ 1 year with serum concentrations ≤ 20 µg/dl\(^1\)

<table>
<thead>
<tr>
<th>Level of importance as a public health problem</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>≥ 2 - &lt; 10%</td>
</tr>
<tr>
<td>Moderate</td>
<td>≥ 10% - &lt; 20%</td>
</tr>
<tr>
<td>Severe</td>
<td>≥ 20%</td>
</tr>
</tbody>
</table>

\(^1\) Source: WHO/UNICEF, 1995

Dietary assessment:
Simpler methods than the ones described above are needed to assess community vitamin A status and to plan and monitor diet-based interventions, particularly if they are intended gradually to phase out "short-term" measures such as universal distribution of vitamin A supplements (Greiner, 1992; ACC/SCN, 1997). Perhaps the most promising method for these purposes is the food frequency method (FFM), which counts how often certain foods are eaten over a given period of time, ignoring amounts. This type of method is therefore not as accurate as other techniques for determining whether an individual is deficient in a specific nutrient. However, research has shown that estimating the exact amount of nutrient intake in individuals is not always necessary to predict the prevalence of inadequate nutrition in a community (Block, 1982; Hernandez-Avila \textit{et al.}, 1988). General eating habits are easier to remember and, therefore, more reliably reported than specific quantities of foods. An advantage of the seven-day FFM is that it captures eating patterns over a week, which may include important dietary days, such as religious days, holidays and market days. FFMs have previously been shown successfully to identify associations between intake of food and disease (Willett \textit{et al.}, 1985).

Helen Keller International Food Frequency Method:
One FFM developed in recent years and coming into frequent use (Paracha and Jamil, 2001; Persson, 1997), and even in national surveys (Malyavin \textit{et al.}, 1996; Newsome \textit{et al.}, 1999) is the Helen Keller International food frequency method (HKI FFM) (Rosen \textit{et al.}, 1993). The HKI FFM asks respondents how many days in the last week the child consumed the foods listed on a predesigned FFQ (see \textbf{figure 2}). Only major sources of vitamin A are taken into consideration (≥100 RE), though some attention are given to other foods. Information on amounts consumed is excluded and thus it is not intended to determine whether an individual is deficient in vitamin A. If VAD is found to be a problem, the results generated from this method also provide suggestions on what types of dietary interventions may be most appropriate. Questions on for example home garden activities and vitamin A capsule distribution can also be added to the questionnaire to obtain information in support of the dietary findings and to facilitate planning for a later intervention.
The HKI FFQ has been designed to facilitate easy tabulation of data. For each vitamin A-rich food item that comes from an animal source, the questionnaire has a circle, where the interviewer must record the number of days eaten. Vitamin A fortified foods also have a circle. Similarly, for each vitamin A-rich food item that comes from a plant source, the questionnaire has a square. DGLVs are included in the analysis as a single food group (see figure 2).

For each questionnaire:
- The total number of days written in the circles (a) represent the total frequency of consumption of animal sources
- The total number of days written in squares (b), represent the total frequency of consumption of vegetables
- (b) is divided by 6, yielding c, the adjusted frequency of consumption of vegetables
- (a) and (c) are added together, yielding the weighted total consumption (animal + vegetables)

Average scores can then be calculated for each community. A community is considered to have a vitamin A deficiency problem if it falls below either of two threshold values:

- \( \leq 4 \) days per week for mean frequency of consumption of animal sources of vitamin A,
- or
- \( \leq 6 \) days per week for mean frequency of weighted total consumption of animal and vegetable sources of vitamin A

If at least 70% of the surveyed communities are not above both threshold values, then VAD is likely to be a public health problem in the entire survey area.

A community is defined as having a problem of public health significance when \( \geq 15\% \) of the child population between 1 and 5 years of age have a serum retinol level of \( < 70 \) µmol/litre (recommended by the International Vitamin A Consultative Group). In a three-country validation study, the HKI FFM correctly classified 11 of 15 (73.3%) communities as having or not having a VAD-problem (Sloan et al., 1997). However, 43% of those communities without VAD were incorrectly identified as having it. One possible reason for this lack of specificity could be that certain key sources of vitamin A such as milk were left out. HKI justifies this on the assumption that animal milk as served to the children is too diluted and that breast milk is a minor source of vitamin A after the first year.

In sum, there is a need for testing and evaluating the HKI FFM in settings with other food habits than in the countries included in the validation study.
Figure 2. The Helen Keller International Food Frequency Questionnaire

HKI Food Frequency Method
Food Frequency Questionnaire

For Supervisor

Animal = ..................(a)
Plant = ...............(b) + 6 = ..................(c)
Weighted Total (a + c) = ..................(d)

Questionnaire Number

Date. ........................................ Community ........................................

Introductory questions to select child (pretest carefully & reword as needed):

1a. What are the names of your children who are one through five years old? (12-71 months)
child 1. .................. (name)
child 2. .................. (name)
child 3. .................. (name)
child 4. .................. (name)
child 5. .................. (name)

1b. What are their ages?
(none years)
(none years)
(none years)
(none years)
(none years)

Note to interviewer: Randomly select one child from the list above.
Circle the child’s name and fill in the blank for child’s name below.
Proceed to interview the caretaker about the selected child only.

2. Is .................. (child’s name) a boy or a girl? .................. (girl) .................. (boy)

Note to interviewer: For each food listed in the table below, ask the following question in the order
that the food items are listed:

3. How many days, in the past seven days, did .................. (name of selected child)
eat .................. (specific food item)?

<table>
<thead>
<tr>
<th>Name of food item</th>
<th>Number of days eaten per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main staple food (such as rice, cassava, tortillas, etc.; select only one)</td>
<td></td>
</tr>
<tr>
<td>Spicy, hot peppers</td>
<td></td>
</tr>
<tr>
<td>Dark green leafy vegetables (DGLVs as a food group)</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td></td>
</tr>
<tr>
<td>Carrots</td>
<td></td>
</tr>
<tr>
<td>Ripe mango</td>
<td></td>
</tr>
<tr>
<td>Dark yellow or orange squash (includes pumpkin)</td>
<td></td>
</tr>
<tr>
<td>Spinach (or other DGLV)</td>
<td></td>
</tr>
</tbody>
</table>

Continued.
### HKI Food Frequency Method (continued)

<table>
<thead>
<tr>
<th>Name of food item</th>
<th>Number of days eaten per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ripe papaya</td>
<td></td>
</tr>
<tr>
<td>Noodles (or other staple food)</td>
<td></td>
</tr>
<tr>
<td>Eggs with yolk</td>
<td>c</td>
</tr>
<tr>
<td>Small fish (liver intact)</td>
<td></td>
</tr>
<tr>
<td>Peanuts (or other legume or meat)</td>
<td></td>
</tr>
<tr>
<td>Yellow or orange sweet potato or yam</td>
<td></td>
</tr>
<tr>
<td>Chicken or other fowl (or other meat or legume)</td>
<td></td>
</tr>
<tr>
<td>Amaranth leaves (or other DGLV)</td>
<td></td>
</tr>
<tr>
<td>Any kind of liver</td>
<td>c</td>
</tr>
<tr>
<td>Sweet potato leaves (or other DGLV)</td>
<td></td>
</tr>
<tr>
<td>Beef (or other red meat or pork)</td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td></td>
</tr>
<tr>
<td>Lentils (or other legume or meat)</td>
<td></td>
</tr>
<tr>
<td>Red palm oil</td>
<td></td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>c</td>
</tr>
<tr>
<td>Foods cooked in oil</td>
<td></td>
</tr>
<tr>
<td>Apricots (or other plant source rich in vitamin A)</td>
<td></td>
</tr>
<tr>
<td>Coconuts (or other fat or oil)</td>
<td></td>
</tr>
<tr>
<td>Weaning food fortified with vitamin A (or other food fortified with vitamin A)</td>
<td></td>
</tr>
<tr>
<td>Margarine fortified with vitamin A (or other food fortified with vitamin A)</td>
<td></td>
</tr>
</tbody>
</table>

- These food items can be replaced with similar foods that are locally available.

- Animal sources of vitamin A
- Plant sources of vitamin A

Interviewer Name .................................................................
2.5 Improving vitamin A status in communities

2.5.1 Background
At the International Conference on Nutrition, jointly convened by the FAO and the WHO in 1992, it was declared that all efforts should be made to eliminate VAD before the end of that decade. In the Plan of Action, it was stated that in low-income countries, first priority should be given to dietary-based strategies (FAO/WHO, 1993). These include promotion of increased production and consumption of inexpensive micronutrient rich foods such as DGLV and YOFV. It can also include husbandry activities, e.g. raising cattle and poultry, and fisheries. The advantages with the long-term, dietary strategies are that they reach everybody, benefit women, provide many nutrients and may be sustainable; disadvantages are that they take some time and require some effort to reinforce (FAO/ILSI, 1997). High cost of this approach per beneficiary is something said to be a disadvantage but costs can be low with efficient implementation on a large scale (Greiner and Mitra, 1995).

However, in the Plan of Action it was also stated that in severely deficient populations, supplementation with vitamin A may be required to reinforce dietary approaches, but “supplementation should be progressively phased out as soon as micronutrient-rich food-based strategies enable adequate consumption of micronutrients”. Thus, in many countries, vitamin A is given biannually to children from the age of 6 months to the age of five years. The dose is 200,000 international units (IU), except for the 6-12 month olds whose dose should be 100,000 IU. In addition, it is recommended to supplement mothers 200,000 IU within 8 weeks postpartum (IVACG, 1998). The advantages of short-term supplementation strategies are that the impact is rapid, and they are simple. The major drawbacks are that they may reach only a small part of the society, can reach the wrong group (i.e. young infants and pregnant women), coverage rates are often too low to reach the poorest and most isolated groups, they provide only a single nutrient, and they are unsustainable. However, coverage rates have in many countries increased dramatically when vitamin A capsule (VAC) distribution was linked to national immunisation days (Myint Zin et al., 2001; Panagides et al., 2001). For example in Bangladesh, coverage rates increased from 46% in 1982 and 35% in 1989 to over 85% in 1995 and 1996 (Shahjahan et al., 1997).

Some countries are also fortifying foods with vitamin A. It is useful if the vehicle is affordable, consumed by the target group, and variability in consumption is low among consumers. The risk of toxicity has also to be taken into consideration (FAO and ILSI, 1997). It is also important that the vehicle is stable under proper conditions of storage and use. Monosodium glutamate, used in cooking throughout Asia, was the subject of a fortification trial in Indonesia. Although it provided adequate vitamin A, the yellowish discoloration of the monosodium glutamate was found to be a problem for acceptability on a wider scale (Solomons, 1995). In most countries, margarine is fortified, though it may
not reach the poorest group. In Central America, sugar is successfully used as a vehicle and in Guatemala it was found to be the most cost-effective strategy (Phillips et al., 1996). In the Philippines, approximately 18 fortified products are now available, such as noodles, cooking oil, infant cereals and sardines (Marero, 1999).

The FAO/WHO (1993) recommendations on giving priority to dietary strategies were based on the general opinion at that time that it is possible to improve individual and community vitamin A status mainly with plant sources of vitamin A. The body of research supporting this is summarised in section 2.5.2-2.5.4.

2.5.2 Cross-sectional studies and case-control studies
In cross-sectional studies, the status of an individual with respect to the presence or absence of both exposure (e.g. vitamin A intake) and disease (e.g. VAD) may be assessed at the same time. In a case-control study, the investigator selects individuals on the basis of whether or not they have disease (e.g. VAD) and then determines their previous exposure (Hennekens and Buring, 1987). The major disadvantage with both cross-sectional studies and case-control studies is that they do not test causality. For example, it is not possible to determine whether or not the difference between cases and controls is the cause of the disease among cases, or if the difference is the result of the disease. Another disadvantage of case-control studies is the difficulty in selecting a satisfactory control group. However, they are very good for generating hypothesis, which later can be tested with cohort studies or experimental studies (Ahlbom and Norell, 1990).

There are several cross-sectional studies on the relationship between vitamin A intake and status. In Indonesia, logistic regression was used to predict the chance for a serum retinol greater than the observed median ($1.37 \mu\text{mol/L}$) among women with a child $\leq 24$ months. A high intake of both plant sources animal sources and ownership of a home garden was associated with an increased chance (de Pee et al., 1998). A study among female adolescent garment factory workers in Bangladesh found a strong relationship between serum retinol levels and frequency of intake of DGLV (Ahmed et al., 1997). In the national vitamin A survey in Bangladesh, conducted in 1997-98, homestead gardening was associated with a lower risk of clinical VAD in both pre-school children and mothers. It was also shown that for pre-schoolers who did not receive a high dose of vitamin A, a presence of home garden was protective against XN. In addition, among women, subclinical VAD (below the median and below 1.05 $\mu\text{mol/L}$) was associated with a low total intake of vitamin A (HKI/IPHN, 1999).

There are also plenty of case-control studies which have examined the relationship between diet and VAD. In Indonesia, $\beta$-carotene contributed 1/3 of
the RDI of vitamin A in healthy children (2-5 years old), and 1/5 in malnourished children of whom some were night blind, while protein and energy consumption were comparable (Blankhart, 1967). Another study from Indonesia found that controls consumed eggs, fish, DGLV, carrots, carotenoid rich fruits and breast milk more often than did children with corneal xerophthalmia (Tarwotjo et al., 1982). A third study from Indonesia found that a low consumption of DGLV, yellow fruits and eggs was related to an increased risk of xerophthalmia (Mele et al., 1991). A study in Tanzania found that children with xerophthalmia consumed DGLV, whole milk and butter less frequently than did controls (Pepping et al., 1989). Results from a food consumption survey in Sudan within a large vitamin A supplementation trial found that dietary intake of vitamin A and carotene were both strongly and inversely related to xerophthalmia and mortality (Fawzi et al., 1993; Fawzi et al., 1994). A study on preschool children in Bangladesh found that levels of serum retinol were higher with increased consumption of carotenoid rich foods (Hussain and Kvåle, 1996).

2.5.3 Community-based interventions and social marketing campaigns
Community-based interventions aimed at improving vitamin A status may promote cultivation or consumption of vitamin A rich foods or both. Social marketing campaigns usually promote increased consumption of only certain vitamin A rich foods. There are several studies which have evaluated both consumption and vitamin A status. However, in most cases, changes in the intervention group/area were not compared with changes in a control group/area. Thus, it is very difficult to draw any firm conclusions whether the changes were due to the intervention per se, or secular trends, such as changes in economic growth.

In Tamil Nadu, India, papaya and drumstick leaves were provided together with nutrition education. After one year, β-carotene content of the children’s diets had improved as well as the serum retinol levels (Chandrasekhar and George, 1993). No control area was included. Evaluation of a large social marketing campaign of ivy gourd in Thailand, reported both an increased production and consumption. However, no clear impact on vitamin A status was found (Smitasiri et al., 1993). In Bangladesh, an evaluation of a large home gardening program found an increase in vegetable and energy consumption, a small reduction of night blindness and an increase in income compared to controls during the study period (HKI/AVRDC, 1993). The vitamin A intake in this population derived almost completely from plant sources and logistic regression analyses showed that maternal vitamin A intake was determined by type of home garden, the total quantity of carotenoid-rich foods and the number of fruits and vegetables (Bloem et al., 1996). Another nutrition education program in Bangladesh compared prevalences of night blindness prior to the intervention (in 1986), after three years of intervention (in 1989) and three years after the termination of active intervention (in 1992). It showed that
prevalence of night blindness in children decreased from 1986 to 1989 and rose from 1989-1992, but remained significantly lower in 1992 than in 1986 (Hussain and Kvåle, 1996). However, there was no control group. Greiner and Mitra (1995) studied a similar nutrition education program in a nearby area in Bangladesh to which gardening activities were added. They found substantial increases in consumption of DGLV among young children but no change in X1B compared to control area (Greiner and Mitra, 1995). Finally, in Central Java, Indonesia, a social marketing campaign promoting an increased consumption of eggs and DGLV found an increased consumption of both eggs and vegetables 9 months after the initiation of the campaign. In addition, vitamin A status in both mother and child increased and was related both to egg consumption and total vitamin A intake (de Pee et al., 1998).

2.5.4 Experimental studies
Characteristic for the experimental design is that exposure status of each participant is assigned by the investigator. When participants are allocated to a particular exposure group (e.g. diet) at random, it achieves on average control of other factors that may affect the outcome (e.g. retinol concentration) (Hennekens and Buring, 1987). Many of the intervention studies which have been performed in developing countries have been extensively reviewed by de Pee & West (1996). One of the first studies conducted is the one by Roels and colleagues in boys aged 9-16 years old in Ruanda. It showed that serum concentrations of both retinol and carotene increased after a 31-day consumption of carrots and purified carotene, together with fat. However, if fat was excluded the increase in serum retinol was not significant. However, the sample size was 4-5/group (Roels et al., 1958). Seven studies followed from India. All of them found that serum retinol concentrations increased after consumption of DGLV (Pereira and Begum, 1968; Lala and Reddy, 1970; Devadas et al., 1978; Devadas et al., 1980; Jayaran et al., 1980; Wadhwa et al., 1994), carrots (Wadhwa et al., 1994) or papaya (Devadas et al., 1980; Wadhwa et al., 1994). The sample size varied from 5-61 per group and the duration of the interventions ranged from 15 days to 3 months. Wadhwa and co-workers studied children aged 7-12 years old and the other studies included preschool children. The mean baseline serum retinol was low, ranging from 12-21 µg/ml. Devadas and co-workers (1980), Jayarajan and co-workers (1980), Wadhwa and co-workers (1994) all included a negative control group. Jayaran and co-workers (1980), found a greater increase in serum retinol when 5-10 g oil was added to the supplement. Only one of the other studies reported on the fat content of the supplement (Lala and Reddy, 1970).

Hussein & El-Tohamy reported on two studies. Their first study (1989) looked at boys in Egypt aged 6-13 years and found that serum retinol concentrations increased as much after 21 servings of spinach (3.7 mg β-carotene/serving) and carrots (2.4 mg β-carotene/serving) as after a megadose of vitamin A. However, the sample size was <10 per group and no negative control group was included.
In their second study (1990) (boys aged 11-13 years), they found that 150 g carrots/day for two weeks resulted in an increase in serum retinol, while 150 or 280 g spinach/day increased serum carotene but not retinol.

Muhilal and Karyadi (1977) supplemented children aged 3-5 years old with either DGLV (n=40) containing 1.9 mg β-carotene or salt fortified with vitamin A (n=60) for 75 days. No information about fat content of the supplement was given and no negative control group was included. Their mean baseline retinol levels was 19-20 µgram/dl. An increase in serum retinol was only detected for the latter group. However, serum β-carotene increased in both groups.

To conclude, many of the older intervention studies described above suffer from methodological constraints, thus leaving the findings questionable. This includes:

- Not including a negative control group
- A very small sample size, thus providing inadequate statistical power to test the null hypothesis
- No information about drop-outs
- No information about daily diet
- No information about fat content of the supplement.

2.5.5 The basis for the current conversion factor of 6 µg β-carotene to 1 µg retinol

The recommendations made by FAO/WHO (1993) in the Plan of Action on giving priority to dietary strategies based on the belief at that time that 6 µg β-carotene is equal to 1 µg retinol (FAO and WHO, 1967).

The FAO/WHO committee in 1967 based its equivalency ratio on two assumptions:

1) that the mass ratio of the maximum conversion of β-carotene in oil, its most bioavailable form, to vitamin A in vivo was 2:1, and
2) that the bioavailability of β-carotene from foods on average was 1/3 that of β-carotene in oil. Thus, 2 x 3 = 6 µg β-carotene per µg retinol.

Other common carotenoids, like α-carotene and β-cryptoxanthin were considered to be half as active as β-carotene. Later, cis-isomers of β-carotene was considered to be half as active as the all-trans counterpart. The bioavailability of carotene from various food sources were in the older studies investigated in basically two different ways.

1. By looking at their effectiveness in maintaining serum retinol and carotene, or restoring dark adaptation. For example, it was found that carotene from carrots was less effective in restoring dark adaptation than carotene in oil
Vitamin A intake, status and improvement using the dietary approach

(Callison and Orent-Keiles, 1947). However, the preparation method was found to be important: cooked carrots were found to be equally effective as blanched carrots (Callison and Orent-Keiles, 1947), while another study found that grated raw carrots increased serum carotene levels but cooked carrots not (van Zeben, 1946). Leitner and co-workers (1964) found that it was possible to increase serum levels of carotene and retinol by feeding large amounts of carrots and spinach containing 17 mg carotene/day for three months.

2. By comparing the bioavailability of β-carotene from various sources by studying apparent proportion absorbed, which is calculated by subtracting the amount of carotene in faeces from the amount consumed and dividing the difference by the amount consumed. In adults, a number of such studies was performed in Europe between 1935 and 1950 while in children, there was a number of such studies conducted in Ruanda, India and Indonesia between 1960 and 1980. Most of these studies have been reviewed by Hume & Krebs (1949) and by Rodriguez & Irwin (1972). They show that absorption of carotene ranged from 30-99% in oil, 1-60% in cooked carrots, 1-20% from raw carrots without fat and 25-50% with fat, 46-77% from papaya and 5-77% from vegetables with and without fat. In addition, James & Hollinger (1954) reported an absorption of carotene from sweet potato of 46% and Rao & Rao (1970) reported an absorption of β-carotene from carrots, DGLV and papaya of 81%, 76% and 90%, respectively.

It is possible both to overestimate and underestimate absorption by studying apparent absorption. Overestimation can happen when carotene extraction from faeces is incomplete, or if the assumption that carotenoid absorption is represented by the total amount of carotenoids not found in the faeces is incorrect (van Vliet, 1996). Studies investigating the absorption of β-carotene and retinol using ¹⁴C-labelled compounds found that 8-17% of the ingested β-carotene and 7-41% of ingested retinol was recovered in the lymph (Goodman et al., 1966; Blomstrand and Werner, 1967). This suggests that a significant proportion of β-carotene and retinol is metabolised in the gut and is not available for absorption. Underestimation can happen 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.

Given that all the studies reviewed above were carried out with a small number of subjects and that the results were very variable, the expert groups of FAO/WHO (1967) concluded that it is not possible to predict the bioavailability accurately.

2.5.6 Research questioning the conversion factor 6

Since the results of studies conducted in Guatemala (Bulux et al., 1994) and Indonesia (de Pee et al., 1995; de Pee et al., 1998) were published, the conversion factor of 6 µg β-carotene to 1 µg retinol has been seriously
questioned. Bulux and colleagues (1994) supplemented children aged 7-12 years old for 20 days with either 50 g carrots together with 10 g fat, with retinol or with purified β-carotene. The mean baseline serum retinol concentration was 34 µgram/dl, the sample size ranged from 16-20 per group and they also included a negative control group. There was no increase in serum retinol concentrations in any of the groups, and only the group receiving pure β-carotene increased their serum β-carotene. de Pee and colleagues in their elegant studies in Indonesia, included both a negative and a positive control group. The first one (1995), on lactating women with a low haemoglobin concentration (<130 g/L) did not find any impact on serum retinol after a 12 week supplementation with DGLV and carrots containing 3.5 mg β-carotene together with 8 g of fat, while the same amount from an enriched wafer did improve vitamin A status (n=53-62/group). Their baseline serum retinol levels ranged from 0.84 to 0.89 µmol/L. Also, the change in serum β-carotene was very small in the group receiving DGLV, 0.03 µmol/L compared to 0.73 µmol/L in the group receiving the enriched wafer. However, the median intensity of infection with the intestinal parasite Ascaris Lumbricoides was high, 13,020 eggs per gram (epg), which has been given as a plausible explanation for the non-response in the vegetable group (de Pee et al., 1995). Their second study (1998) included anaemic children aged 7-11 years old and compared the impact on vitamin A status of daily supplementation with carotenoids having 509 RE from fruits (n=49), 684 RE from DGLV and carrots (n=45) for 9 weeks. The mean baseline retinol levels were 0.69 to 0.73 µmol/L, respectively. The increase in serum retinol was 0.12 and 0.07 µmol/L, respectively in the two groups, and thus the author concluded that fruits such as mangoes, papaya and sweet potato are better than vegetables in increasing serum retinol. The increase in serum β-carotene was 0.14 µmol/L in the vegetable group compared to 0.52 in the fruit group. Also in this study, the median Ascaris Lumbricoides intensity was high, 4720 epg. Suggestions have therefore been made to change the estimated conversion factor 6 to 12 for β-carotene coming from YOFV and 26 for DGLV and carrots (West and Hautvast, 1997).

Among several explanations for the poor response DGLVs had on serum retinol concentrations in these studies, one of the most likely is the high baseline retinol status among the subjects (de Pee et al., 1995). However, in one of the studies with poor DGLV response (de Pee et al., 1998) the mean baseline retinol concentration was around 0.70 µmol/L, the cut-off for a low vitamin A status (WHO/UNICEF, 1996). Another cause for poor response could be intestinal parasites, in particular Ascaris lumbricoides. A supplementary vegetable feeding trial in Indonesia found that serum retinol concentration increased more in highly worm infected children if they were dewormed first (Jalal et al., 1998). It has been postulated that “a high parasite load may make the freeing of β-carotene more difficult from a complex matrix (vegetables)” (de Pee et al., 1995).
Further studies are thus needed to elucidate the role of these factors, in particular *Ascaris lumbricoides*, on the bioavailability of plant sources of vitamin A.

### 2.6 Health and vitamin A deficiency in Indonesia, Bangladesh and India

The world region worst affected by malnutrition is South Asia. Half of the world’s malnourished children are to be found in just three countries - Bangladesh, India and Pakistan. This is partly because of the huge size of the populations (India alone has twice as many people as all of sub-Saharan Africa), but also because the proportion of people affected, in most cases higher than in sub-Saharan Africa. There are several theories as to why this is so (UNICEF, 1997).

Approximately 1/3 of the infants in India and Bangladesh are born with birth weights below 2,500 grams. During pregnancy itself, most women in South Asia gain little more than 5 kilos whereas most women in Africa come closer to 10 kilos, the average of what women should gain during pregnancy (UNICEF, 1997). The nutritional status of adults, particularly women, is also much lower in South Asia than in Africa. Prevalence of underweight in children under 5 years of age in Bangladesh and India is approximately 50% compared to 30% for sub-Saharan Africa (UNICEF, 2001).

#### 2.6.1 Indonesia

With a population of 209 million, Indonesia is the fourth most populous country in the world after China, India and the US. The country consists of 13,600 islands of which 6,000 are inhabited by people. Although a large country, almost 2/3 of the population live on Java, with an area of 132,190 square km, giving it a population density of 813 persons/square km (in 1989). The first two studies in this thesis were conducted in Purworejo District, Central Java (see figure 3).

Indicators of demography, health, nutrition and economy are shown in table 5. During 1990-97, the economy of Indonesia improved substantially. For example, the gross national product per capita (GNP) increased from US$ 561 to 1,089 (MOH, 1997). Unfortunately, starting August 1997, the country experienced a radical and rapid deterioration in its economy as part of the financial crisis that severely affected many Asian countries (Jakarta Post, 1998).

*Dietary intake of vitamin A:*

In Central Java, vitamin A intake data were collected in five rounds by a nutritional surveillance system between December 1995 until January 1997.
Figure 3. Study locations and vitamin A deficiency situation (from information available to WHO by the year 2000). The designations do not necessarily imply VAD is uniformly distributed throughout each country (with permission from Sight & Life)
This showed that the median vitamin A intake of mothers increased from 335 to 371 RE/day, and that for children increased from 130 to 160 RE/day (de Pee et al., 1998). The majority of the vitamin A intake, 80%, came from plant foods. Population-based data from other islands, South Sulawesi and South Kalimantan, showed a vitamin A intake among preschoolers of 470 RE and 350 RE, respectively, and among their mothers it was 870 RE and 520 RE, respectively. However, the proportion of the vitamin A coming from plant sources was much greater in Sulawesi, 74-86% compared to 18-53% in Kalimantan (Martini et al., 1999).

**Vitamin A deficiency:**
Over the past 20 years, much of the pioneering work in vitamin A control program development and research has taken place in Indonesia. This includes evaluation of the mortality effects of a vitamin A supplementation trial (Beaton et al., 1993). A large-scale national prevalence survey (1977-78) of children aged 0-5 years found an overall X1B prevalence of 1.0% and corneal xerophthalmia of 0.06%. Between 1983 and 1990, many of the provinces earlier identified as having the largest prevalences of xerophthalmia were re-surveyed. A considerable reduction was noted. Later studies in several provinces (1991) showed a total xerophthalmia rates of <1.0%, but biochemical studies showed that >50% of those surveyed had serum retinol levels < 0.70 µmol/l (WHO/UNICEF, 1996). The prevalence of low serum retinol (< 0.70 µmol/l ) among pregnant, lactating and non-pregnant non-lactating women from rural East Java was 66%, 51% and 58%, respectively (Kusin et al., 1980), and 34% and 18% among pregnant (Suharno et al., 1992) and lactating women, respectively (Dijkhuizen et al., 2001) in West Java.

Preliminary findings of data collected after the onset of the economic crisis, which started approximately in mid-1997, showed that VAD was still highly prevalent among preschoolers and their mothers, and that the situation was far worse in urban slums than in rural areas. In addition, it showed an increase in the prevalence of VAD among mothers since the start of the crisis (HKI, 1998).

**Strategies for the prevention of VAD:**
The Government of Indonesia has a vitamin A policy and an operational plan to address VAD nation-wide. Micronutrient interventions form a part of the Government’s five-year development plans (Repelita) under the Minister of Health’s Nutrition Directorate (MOH and WHO, 2000). The strategies for VAD control that have been adopted include 1) continued high-dose vitamin A supplementation to preschool children 6 months to 5 years old through the Posyandu (health service post), 2) high-dose vitamin A supplementation to postpartum mothers (MOH, 2000; MOH et al., 2000), 3) food fortification (not yet implemented) and 4) food diversification and nutrition education (WHO/UNICEF, 1996). Social marketing campaigns have been implemented to
increase the consumption of vitamin A rich foods (DGLV’s and eggs) in several areas such as South Sulawesi and South Kalimantan (HKI, 1998) and Central Java (de Pee et al., 1998). In the last five years, some manufactures have started fortifying instant noodles with vitamin A, iron and B-vitamins. Powdered and condensed milk and some brands of margarine are also fortified with vitamin A.

2.6.2 Bangladesh
Bangladesh is a small country bounded by India in the west, north and east, by Burma in the south-east, and by the Bay of Bengal in the south (figure 3). It has a surface area of 143,988 square kilometres (1/3 of Sweden). The population is approximately 127 million people. The population density is 882 per square kilometre (20 in Sweden). The annual population growth rate has declined rapidly in recent decades and between 1990-97 was only 1.6% (UNICEF, 2001). Bangladesh experiences four seasons. Summer lasts from April to May, the rainy period stretches from June to September and the “winter” is from October to January. The spring lasts from February to March. The country is divided into six divisions. Each division consists of several districts and each district is divided into thanas (subdistricts). Studies 3, 5-6 in this thesis were conducted in Thakurgaon District and Panchargar District in northern Bangladesh, both of which belong to Rajshahi Division. Dhaka is the capital of Bangladesh. Demographic, health, nutritional and economic indicators are shown in table 5. Under-five mortality has dropped from 247 in 1960 to 89 in 2000. The female literacy rate has increased from being 17% in 1980 to 48% in 2000.

Dietary intake of vitamin A:
Vitamin A intake was found to be inadequate in all seasons but was lowest in August and highest in October-November (Hassan et al., 1985). The nutrition survey of 1981-82 found that the mean intake of vitamin A was 38-78%, 68% and 20-58% of the recommended daily intake for children aged 1-6 years, 7-9 years and 10-19 years old, respectively. Interestingly, studies have shown that vitamin A intake does not always show consistent association with socio-economic status (Zeitlin et al., 1992; Bloem et al., 1996). Instead, seasonal low prices and home availability both in terms of quantity and number of fruits and vegetables grown in the garden seem to be more important.

Vitamin A deficiency:
VAD was previously identified as a public health problem in Bangladesh (Cohen et al., 1985) and with 3.53% of children under six years old suffering from XN, it was estimated that 30,000 children became blind each year due to VAD (HKI/IPHN, 1985). In addition, 14,725,000 persons were at risk of VAD. The prevalence of XN among children receiving and not receiving VAC was
2.65% and 4.37%. The Bangladesh National Vitamin A survey in 1997 found that only 0.67% of children 6-59 months old suffered from XN (ACC/SCN, 1998). The prevalence of XN among those who received VAC was 0.58% compared to 1.05% for the children who did not receive VAC. Based on these findings, it was concluded that the prevalence of xerophthalmia is no longer a problem of public health significance. However, the prevalence of XN among women during most recent pregnancy was 6.8%. Since the prevalence of XN decreased also among children not receiving VAC, it is not likely that VAC alone was responsible for the long-term improvement of the vitamin A status in Bangladesh. Instead, it is likely that both the VAC-program and the improvement of dietary intake of vitamin A in part through large scale promotion of home gardens was responsible. A third factor which may explain the decrease the prevalence of XN is that the GNP per capita average annual growth rate has increased from -0.3 between 1965-80 to 6.6% between 1990-99 (UNICEF, 2001). The magnitude of VAD among older children is not fully known, since data for that age group is scarce (Ahmed, 1999), but it may be a neglected problem. In the survey of 1981-82, prevalence of night blindness was only 0.3% in boys and none was found in girls, aged 5-14 years old. However, a study of 5,420 children aged 0-15 years in northern Bangladesh in 1992 showed a prevalence of night blindness of 1.9% (Hussain et al., 1995). Saha and colleagues (1991) in their study found that about 1.4% of males aged 5-14 years in tube well areas and 7.9% of the same age group in non tube-well areas had night blindness. Among the females it was 0.7% and 1.4%, respectively.

Very limited information is available on serum retinol in older children. Recent studies on female adolescent garment worker show that 14% had less than 20 µgram/dl, indicating a public health problem (Ahmed et al., 1997). A study in Rangpur District found that the mean serum retinol levels of children 7-8 and 9-15 years old were 17.6 µgram/dl and 25.2 µgram/dl in children with XN and 22.4 µgram/dl and 29.2 µgram/dl in children without XN (n=92) (Hussain and Kvåle, 1996).

Programs to prevent VAD in Bangladesh
In 1973, the Government of Bangladesh initiated VAC distribution for all rural children under six years old. Later distribution was extended to children in urban slums, mainly by non governmental organisations (NGO). From 1973 to 1994, VAC distribution was biannually during March-April and September-October as part of the government’s Nutritional Blindness Prevention Program. The government’s health workers distributed VAC through door to door visits. Although government monitoring reported coverage rates of 70% and 89% during the period 1982 to 1990, independent surveys showed that coverage rates actually were 46%, 37%, and 35% during 1982/83, 1987/88 and 1989, respectively and between 42-55% from 1990 to 1994. From 1995 the strategy has changed to administration at outreach centres on special days, once with national immunisation days for the eradication of polio and another through
organising national vitamin A week for children aged 12-71 months. Social mobilisation, involvement of mass media and utilisation of local resources were some key aspects in the changed strategy. The coverage rates increased to over 85% in 1997 and to over 70% in 1998 (HKI/IPHN, 1998).

From 1982-1996, home garden activities took place in the northern region of Bangladesh through the Nutrition and Blindness Prevention Program, which later became the Comprehensive Nutrition and Blindness Prevention Program. The NGO Worldview International Foundation was responsible for the implementation of the programs. Six districts were covered by these large-scale projects with a total population coverage above 9 million (Greiner and Mannan, 1999). HKI has implemented a home gardening project since 1993, reaching approximately 244,000 families.

| Table 5. Indicators of demography, health, nutrition and economy in Bangladesh, India, Indonesia and Sweden¹ |
|---|---|---|---|---|
| | Bangladesh | India | Indonesia | Sweden |
| Area (Square km) | 143,988 | 3,287,263 | 1,904,600 | 449,964 |
| Total population (million) | 127 | 998 | 209 | 8.9 |
| Population density (persons/square km) | 882 | 304 | 813(Java) | 20 |
| Crude birth rate (per 1000 population) | 28 | 25 | 22 | 10 |
| Vitamin A supplementation coverage | 73 | 25 | 64 | -- |
| Population growth rate | 1.6 | 1.8 | 1.5 | 0.4 |
| % with measles vaccination | 66 | 55 | 71 | 96 |
| Infant mortality rate | 58 | 70 | 38 | 3 |
| Under-five mortality rate | 89 | 98 | 52 | 4 |
| Total adult literacy rate (% literate >15 years) | 56 | 58 | 88 | -- |
| Adult literacy rate, females as % of males | 76 | 62 | 87 | -- |
| Life expectancy at birth | 58 | 63 | 66 | 79 |
| Income per capita per annum (US$) | 370 | 450 | 580 | 25,040 |
| Maternal mortality | 440 | 410 | 450 | 5 |
| Fertility rate | 3.0 | 3.0 | 2.5 | 1.6 |
| Prevalence of underweight in under 5’s stunting | 56 | 53 | 34 | -- |
| wasting | 55 | 52 | 42 | -- |
| % of infants with low birth weight | 18 | 18 | 13 | -- |
| % of children still breastfeeding at 20-23 mo. | 30 | 33 | 8 | 5 |

¹ Source: The state of the World’s children 2001
2.6.3 India
India has the second largest population in the world with 998 million people. The surface area is 3,287,263 sq km, making it the fifth largest country in the world, or eight times bigger than Sweden. It has a common border with Pakistan in the west, Bangladesh in the east and Nepal and China in the north (see figure 3). Each of the 23 states consists of several districts and each district is divided into talukas (blocks/sub-districts). The fourth study included in the thesis was conducted in the rural area of Pune District, Maharashtra State. Indicators of demography, health, nutrition and economy are shown in table 5. Under-five mortality has dropped from 236 in 1960 to 98 in 2000. The female literacy rate has increased from 25% in 1980 to 44% in 1999.

Dietary intake of vitamin A
The average intake of vitamin A from the diet was estimated in a survey conducted in 1988-90. It was found to be 294 RE per person and day, which is an improvement from the previous survey in 1975-79 when the intake was 257 RE. However it is still much below the Indian RDI of 600 RE/day. The average intake of DGLV was 11 g per person and day (RDI, 40 g) and the consumption of milk and milk products was 96 gram (RDI, 150 g) (FAO/WHO, 1992).

Vitamin A deficiency
According to the WHO criteria, VAD, is a significant health problem in India (WHO/ UNICEF, 1996). In 1996, 88 million children below 4 years of age were at risk of VAD (Government of India, 1996). During the last two decades there has been a decline in blindness due to VAD (Government of India, 1995). However, the estimated incidence of corneal xerophthalmia among pre-school children is about 30,000 cases per year, of which nearly half would result in permanent blindness (ACC/SCN, 1992). “Milder” forms of clinical VAD remain a problem; the National Survey of Blindness, 1986-89 revealed that 5-7% of all children under 5 years suffer from varying degrees of VAD exhibited through ocular manifestations. In the state of Maharashtra the proportion of children with Bitot’s spot was 1.78% (Government of India, 1996). Biochemical data are available from a survey in Tamil Nadu (1988-90) on children 0.5 to 5 years old. These data show that 29.5% of these children had serum retinol levels between 0.35-0.70 µmol/l and 8% had < 0.35 µmol/l (ACC/SCN, 1992).

Strategies for the prevention of VAD
The Government of India has a vitamin A policy and an operational plan to address vitamin A deficiency nation-wide. The official policy is to give 5 mega-doses of vitamin A to each child between 9 months and 3 years of age. The first dose is 100,000 I.U and is given along with measles immunisation. The second to fifth doses are 200,000 I.U. and are given every six months. The
program targets children with access to governments services, either through primary health care or the Integrated Child Development Services (Ministry of Health & Family Welfare Government of India, 1991). However, the number of children reached by the program was estimated to be one quarter of the total eligible population in 1998 (UNICEF, 2000). Problems include workers not being clear on the protocol for vitamin A distribution and irregularities in supply (Vijayaraghavan, 1995). Based on the recommendations of a review committee, the program is now being modified to improve the outreach to the target population (ACC/SCN, 1992). The issue of providing vitamin A supplements to lactating mothers will be explored (Government of India, 1996).

Home gardening and education programs are strategies which have been recommended (Government of India, 1996). These have been initiated in three states, (ACC/SCN, 1992) but Maharashtra State has not been included. Effort has also been made to find a suitable vehicle for fortification with vitamin A. Several foods are currently being fortified, including milk and margarine. However, they do not reach most of the population in need.
3. AIM OF THE STUDIES

3.1 Overall aim
The overall aim of the studies presented in this thesis is to examine whether individual and community vitamin A intake and status can be reliably assessed with simple dietary methods, to examine whether vitamin A intake and status are adequate in the studied populations, and to evaluate if vitamin A status can be improved by increasing the consumption of vegetables rich in β-carotene.

3.2 Specific objectives
- To investigate whether individual vitamin A intake can be reliably assessed in a developing country such as Indonesia with the 24-h recall method. Further, to describe intra-individual and inter-individual variability in vitamin A intake and other nutrients among pregnant women in Indonesia. These estimates of variation are also used to show the implications of using different number of days for estimating true average intake, as well as relationships between dietary intake and health outcomes (paper 1).

- To quantify vitamin A intake and to identify risk groups for a low vitamin A intake among pregnant women in Indonesia. To elucidate whether the proportions of vitamin A coming from different sources (animal, fortified, fruits and vegetables) vary among groups of different socio-economic status (paper 2).

- To investigate whether the simplified “Helen Keller International Food Frequency Method”, used to assess community risk of vitamin A deficiency, is applicable to countries like India and Bangladesh, even though it excludes breastmilk and animal milk (papers 3 and 4).

- To explore the relationship between biochemical indicators of vitamin A, iron status and the intestinal helminths *Ascaris lumbricoides* and *Ancylostoma duodenale* (hookworm) in primary school children of Bangladesh (paper 5).

- To investigate whether it is possible to improve vitamin A status with dark green leafy vegetables in school children who are moderately vitamin A deficient, and free of *Ascaris lumbricoides* (paper 6).
4. SUBJECTS AND METHODS

4.1 Variability in nutrient intake (paper 1) and vitamin A intake during pregnancy (paper 2)

Design
This study was conducted in Purworejo District, Central Java, Indonesia, which consists of 16 subdistricts and 494 villages. The total population is 750,000. According to the 1990 census, the infant mortality rate in Purworejo was 52 per 1000 births and the fertility rate was 3.1 per woman (Nurdiati et al., 1998). Since 1994, the Faculty of Medicine at Gadjah Mada University has been operating the Community Health and Nutrition Research Laboratories (CHN-RL) in this area to support the Ministry of Health in developing and implementing a community health and nutrition surveillance program. A two-stage cluster sampling method was used to select a 10% sample of households representative of the district. From 1994 to 1998, each household were visited every 3rd month for data collection. However, to identify new pregnancies, households with women likely to be pregnant (i.e. married women of reproductive age) were visited monthly. Between April 1996 and October 1998, a cohort of 846 women in early pregnancy was recruited for a study on nutritional status during reproduction. Within this framework, dietary data were collected among a subsample of 493 women, with dietary data in at least one trimester. In study 1, only women with six complete 24-h recalls per trimester were included. However, they were only excluded from analyses for those trimesters where fewer than six recalls were completed. This caused the total study sample to drop from 493 to 451.

Dietary assessment
In each trimester, a minimum of three and a maximum of six 24-h recalls were used to estimate the dietary intake of the individual women. However, for the majority (82%, 90% and 84% in trimesters 1, 2 and 3, respectively), six recalls were obtained. These replications were equally distributed among the five different days of the Javanese calendar on non-consecutive days. Detailed descriptions of all foods, beverages, and vitamin and mineral supplements consumed between 00.00 a.m. and midnight the previous day, as well as cooking methods were recorded. Quantities were estimated using seven household utensils and approximately 20 types of food models, e.g., fish, tomato, banana and meat. The average weight of each type of food equivalent to the portion sizes and fitting into the household utensils were estimated to the nearest gram. To calculate nutrient intakes, a computer program (Inafood) was developed, which mainly uses the Indonesia nutrient composition data base.

The data were collected by 22 trained female interviewers and each respondent was interviewed at home by the same interviewer for all measurements. The interview lasted one to two hours.
**Other covariates**
Socio-economic and demographic information for the women were retrieved from the surveillance data collected between August and October 1997. In addition, we used information on home garden activities and livestock ownership that was collected in 1997-1998. Information on height and mid-upper-arm circumference (MUAC) was also used.

**Main statistical analyses**

*Variability in nutrient intake (paper 1):*
Variance components are reported as absolute values, ratios and coefficients of intra- (within) individual variation ($CV_w$). The Variance Components procedure in SPSS was used to calculate the absolute values, and Equation 1 shows how $CV_w$ was calculated, where $s_w$ = square root of the estimated intra-individual variance.

$$CV_w = \frac{s_w}{\text{mean intake (of that nutrient)}}, \quad (1)$$

For the second goal, values of $CV_w$ were used to illustrate the required number of recalls per individual for the various nutrients (Willett, 1990) (Equation 2):

$$n = \left(\frac{Z_D CV_w}{D}\right)^2, \quad (2)$$

where $n$ = number of replicate days required, and $Z_\alpha$ = the normal deviate for the percentage of times a confidence interval (e.g. $Z_\alpha = 1.96$ for 95% confidence) should cover the “true” average mean intake of an individual. Finally, $D$ = half length of the confidence interval as percentage of the mean.

In addition, the values of variance ($s^2_b$ and $s^2_w$) were used to illustrate the error in a regression analysis designed to look at the relation between dietary intake and some hypothetical outcome, with observed dietary intake as an independent variable. The error is measured by the ratio of the observed-to-true slope coefficients (Beaton *et al.*, 1979), using Equation 3:

$$b_0/b_t = \frac{r s^2_b}{(r s^2_b + s^2_w)}, \quad (3)$$

where $b_0 = \text{observed regression coefficient and } b_t = \text{true regression coefficient,}$
$r = \text{number of replications per individual, } s^2_b = \text{estimated inter-individual variance component}$ and $s^2_w = \text{estimated intra-individual variance component.}$

*Vitamin A intake during pregnancy (paper 2):*
Kruskal-Wallis and Mann-Whitney $U$-test were used for testing differences in median intake of vitamin A. Univariate logistic regressions were used to examine the relationships between vitamin A intake and other individual
characteristics in the second and third trimesters. Variables significant in the univariate logistic regressions were included in multivariate logistic regressions, where possible associated factors were evaluated simultaneously. The logistic regression analyses were constructed with vitamin A intake as a dichotomous variable (vitamin A intake < FAO/WHO (1988) basal RDI (370 RE) vs. ≥ FAO/WHO basal RDI).

4.2 Vitamin A intake where sustained breastfeeding is common (paper 3)

**Design**
Forty children from five villages in rural Thakurgaon District, Bangladesh, selected by quota sampling, were studied in November and December, 1996. The age of the children were one to three years old, with a mean age of 18 months and equal numbers of one- to two-year-olds and two- to three-year olds. There were also an equal number of boys and girls in the sample. The consumption of breastmilk over a 9-hour period was quantified by the test-weighing technique, i.e. children were weighed immediately before and after all feedings from 9 a.m. to 6 p.m on a UNICEF beam balance accurate to 5g. From these data, the 12-hour intake was derived as 1.33 times the 9-hour intake and the 24-hour consumption was extrapolated using the correction factor 12-hour/0.53, which had been estimated earlier for Bangladeshi women (Brown, 1982).

**Main statistical analyses**
The independent sample t-test was used to study differences in breastmilk intake between groups.

4.3 Vitamin A intake where animal milk is a part of children’s diet (paper 4)

**Design**
The study was performed in seven villages of Purandar subdistrict in Pune District, Maharashtra State, India, from May to July 1996. Approximately 50 households per village with children aged 12-72 months were surveyed. Within each of these households, one mother /caretaker was randomly selected. After the mother was selected, one of her children aged 12-72 months was chosen randomly. The total sample included 345 children with a mean age of 38 months, and with 48% girls. The majority of them, 79%, belonged to the Maratha caste and the remaining respondents, 5%, belonged to the lower castes and 16% to other castes.
Vitamin A intake, status and improvement using the dietary approach

Dietary assessment
Dietary information for each child was obtained through interviews with the mother/ caretaker using the HKI FFM (see section 2.4.3, page 23).

Qualitative assessment on milk consumption
Three group discussions and 10 key informant interviews were held to obtain information on children’s milk drinking. Questions included how milk is fed to young children, e.g. if the cream is separated or mixed with the rest, how the milk is diluted and approximately how much milk is usually given.

Fat analyses
To confirm qualitative data, nine samples of cow milk as consumed by young children were analysed for fat concentration.

Main statistical analyses
Mann-Whitney \( U \)-test and Kruskal-Wallis analyses of variance were used to compare differences in frequency of milk consumption, and the chi-square test was used to compare differences in milk cattle ownership.

4.4 Relationships between vitamin A, iron and helminths (paper 5) and the effect of DGLVs on vitamin A status in primary school children (paper 6)

Design
Primary school children from grades 3-5 at one rural school in Panchargar District and another in Thakurgaon District, Bangladesh, were enrolled in a dietary intervention, between March to June, 1998. Prior to the intervention, all children in grades 3-5 from the two schools were screened for intestinal helminths. Out of 302 children screened, 47\% (143) were found to be positive and to complete the desired sample size for the intervention, these 143, plus 25 children with an unhygienic toilet were selected. After four of the children dropped out, the final sample at the start of the intervention was 164 children. All of them were given a single supervised 500 mg dose of Mebendazole 2 weeks before initiation of the supplementation trial. Due to a decision not to have a second blood test (n=40), two cases of xerophthalmia, presence of acute infection (n=5), absence from school (n=7), the number of children still included at the end of the intervention was 110 (see figure 4). Before and after the intervention, information on height, weight and dietary intake was collected from all 110 participants, and a blood sample was taken. In addition, a second stool sample was collected at the end of the study.
**Experimental diet**
Each child received 1 meal/d, 6 d/wk for 6 wk. The only difference in the meals given to the three groups was in the source and amount of vitamin A (see table 6). In each school, meals were prepared by 3-5 village health workers supervised by a nutritionist from Dhaka University. Recipes were developed on a weekly basis after discussion with the health workers and teachers. The recipes listed, for each ingredient, the amount to be purchased and the weight to be cooked, excluding roots and peels. The correct measured amount of vegetables was put on the plates for each group. The food was served around noon and each group ate in a separate room. The village health workers provided the meals and together with the teachers supervised consumption, keeping records of attendance and leftovers for each child. Approximately half of the time, consumption was directly supervised by the nutritionist, to make sure that all instructions were being followed.

**Figure 4. Sample selection**

![Sample selection diagram]

**Table 6. Ingredients in the vegetable supplements given to the three treatment groups**

<table>
<thead>
<tr>
<th></th>
<th>DGLV group</th>
<th>Sweet pumpkin group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lal shak</td>
<td>Sweet pumpkin</td>
<td></td>
<td>Potatoes (Solanum tuberosum): 100 g, 2 d; lau (Lagenaria vulgaris): 100 g, 2 d; Jhinga (Luffa acutangula): 100 g, 1 d; Chal kumra (Benincasa hispida): 100 g, 1d</td>
</tr>
<tr>
<td>(Amaranthus gangeticus): 100 g, 2 d; Palang shak (Spinacia oleracea): 100 g, 1 d; Pui shak (Basella alba): 100 g, 2 d; Kalmi shak (Ipomoea reptans): 100 g, 1d</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a The amount given to each child/day and the number of days per week the vegetable was given
**β-carotene and fat content the food supplements**

The β-carotene values for DGLV were estimated from earlier analyses (HPLC) done in Bangladesh (Rahman *et al.*, 1990), using the values for the cooking method most similar to the one in the present study. To provide at least 3.5 mg β-carotene daily, we fed the children approximately 100 g of DGLV daily (Table 1). Given the average weekly amounts of each type of DGLV used, the average amount of β-carotene fed was 4.4 mg per person per day. The estimated β-carotene content of the local sweet pumpkin was 0.75 mg/100 g, yielding approx. 1.5 mg per person per day. In the control group, the β-carotene content on average was 0.013 mg per person per day. The fat content of the vegetable dishes was approximately the same for all three groups, 8 g/portion.

**Dietary interviews**

A questionnaire was developed to obtain information on frequency of consumption of dietary sources of vitamin A and on meat intake. In addition, the children were asked about the presence or absence of various symptoms related to vitamin A status, such as fever, XN, diarrhoea and acute respiratory infection, as well as standard of housing (type of roof, wall, floor and toilet).

**Anthropometry**

The children’s weight was measured to the nearest 0.2 kg on a digital scale (Soehnle, Germany). They were measured barefoot and wearing only light clothing. Height was measured to the nearest 0.1 cm using a stadiometer (Somatomètre, Inter 16, CMS Equipment Ltd, England) mounted on a wall.

**Analytical methods**

Serum retinol and β-carotene levels were determined by the HPLC procedure at the Institute of Nutrition and Food Sciences, Dhaka University, Bangladesh. The inter-assay and intra-assay variations for serum retinol were 2.7% and 2.6%, respectively. The inter-assay and intra-assay variation for serum β-carotene were 5.7% and 4.0%, respectively.

CRP was analysed by a turbometric method using a polymer on a Hitachi 911, at the Department of Clinical Chemistry, Uppsala University Hospital. The coefficient of variation was 10% at 20 µg/L. Levels > 10 µg/L were taken as an indicator of acute infection, and these children were excluded from analysis.

The haemoglobin concentration was determined on site using the Hemocue method (HemoCue, Inc, Ängelholm, Sweden) on venous blood.

Stool samples were examined using Stoll’s dilution egg count technique (Suzuki, 1981). The total number of each type of helminth egg was counted.
microscopically and expressed as number of eggs per gram of stool by multiplying by a factor 200.

**Main statistical analyses**

*Relationships between vitamin A, iron and helminths (paper 5):* Pearson’s correlation test was used to study the association between serum retinol and \( \beta \)-carotene. Chi-square tests, independent sample \( t \)-tests and analysis of variance (ANOVA) were used to assess the effect of certain differences between groups. Multiple regression analysis was used to study the relationship between iron status and the explanatory variables retinol and hookworm.

*The effect of DGLVs on vitamin A status in primary school children (paper 6):* Changes in serum retinol and \( \beta \)-carotene within groups were tested with the paired \( t \)-test. Differences in changes of the same indicators between groups were examined by ANOVA. Multiple regression analysis was used to compare changes in retinol and \( \beta \)-carotene concentrations between groups using dummy variables for the three supplementation groups to control for baseline levels of retinol, \( \beta \)-carotene and *Ascaris lumbricoides* load.
5. RESULTS

5.1 Vitamin A intake

*Characteristics of women*
Basic characteristics of the sample women are shown in table 7. The mean ± SD age of the study sample was 28.8 ± 5.4 years and the mean parity was 1.6 ± 1.4. Thirty-nine percent worked with agriculture and 22% had ≥10 years of education.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD or %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.8 ± 5.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>149.9 ± 4.9</td>
</tr>
<tr>
<td>Mid-upper-arm circumference at 5 months (cm)</td>
<td>25.2 ± 2.6</td>
</tr>
<tr>
<td>Parity (number of children)</td>
<td>1.6 ± 1.4</td>
</tr>
<tr>
<td>Education (%)</td>
<td></td>
</tr>
<tr>
<td>0-6 years</td>
<td>56</td>
</tr>
<tr>
<td>7-9 years</td>
<td>22</td>
</tr>
<tr>
<td>≥10 years</td>
<td>22</td>
</tr>
<tr>
<td>Urban residence (%)</td>
<td>9</td>
</tr>
<tr>
<td>Occupation (%)</td>
<td></td>
</tr>
<tr>
<td>non-agricultural</td>
<td>20</td>
</tr>
<tr>
<td>agricultural</td>
<td>39</td>
</tr>
<tr>
<td>housewife/unemployed</td>
<td>41</td>
</tr>
<tr>
<td>Uses closed latrine (%)</td>
<td>53</td>
</tr>
<tr>
<td>Uses water from tap, well or pump (%)</td>
<td>90</td>
</tr>
<tr>
<td>Owns home garden with vegetables (%)</td>
<td>69</td>
</tr>
<tr>
<td>Owns chickens</td>
<td>86</td>
</tr>
</tbody>
</table>

5.1.1 Methodological aspects of dietary assessment (paper 1)

*Variance components:*
Intra and inter variance components for the nutrient intake of the Indonesian women are shown by trimester in table 8. In all three trimesters, the ratio of intra- to inter-individual variation was <1.0 for energy and carbohydrates. Also in all trimesters, the ratios were greatest for the micronutrients (iron, vitamin A). For most nutrients, the ratios were lowest in the first trimester.

Values of CV_w are shown in table 9. For most nutrients, the CV_w was highest in the first trimester and lowest in the third. However, the differences in CV_w between the trimesters were not large.
Table 8. Intra and inter individual variance components in trimester 1 (n=122), 2 (n=406) and 3 (n=356) in dietary intake data of pregnant women in Indonesia

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean ± SD (Per day)</th>
<th>Variance</th>
<th>Intra</th>
<th>Inter</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>6730 ± 2466&lt;sup&gt;1&lt;/sup&gt;</td>
<td>180801</td>
<td>318597</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7925 ± 2132&lt;sup&gt;2&lt;/sup&gt;</td>
<td>180031</td>
<td>229724</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8235 ± 2027&lt;sup&gt;3&lt;/sup&gt;</td>
<td>152872</td>
<td>210182</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>38.6 ± 17.1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>323</td>
<td>238</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43.1 ± 15.9&lt;sup&gt;2&lt;/sup&gt;</td>
<td>305</td>
<td>203</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45.4 ± 15.8&lt;sup&gt;3&lt;/sup&gt;</td>
<td>267</td>
<td>205</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>282 ± 104&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5643</td>
<td>9967</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>334 ± 90.9&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5793</td>
<td>7422</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>347 ± 85.7&lt;sup&gt;3&lt;/sup&gt;</td>
<td>5340</td>
<td>6698</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>39.6 ± 16.3&lt;sup&gt;1&lt;/sup&gt;</td>
<td>191</td>
<td>233</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.0 ± 13.7&lt;sup&gt;2&lt;/sup&gt;</td>
<td>214</td>
<td>151</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.9 ± 13.6&lt;sup&gt;3&lt;/sup&gt;</td>
<td>224</td>
<td>149</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>Iron&lt;sup&gt;4&lt;/sup&gt; (mg)</td>
<td>0.99 ± 0.23&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.0598</td>
<td>0.0411</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.08 ± 0.18&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.0474</td>
<td>0.0255</td>
<td>1.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.13 ± 0.21&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.0618</td>
<td>0.0357</td>
<td>1.84</td>
<td></td>
</tr>
<tr>
<td>Vitamin A&lt;sup&gt;4&lt;/sup&gt; (µg)</td>
<td>2.29 ± 0.38&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.298</td>
<td>0.0955</td>
<td>3.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.36 ± 0.38&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.288</td>
<td>0.0890</td>
<td>3.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.41 ± 0.34&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.260</td>
<td>0.0756</td>
<td>3.44</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Data from trimester 1.<n
<sup>2</sup>Data from trimester 2.<n
<sup>3</sup>Data from trimester 3.<n
<sup>4</sup>Due to skewed values, log-transformed data shown.

Table 9. Number of 24-hour recalls needed to estimate true average intake of pregnant women in Indonesia

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>CV&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;, in trimester</th>
<th>Number of replicate days needed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Energy</td>
<td>0.26</td>
<td>0.22</td>
</tr>
<tr>
<td>Fat</td>
<td>0.47</td>
<td>0.41</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>0.27</td>
<td>0.23</td>
</tr>
<tr>
<td>Protein</td>
<td>0.35</td>
<td>0.31</td>
</tr>
<tr>
<td>Iron&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>Vitamin A&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.24</td>
<td>0.23</td>
</tr>
</tbody>
</table>

<sup>1</sup>Coefficient of variation and calculated from <i>s</i><sub>1</sub>/mean intake (of that nutrient), where <i>s</i><sub>1</sub> = square root of the estimated intra-individual variance.<n
<sup>2</sup>Half length of the confidence interval as percentage of the mean.<n
<sup>3</sup>Range indicates the number of days needed based on the CV<sub>1</sub> in trimester 1-3.<n
<sup>4</sup>Due to skewed values, log-transformed data shown.
Dietary intake in regression analyses:
Relationships between dietary intake and health outcomes are often evaluated with regression analyses. The ratio of the observed-to-true regression slope as a function of the number of replicates, with the different nutrients as the independent variable, is shown in table 10. The results are based on the second trimester, but similar results were found also for the other two trimesters. The attenuation of the true regression coefficient with decreased replicates was greatest for the micronutrients. The regression coefficient for energy and carbohydrates did not change substantially by reducing the number of d from six to three.

Table 10. Ratio of the observed-to-true regression slope with the various nutrients as independent variables, given different numbers of replicates
for pregnant women in Indonesia

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Number of replications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Energy</td>
<td>0.96</td>
</tr>
<tr>
<td>Fat</td>
<td>0.93</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>0.96</td>
</tr>
<tr>
<td>Protein</td>
<td>0.93</td>
</tr>
<tr>
<td>Iron(^2)</td>
<td>0.92</td>
</tr>
<tr>
<td>Vitamin A(^2)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

1 Trimester 2 only.
2 Due to skewed values, log-transformed data shown.

5.1.2 Vitamin A intake during pregnancy (paper 2)
The median (25th-75th percentile) vitamin A intake in trimesters 1, 2 and 3 were 363 (211-595), 427 (232-653) and 430 (430-254) RE, respectively. The proportion consuming less than the Indonesian RDI of 700 RE for pregnant women was high in all trimesters; 83% in first trimester, 78% in second trimester and 76% in the third trimester.

Results from bivariate analyses of vitamin A intake (total as well as from different sources) by women’s education level are shown in table 11. In general, the proportion of vitamin A coming from animal foods and fortified sources increased with increasing maternal education and concurrently the proportion coming from vegetables decreased.

The logistic regression model for the risk of having a vitamin A intake below 370 RE (which included 44% of the women) in trimester 3 is shown in table 12. Univariate analyses showed an increased risk for low vitamin A intake with lower energy intake, as well as with a vitamin A intake below basal RDI in the second trimester. In addition, several socio-economic indicators were associated with an increased risk of low vitamin A intake (lower education level, having a house with a soil floor and not owning a motor bike). Also, a height in the lowest quartile (<146.5 cm) and parity of ≥3 children were
associated with a greater risk of a low vitamin A intake. Surprisingly, growing and consuming vegetables and owning chickens were not associated with vitamin A intake in either trimester. The association between vitamin A intake, and education level, total energy intake and having a vitamin A intake below basal RDI of 370 RE in the second trimester remained significant after adjusting for other factors. The same risk factors plus having no motorbike remained significant also in the second trimester.

Table 11. Vitamin A intake and proportion from fortified, animal and plant foods by mothers’ education levels and trimester

<table>
<thead>
<tr>
<th>Source (%)</th>
<th>Trimester/ Education level</th>
<th>Total intake (RE)¹</th>
<th>Animal</th>
<th>Fortified foods²</th>
<th>Fruits</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trimester 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-6 yrs (n = 82)³</td>
<td>289³ (174-506)</td>
<td>22</td>
<td>4³</td>
<td>14</td>
<td>60³</td>
</tr>
<tr>
<td></td>
<td>7-9 (n = 37)</td>
<td>307³ (176-459)</td>
<td>25</td>
<td>10³</td>
<td>16</td>
<td>49³</td>
</tr>
<tr>
<td></td>
<td>≥10 yrs (n = 33)</td>
<td>652³ (406-944)</td>
<td>22</td>
<td>15³</td>
<td>15</td>
<td>48³</td>
</tr>
<tr>
<td></td>
<td>Trimester 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-6 yrs (n = 250)</td>
<td>322³ (196-536)</td>
<td>19</td>
<td>3²</td>
<td>13</td>
<td>65³</td>
</tr>
<tr>
<td></td>
<td>7-9 (n = 99)</td>
<td>451³ (266-778)</td>
<td>24³</td>
<td>6³</td>
<td>16</td>
<td>54³</td>
</tr>
<tr>
<td></td>
<td>≥10 yrs (n = 99)</td>
<td>624³ (414-902)</td>
<td>26³</td>
<td>11³</td>
<td>13</td>
<td>50³</td>
</tr>
<tr>
<td></td>
<td>Trimester 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-6 yrs (n = 220)</td>
<td>353³ (209-602)</td>
<td>18³</td>
<td>3³</td>
<td>11</td>
<td>68³</td>
</tr>
<tr>
<td></td>
<td>7-9 (n = 99)</td>
<td>463³ (267-678)</td>
<td>26³</td>
<td>6³</td>
<td>12</td>
<td>56³</td>
</tr>
<tr>
<td></td>
<td>≥10 yrs (n = 100)</td>
<td>558³ (349-887)</td>
<td>25³</td>
<td>10³</td>
<td>11</td>
<td>54³</td>
</tr>
</tbody>
</table>

¹ Median (25th and 75th percentile)
² Also include supplements (number of women taking supplements containing vitamin A, n = 2, 4 and 9 in trimester 1-3, respectively).
³ Values with different superscript letters within a column and trimester are significantly different, P < 0.05 (ANOVA and Bonferroni post-hoc test for testing proportions and Kruskal-Wallis and Mann-Whitney U-test as post-hoc test for testing medians)
Table 12. Risk factors for a low vitamin A intake (<basal RDI, 370 RE) in trimester 3 (n=420)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>N</th>
<th>Level</th>
<th>Univariate analyses</th>
<th>Multivariate analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A intake &lt;370 RE in trimester 2</td>
<td></td>
<td></td>
<td>OR(^1) (95% CI(^2))</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Vitamin A intake 223 No 1</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin A intake 170 Yes 2.84 (1.88, 4.30)</td>
<td></td>
<td></td>
<td>2.16 (1.36, 3.42)</td>
<td></td>
</tr>
<tr>
<td>Energy intake in trimester 3 84 582-773 1</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Energy intake in quintiles (kJ) 84 523-581</td>
<td></td>
<td></td>
<td>1.88 (0.90, 3.94)</td>
<td></td>
</tr>
<tr>
<td>Energy intake in quintiles (kJ) 84 376-454</td>
<td></td>
<td></td>
<td>2.75 (1.31, 5.78)</td>
<td></td>
</tr>
<tr>
<td>Energy intake in quintiles (kJ) 84 lowest-375</td>
<td></td>
<td></td>
<td>4.75 (2.24, 10.10)</td>
<td></td>
</tr>
<tr>
<td>Height &gt; lowest quartile, 146.5 cm 318 Yes</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Height &gt; lowest quartile, 146.5 cm 101 No</td>
<td></td>
<td></td>
<td>1.64 (1.05, 2.57)</td>
<td></td>
</tr>
<tr>
<td>Parity 346 0-2 1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Parity 73 ≥ 3 2.36 (1.41, 3.97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education (years) 100 ≥ 10 1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Education (years) 99 7-9 1.53 (0.84, 2.76)</td>
<td></td>
<td></td>
<td>1.28 (0.64, 2.56)</td>
<td></td>
</tr>
<tr>
<td>Education (years) 220 0-6 2.83 (1.70, 4.70)</td>
<td></td>
<td></td>
<td>2.26 (1.16, 4.39)</td>
<td></td>
</tr>
<tr>
<td>Ownership of Motorbike 82 Yes 1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ownership of Motorbike 337 No 1.70 (1.03, 2.82)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of floor 130 Ceramic/tile 1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Type of floor 157 Cement/Brick 1.45 (0.90, 2.33)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of floor 132 Soil 1.66 (1.01, 2.71)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Odds ratio
\(^2\) Confidence interval
5.2 Vitamin A status

5.2.1 Helen Keller International Food Frequency Method (paper 3 and 4)

In rural Thakurgaon, Bangladesh, the mean nine-hour intake of breast milk for the group 12-23 months of age was 215g and at 24-36 months it was 123g (table 13). The mean suckling time in the younger and older age group was 79 and 65 minutes, respectively. The mean frequency of breast feeding was 6.8 and 5.7 times, respectively. Using the correction factors 1.33 x 9-hour and 12-hour/0.53, the estimated mean 24-hour intake would be 548 g for the 12-23 month-old group and 312 g for the 24- to 36-month-old group.

Vitamin A concentration in breast milk of deprived mothers of Bangladesh has been found to be 30 RE/100g. Then the total intake of vitamin A from breast milk given to the younger and older age group would be 164 RE/24-hour and 93 RE/24-hour, respectively, corresponding to approximately 40% and 20% of the safe RDI of 400 RE of vitamin A for each age group.

Table 13. Measured nine-hour breastmilk intake (g) according to age group (mean ± SD)

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Boys</th>
<th>Girls</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-23</td>
<td>216 ± 132</td>
<td>221 ± 134</td>
<td>218 ± 129a</td>
</tr>
<tr>
<td>24-36</td>
<td>136 ± 123</td>
<td>111 ± 91</td>
<td>124 ± 106</td>
</tr>
<tr>
<td>Total</td>
<td>176 ± 131</td>
<td>166 ± 125</td>
<td>171 ± 126</td>
</tr>
</tbody>
</table>

a. p = .015 (t-test)

Basic characteristics of the children in rural Pune, India, are shown in table 14. The mean (SD) age was 38 (16) months.

Table 14. Basic characteristics of the children in rural Pune

<table>
<thead>
<tr>
<th>Village</th>
<th>Number of children</th>
<th>Boys (%)</th>
<th>Girls (%)</th>
<th>Mean age (months) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rajewadi</td>
<td>48</td>
<td>26 (54.2)</td>
<td>22 (45.8)</td>
<td>30 ± 12</td>
</tr>
<tr>
<td>Shingapur</td>
<td>61</td>
<td>30 (49.2)</td>
<td>31 (50.8)</td>
<td>40 ± 18</td>
</tr>
<tr>
<td>Wagapur</td>
<td>43</td>
<td>20 (46.5)</td>
<td>23 (53.5)</td>
<td>42 ± 19</td>
</tr>
<tr>
<td>Malshiras</td>
<td>60</td>
<td>40 (66.7)</td>
<td>20 (33.3)</td>
<td>35 ± 13</td>
</tr>
<tr>
<td>Guroli</td>
<td>49</td>
<td>16 (32.7)</td>
<td>33 (67.3)</td>
<td>38 ± 15</td>
</tr>
<tr>
<td>Tekawadi</td>
<td>34</td>
<td>22 (64.7)</td>
<td>12 (35.3)</td>
<td>40 ± 19</td>
</tr>
<tr>
<td>Amble</td>
<td>50</td>
<td>27 (54.0)</td>
<td>23 (46.0)</td>
<td>40 ± 16</td>
</tr>
<tr>
<td>Total</td>
<td>345</td>
<td>181 (52.5)</td>
<td>164 (47.5)</td>
<td>38 ± 16</td>
</tr>
</tbody>
</table>

The mean frequency of intake of animal milk was 5.3 days per week and 67% of the children consumed milk every day during the last week. The highest consumption, 6.3 days/week, was found in the youngest age group, 1.0-2.0 years (table 15). The key informant interviews and group discussions suggested that mostly cow milk, but also buffalo milk was consumed.
Most people added two parts of water to five parts of milk, giving a mixture which was nearly 30% water. The fatty part of the milk is removed only on rare occasions when ghee (clarified butter) or curd is made. The amount of diluted milk consumed at one time is 50-200 ml, depending on how much milk is available within the household and the child’s desire.

Table 15. Milk consumption by age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number of children (%)</th>
<th>Mean intake ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-23</td>
<td>70 (20.3)</td>
<td>6.3*** ± 1.9</td>
</tr>
<tr>
<td>24-35</td>
<td>72 (20.9)</td>
<td>5.2 ± 2.9</td>
</tr>
<tr>
<td>36-47</td>
<td>83 (24.1)</td>
<td>5.4 ± 2.7</td>
</tr>
<tr>
<td>48-59</td>
<td>62 (18.0)</td>
<td>4.5 ± 2.9</td>
</tr>
<tr>
<td>60-72</td>
<td>58 (16.8)</td>
<td>4.8 ± 2.7</td>
</tr>
<tr>
<td>Total</td>
<td>345</td>
<td>5.2 ± 2.7</td>
</tr>
</tbody>
</table>

*** P = 0.001 (Mann-Whitney U-test)

The median fat concentration in the nine samples was 3.7% (range 2.4-6.6%). According to Indian Food Composition tables, whole milk contains 53 RE/100g when fat concentration is 4.1%, giving a vitamin A concentration of 48 RE, “as consumed” by the children. Assuming milk is consumed 5.3 days/week, the daily intake of vitamin A from diluted animal milk would then range from 18 to 72 RE, corresponding to 5-18% of the “safe” RDI (400 RE) of vitamin A for children 1-5 years old.

5.2.2 Relationships between vitamin A, iron and helminths in children (paper 5)

Basic characteristics of the children are shown in table 16. The mean serum retinol concentration was 26.7 µg/dl and 20% had a level <20 µg/dl. In addition, 31% were anaemic, 30% suffered from iron deficiency and 14% from iron deficiency anaemia. There was a strong positive association between serum β-carotene and serum retinol (r=0.44, p<0.001).

Children with serum retinol concentrations >30 µg/dl had significantly higher mean haemoglobin and median serum ferritin concentrations than had children with serum retinol levels <20 µg/dl (P<0.01, table 17). No association was found between severity of infection with *Ascaris lumbricoides* and the levels of serum retinol, but those who were positive for hookworm had significantly lower haemoglobin (p<0.05) and ferritin levels (p<0.01). Multiple regression analyses showed that vitamin A and hookworm had a strong and independent association with log-transformed serum ferritin status. However, there were no evidence that hookworm had a greater impact on iron status among children with lower vitamin A status (interaction term hookworm x serum retinol, P>0.05).
Table 16. Anthropometric and biochemical measures and helminth infections in 164 Bangladeshi school children

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>25-75 Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>131.9 ± 8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>25.5 ± 5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/H (Z-score)*</td>
<td>-1.27 ± 0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biochemical†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>12.1 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>20.5</td>
<td>11-32</td>
<td></td>
</tr>
<tr>
<td>Retinol (µg/dL)</td>
<td>26.7 ± 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-carotene (µg/dL)</td>
<td>4.9</td>
<td>3.4-7.9</td>
<td></td>
</tr>
<tr>
<td><strong>Helminths</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascaris (eggs/g)</td>
<td>1600</td>
<td>600-4450</td>
<td></td>
</tr>
<tr>
<td>Hookworm (eggs/g)</td>
<td>0</td>
<td>0-250</td>
<td></td>
</tr>
</tbody>
</table>

* n=136
† n=159

Even though children with elevated CRP levels (>10 µg/L) were excluded, children reporting fever in the past week had a significantly higher serum ferritin than those not reporting fever, whereas haemoglobin concentrations were the same. In addition there was a non-significant trend (P=0.09) towards lower serum retinol values for those reporting fever, compared to those not reporting it.

Table 17. Biochemical, anthropometric and helminthic measures by serum retinol concentration

<table>
<thead>
<tr>
<th>Serum Retinol Concentration</th>
<th>&lt;20 µgram/dl (n=33)</th>
<th>20-30 µgram/dl (n=79)</th>
<th>&gt;30 µgram/dl (n=49)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.7</td>
<td>12.0</td>
<td>12.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>18.3</td>
<td>25.3</td>
<td>27.8</td>
<td>0.033</td>
</tr>
<tr>
<td>Weight</td>
<td>24.4</td>
<td>25.1</td>
<td>27.2</td>
<td>0.052</td>
</tr>
<tr>
<td>Height</td>
<td>129.3</td>
<td>131.7</td>
<td>134.2</td>
<td>0.048</td>
</tr>
<tr>
<td>W/H</td>
<td>-1.38</td>
<td>-1.33</td>
<td>-1.01</td>
<td>0.10</td>
</tr>
<tr>
<td>Ascaris (eggs/g)</td>
<td>4364</td>
<td>5233</td>
<td>2571</td>
<td>0.19</td>
</tr>
<tr>
<td>Hookworm (eggs/g)</td>
<td>206</td>
<td>253</td>
<td>135</td>
<td>0.29</td>
</tr>
<tr>
<td>Meat intake (servings/week)</td>
<td>1.28</td>
<td>2.43</td>
<td>2.15</td>
<td>0.12</td>
</tr>
</tbody>
</table>

5.3 Vitamin A improvement

5.3.1 The effect of DGLVs on vitamin A status in primary school children (paper 6)

Some characteristics of the treatment groups at baseline are shown in Table 18. Among those infected with *Ascaris lumbricoides*, the egg load was 3800 epg in
the DGLV group compared to 2200 epg in the sweet pumpkin and 1800 epg control groups (P=0.08). However, since the cure rate was nearly complete (see below) all groups entered the trial basically ascaris-free. The mean hemoglobin at baseline in the DGLV group was 117 g/L compared to 122 and 120 g/L in the sweet pumpkin and control groups, respectively (P=0.06).

Serum concentrations of β-carotene and retinol at baseline are shown in table 19. Significant increases (P<0.001) in mean serum β-carotene concentrations were seen in all three study groups, with a statistically higher increase (µmol/L) in the DGLV group (0.44, 95% CI: 0.32, 0.55) compared to the control group (0.20, 0.14, 0.26) (P = 0.002).

Table 18. Basic characteristics of the treatment groups at baseline

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>DGLV&lt;sup&gt;a&lt;/sup&gt; (n=37)</th>
<th>Sweet pumpkin (n=36)</th>
<th>Control (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys (n)</td>
<td>18</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Girls (n)</td>
<td>19</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Age (y)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.2 ± 1.3</td>
<td>9.4 ± 1.1</td>
<td>9.2 ± 1.1</td>
</tr>
<tr>
<td>Height (cm)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>131.4 ± 9.8</td>
<td>131.5 ± 8.3</td>
<td>131.0 ± 9.1</td>
</tr>
<tr>
<td>Weight (kg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.6 ± 6.3</td>
<td>25.4 ± 6.1</td>
<td>24.9 ± 5.6</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>117</td>
<td>122</td>
<td>120</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parasitic infection (% with a positive stool)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris</td>
</tr>
<tr>
<td>Hookworm</td>
</tr>
<tr>
<td>No infection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Egg load (epg)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris</td>
</tr>
<tr>
<td>Hook worm</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dark green leafy vegetables  
<sup>b</sup> Mean ± SD. Ages were often estimates.  
<sup>c</sup> Eggs per gram faeces for those with the infection, median (25<sup>th</sup>-75<sup>th</sup> percentile).

The increase in serum retinol (µmol/L) was statistically significant (P = 0.04) only in the DGLV group (mean: 0.066; 95% CI: 0.002, 0.13), but this increase was not statistically different from the increase in the control group (P=0.17, ANOVA). Multiple regression analyses on change in serum retinol concentration, with baseline serum retinol, serum β-carotene, Ascaris lumbricoides-load, weight, and weight change controlled for, did not alter this finding of no difference.
Table 19. Changes in serum β-carotene and serum retinol from baseline (µmol/L) by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Treatment group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DGLV (n=37)</td>
<td>Sweet pumpkin (n=36)</td>
<td>Control (n=37)</td>
<td></td>
</tr>
<tr>
<td>β-carotene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial (^a)</td>
<td>0.18 (0.14-0.28)</td>
<td>0.16 (0.13-0.32)</td>
<td>0.17 (0.098-0.29)</td>
<td></td>
</tr>
<tr>
<td>Change (^b)</td>
<td>0.44 (0.32, 0.55)(^c)</td>
<td>0.32 (0.22, 0.42)(^cd)</td>
<td>0.20 (0.14, 0.26)(^d)</td>
<td></td>
</tr>
<tr>
<td>Retinol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial (^e)</td>
<td>0.85 ± 0.25</td>
<td>0.94 ± 0.21</td>
<td>0.94 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Change (^b)</td>
<td>0.066 (0.002, 0.13)</td>
<td>0.030 (-0.035, 0.094)</td>
<td>-0.017 (-0.07, 0.035)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Median and (25th-75\(^m\) percentile)
\(^b\) Mean; 95% CI in brackets
\(^cd\) Means with different superscript letters are significantly different from each other, P = 0.002 (ANOVA, Bonferroni post hoc test).
\(^e\) Mean ± SD
6. DISCUSSION

6.1 Vitamin A intake (papers 1 and 2)

6.1.1 Methodological aspects of dietary assessment during pregnancy in a developing country (paper 1)

A major finding of this population-based study was that the intra/inter variance ratios were <1.0 for energy and carbohydrates in all three trimesters. However, for all other nutrients they were >1.0 (except for protein in the first trimester). The largest variance ratios were found for the micronutrients, and for vitamin A it was in the order of three. This means that micronutrients are measured with greater error and it will be more difficult to discover associations between micronutrient intake and certain outcomes than is the case for macronutrients.

However, if the purpose of a study would have been to estimate mean intake of individuals with a precision of ± 20% of the true intake, it showed that the current number of replicates (six) per individual would be sufficient not only for energy and carbohydrates, but also for iron and vitamin A. For most nutrients, a tendency towards larger CVw in the first trimester was seen. This could be because the intra-individual variation was largest in the first trimester for the majority of nutrients. The reason behind may have been the fact that many women in the first trimester felt nausea and had a lower nutrient intake.

We only accounted for intra- and inter-variation in nutrient intake. The magnitude of other errors, e.g. nutrient composition data, computer program and errors in recording was not examined. To minimise errors in the nutrient composition data efforts were done to accurately assess nutrient content of foods; details on exact recipes were collected for each woman’s intake. In total over 11,000 local recipes for mixed dishes were collected during the study. Additional examinations of foods not analysed previously were also conducted. In the case of vitamin A, these values were mainly taken from the food composition table of de Pee and Bloem (1999), instead of the Indonesian food composition tables, since the vitamin A values in the former one mainly are based on HPLC analyses, which separate individual carotenoids, compared to spectrophotometric analysis, which gave one value for total carotene. The latter is largely overestimating vitamin A content of plant foods (de Pee, 1996). To validate the Inafood program, total dietary intake of the macronutrients was calculated manually from the nutrient composition tables for four dietary recalls by students at the Nutrition Academy. For energy, complete agreement was obtained. For protein, fat and carbohydrates, differences (expressed as percentage of computed total) were in the ranges of 1-15%, 0-5% and 0-3%, respectively. To minimise errors in recording, data form editing was conducted in the field within a few days and supervisors checked all interviewers periodically. A random sample of 5% of the interviews was repeated later the same day for quality control.
Intra-individual variance may consist of several different components. These include day of the week effect (Hankin et al., 1967; Beaton et al., 1979; McGee et al., 1982; Hartman et al., 1990), sequence of observation (Beaton et al., 1979; Hartman et al., 1990), and seasonal differences (Hartman et al., 1990) and method of data collection (Tarasuk and Beaton, 1991). To minimise the day of the week effect, we included all five days in the Javanese calendar, accounting for market days when dietary habits may change. The variation due to interview occasion was partitioned from the total intra-variance, but was found to be <5% of the total intra variance. The economic crisis in Indonesia, which started approximately in October 1997, could also have contributed to variation in intake. However, we compared the variance ratios of women who completed their pregnancy before the crisis (approximately 50%) with those after, and found them to be quite similar. We did not investigate the effect of seasonality in this study. However, the effect on macronutrient intake in this area is likely to be small.

We are only aware of one other study that has evaluated variance components of pregnant women from the developing world, i.e. Launer and colleagues (1991) from East Java, Indonesia. Their results are derived from months 6-9 in pregnancy among 743 women based on a 3-consecutive-day food weighing method. Though error may be greater (towards underreporting) in measuring individual intake with the 24-h recall method than with the direct weighing method (Block, 1982), recalls are cheaper and simpler, making the method more suitable in larger studies. Further, recent studies (Harrison et al., 2000) including also our women (Winkvist et al., 2001) suggest that underreporting is less of a problem in developing countries. It may also be easier with the 24-h recall method to assess non-consecutive days, which gives a better estimate of the variance ratio (Block, 1982; Tarasuk and Beaton, 1991). Finally, the 24-h recall method does not require literate respondents and is considered suitable for measuring change over time (Tarasuk and Beaton, 1992).

Comparison of results should be made with caution; our study and Launer and colleagues’ used different methods and were done in different areas of Central Java 14 years apart. The socio-economic situation has probably improved, reflected in the higher energy and fat intakes seen among our women. For protein and vitamin A, the variance ratios are comparable (ranging from 0.82-1.50 and 3.12-3.44, respectively for the three trimesters in our study, compared to 1.28 and 3.8, in their study). However, for energy and fat, ours were lower (0.57-0.78 and 1.30-1.50, respectively, compared to 1.35 and 3.24 in theirs). In contrast, the CVw for protein and vitamin A in our study was slightly higher (0.31-0.35 and 0.21-0.24, respectively, compared to 0.25 and 0.19 in their study), but for energy it was similar (0.20-0.26, compared to 0.24 in their study). Similar studies available from Western countries on pregnant women are generally smaller (n = 60-225) and include fewer days than our study and
Launer and co-workers’ from Indonesia. Their intra/inter-variance ratios for energy (1.14-1.4), carbohydrates (1.18-1.2) (Osofsky, 1975; Rush and Kristal, 1982; Nelson et al., 1989) and vitamin A (4.9) (Nelson et al., 1989) were all higher than ours. For protein they were either similar (1.38) (Rush and Kristal, 1982) or higher (1.7-1.9) (Osofsky, 1975; Nelson et al., 1989).

The reasons behind the mostly lower variance component ratios seen in our study could be that in developing countries diets tend to be more monotonous and what people eat is more linked to income than in Western countries, so that even small differences in income are directly reflected in diet. This linkage would tend to increase between-person variation (Willett, 1990), a hypothesis supported by data from India (Hebert et al., 2000).

**6.1.2 Vitamin A intake during pregnancy**

A major finding from our population-based study of women followed throughout pregnancy was that the majority of these Central Java women consumed insufficient amounts of vitamin A during pregnancy. The proportion of women below the Indonesian RDI of 700 RE ranged from 83% in the first trimester to 76% in the last trimester and 58% were below in all three trimesters. Given that the majority of vitamin A came from plant sources, the proportions consuming insufficient vitamin A would have been even greater if we had used the newly suggested conversion factors of 12 and 26 for beta-carotene derived from fruits and vegetables (de Pee et al., 1998; Tang et al., 1999), respectively. In addition, a study by Nurdiati and colleagues (2001) on the same women showed that the level of iron deficiency and iron deficiency anaemia was rather high, 50% and 19%, respectively, in the last trimester. Most pregnant women (70%) were also infected with at least one species of intestinal helminths (61% with *Trichiuris trichiura*, 37% with hookworm and 29% with *Ascaris lumbricoides*. Given the close interrelationship between the status of vitamin A, iron and helminths, this is of great concern for the women’s own health as well as for that of their offspring.

It could be that part of the low vitamin A intake identified was due to underreporting. However, this is not likely since the levels of under-reporting of energy intake among these pregnant women were low. For the three trimesters, ratios of mean energy intake : basal metabolic rate were 1.33, 1.53 and 1.52, respectively and the proportions of women classified as under-reporters were 29.7%, 16.2% and 17.6%, respectively, when level of physical activity as estimated from occupation was taken into account. An inadequate dietary intake due to reported nausea may explain the high proportion of under-reporters in the first trimester (Winkvist et al., 2001). We have no reason to believe that there would be selective under-reporting of vitamin A compared to energy.
A common custom in developing countries is to reduce food intake during pregnancy, especially in the last trimester (Kusin et al., 1984; Djazayery et al., 1992; Hutter, 1996). The reason given for this is to have a small child and thus an easy delivery. However, we did not observe any strong tendency to “eat down” during pregnancy, irrespective of education level.

The possible impact on women’s vitamin A intake of the economic crisis in Indonesia, which started in approximately September 1997, was not explored in this thesis. Preliminary findings show that the mean intake of vitamin A for the whole group did not change over the study period. However, for certain subgroups, such as the rural landless and urban poor, there was a non-significant trend towards a lower intake of vitamin A during the crisis (Th NS Hartini, personal communication).

**Riskfaktors for a low vitamin A intake during pregnancy:**

Women of higher education had a higher total intake of vitamin A, a higher vitamin A density, a higher food intake, a higher intake of vitamin A from animal sources, and they consumed more than thrice the fortified vitamin A of those of lower education (10-15% compared to 3-4%). In addition, the consumption of fortified noodles (the major source of fortified vitamin A in this population) was related to education level. Fortified noodles are slightly more expensive than unfortified noodles. In other parts of Indonesia (South Sulawesi and South Kalimantan) the consumption of noodles fortified with vitamin A has also been shown to be related to socio-economic status (Melse-Boonstra et al., 2000). Further, de Pee and co-workers (de Pee et al., 1998) in their study in Central Java also found that vitamin A intake from animal sources increased with education.

In this study, no association was found between vitamin A intake and ownership of a home garden, which in earlier studies in Indonesia and Bangladesh been associated with a higher intake of vitamin A and a better vitamin A status (de Pee et al., 1998; HKI and IPHN, 1999). However, the indicator a household garden is rather insensitive, since many of the vegetables grown may have been low in carotene or grown in small quantities. Similarly, ownership of chickens, which one could assume would lead to a higher consumption of eggs, was also not related to vitamin A intake. A social marketing campaign to increase egg consumption in Central Java found that most people purchased the eggs that they consumed (de Pee et al., 1998).

Not surprisingly, factors associated with low socio-economic status were related to a particularly low vitamin A intake (< basal FAO/WHO RDI, 370 RE). Having a low education level and not owning a motorbike, as well as a low energy intake and a low vitamin A intake in the previous trimester were all associated with an increased risk of a vitamin A intake below this RDI. A
similar but non-significant trend was seen also for the other socio-economic variables (housing conditions, television ownership).

6.1.3 Implications and recommendations from the study in Indonesia

Our population-based study using the 24-h recall method on women followed throughout pregnancy has generated similar results with a population-based study on pregnant women conducted in the same country using the more accurate weighing method. This has several implications for future research.

It suggests that the usual individual mean intake of several nutrients among pregnant women can be reliably measured using the more practical 24-h recall method. However, for vitamin A at least six replicate measurements are needed.

Secondly, our data on attenuation of the simple regression coefficients of the micronutrients suggest that six replicate 24-h recalls is not enough to find associations, for example between vitamin A intake and vitamin A status, since the observed regression coefficient would be attenuated almost 35%. However, for the macronutrients it should be possible to use dietary intake data from 24-h recalls during pregnancy when evaluating the effect of diet on different outcomes of pregnancy.

Thirdly, our variance component ratios for macronutrients and vitamin A were generally lower than those reported for pregnant women in the industrialised world (Osofsky, 1975; Rush and Kristal, 1982; Nelson et al., 1989). Thus, findings on intra/inter-individual variance derived from the western world should not be generalised to populations in low-income countries.

Fourthly, in our analyses we evaluated the effect of using different numbers of replications on CVw and regression coefficients. It is also possible to increase the sample size when the primary objective of a study is to detect differences between groups of individuals. However, Freudenheim and co-workers (1989) showed that for weaker underlying associations, non-differential misclassification due to intra-individual variability induced bias in the odds ratio that would persist, regardless of sample size.

Fifthly, the nutrient of interest, the primary objectives of the study and the method of analysis to be used should all be taken into account when planning the sample size and number of replicate measures when using the 24-hour recall method. For example, if only intake of vitamin A is of interest, a rather new and simple semi-quantitative 24-h recall method (De Pee and Bloem, 1999) has shown a good correlation between vitamin A intake and serum retinol and may be more suitable.

Given the high proportions of women below the Indonesian RDI for vitamin A in all three trimesters, actions need to be taken to increase vitamin A intake
among all pregnant women, rather than targeted interventions. An earlier campaign (1996) in Central Java, which promoted both eggs and DGLVs was found to be successful in increasing both vitamin A intake and vitamin A status. The latter was linked to both egg consumption and total intake of vitamin A (de Pee et al., 1998). However, there may be a need also for strategies less prone to economic fluctuations, since the expected impact of a similar campaign in other parts of Indonesia was very small due to the economic crisis (Melse-Boonstra et al., 2000). Our results and Melse-Boonstra and co-workers’ show that the foods currently fortified with vitamin A by some manufacturers do not benefit women of lower socio-economic status. Thus we support Melse-Boonstra and co-workers’ recommendation to fortify a food item not related to socio-economic status, such as salt, sugar or mono-sodium-glutamate. Fortification as a strategy to improve the intake of multiple micronutrients may in the near future be implemented in Indonesia (MOH/WHO, 2000). Another important public health strategy would be to provide anthelminthic therapy to infected women in the latter half of pregnancy (de Silva et al., 1999).

6.2 Vitamin A status

6.2.1 Helen Keller International Food Frequency Method (papers 3 and 4)
The aim of these two studies was to find out whether breastmilk and animal milk, as given to children aged one to six years old, contributed significantly to their total vitamin A intake in Bangladesh and India, respectively.

In the study in Thakurgaon, Bangladesh, the mean intake of breastmilk was about 550 g in the 12-23 month age group and 300 g in the 24-36 month age group. Our results are similar to the results of another study in Bangladesh by Brown and colleagues (1982). They estimated 24-hour breastmilk intake to be 563, 501 and 368 g in the age groups 12-17, 18-23 and ≥24 months, respectively. Our estimated levels are also similar to Jelliffe and Jelliffe’s (1978) estimates for poorly nourished populations of 300-500 ml/day at 12-23 months and 270-350 ml/day for children over 24 months. Women in Bangladesh are among the most malnourished in the world with 56% of those of child-bearing age have a body mass index less than 18.5 (Institute of Nutrition and Food Science et al., 1998). However, breastmilk production is only slightly affected by malnutrition. Thus, our results may be generalised to most of South Asia where breastfeeding is common among older children (Greiner, 1999).

We estimated the daily vitamin A intake from breast milk in the younger and older age group to be about 160 and 90 RE/day, respectively, corresponding to approximately 80% and 40% of the basal RDI for vitamin A of 200 RE. This is equivalent to about 25% of the basal RDI for the entire age group one to six years of age covered by the HKI FFM, taking into account these actual percentage being breastfed. In Gaibandah and other districts located in the same
Vitamin A intake, status and improvement using the dietary approach

region as Thakurgaon, 97% of children 12 to 23 months of age, 73% of those 24 to 35 months of age, 32% of those 36 to 47 months of age, and 10% of those 48-59 months of age were still being breastfed (Greiner, 1997), it would then correspond to approximately 10% of the basal RDI for the entire one to six year old age group (Greiner, 1999).

In our study, dietary intake was not assessed and thus the contribution of breastmilk to the total vitamin A intake could not be calculated. However, in other studies in Bangladesh, Brown and colleagues (1982) estimated it to be more than 70% in children 18-30 months of age. Zeitlin and colleagues (1992) estimated that when breastmilk was included, overall dietary intakes for children 25-27 months of age came close to the RDI of 300 RE. They concluded that the only other significant source of vitamin A was seasonally available mangoes.

The frequency of consumption of milk was found to be high among the pre-school children studied in rural India with 67% of them consuming milk every day. In addition the amount of milk consumed per day was rather substantial, ranging from 50-200 ml. The question of dilution was a bit complicated. It may be that people with very few milk animals dilute the milk more. However, all group discussions and key informant interviews led us to believe that the usual level of dilution was one glass of water to one bowl of milk, leading to a product that consist of about 30% water before boiling. However, the fat concentration of the boiled milk was found to be restored to approximately the original fat content of 4%. The effect of boiling on vitamin A has been reported to be low (Renner, 1989). It is also not likely that vitamin A is lost due to light exposure since cooking is performed inside the houses in this area. Thus, given the mean frequency of consumption of 5.3 days per week plus the rather high fat content of the “diluted milk”, the estimated intake of vitamin A from animal milk would range from 18 to 72 RE, depending on the volume consumed, corresponding to 10-36% of the “basal” RDI (200 RE) of vitamin A for children 1-5 years old.

However, it may be that our study population was slightly skewed and that the actual proportion of lower caste people was higher than the 8% in this sample. For example, some might have avoided some of the poorer low-caste people. This could have been caused by the way interviewers chose houses to visit. Then, our estimate of milk intake may be somewhat overestimated for this area.

6.2.2 Relationships between vitamin A, iron and helminths in children (paper 5)

The study found that 20% of the Bangladeshi school children studied were below 20 µgram/dl for serum retinol, a cut-off value defined as “low vitamin A status” and an additional 50% were below 30 µgram/dl and thus at risk. In addition, 31% were anaemic, 30% suffered from iron deficiency and 14% from
iron deficiency anaemia. However, in this study, we only included children in grades 3-5 from two governmental schools, and purposely included all those with intestinal parasites in the sample. These children are thus likely to be worse off than the average school child in the parameters measured. However, school-going children may have a higher nutritional status than those not attending school. In this part of Bangladesh (rural Rajshahi division), 76% of the girls and 78% of the boys aged 6-10 years enrol in school but only approximately 40% eventually reach grade 5 (Bangladesh Bureau of Statistics and UNICEF, 1995). In addition, selection bias may explain similarity in nutritional status between boys and girls, with boys from slightly better-off families being more often sent to private schools.

A study of 242 school children in urban Dhaka, aged 5-12 years found that about 20% of the children had serum retinol levels less than 30 µgram/dl and 4% had less than 20 µgram/dl (Ahmed et al., 1993), which suggests that our study population was more nutritionally deprived. However, the mean serum retinol value of 26.7 µgram/dl found in our study was similar to that seen in a community-based sample of children aged 9-15 years in Rangpur District, where the mean serum retinol levels of children with (n=22) and without (n=21) night blindness were 25.2 µgram/dl and 29.2 µgram/dl, respectively (Hussain and Kvåle, 1996). Nineteen percent of the children in our study reported being night blind. We did not confirm this, but, similar to the case in Rangpur, XN children had significantly lower serum retinol values. The correlation between serum retinol and beta-carotene was relatively high (r=0.44, P < 0.001). Thus, children with a higher serum retinol concentration probably had a higher carotene intake and/or better absorption or bioconversion.

The absence of association between severity of Ascaris lumbricoides and serum levels of retinol could be due to the low burden of Ascaris lumbricoides among the children studied, plus the fact that children were dewormed two weeks prior to the blood collection. Similar to others (Bloem et al., 1989; Ahmed et al., 1993; Wolde-Gebriel et al., 1993; Suharno and Muhilal, 1996), we found a clear association between serum vitamin A concentrations and iron status. Children with low vitamin A status had significantly reduced ferritin and haemoglobin levels. Vitamin A and hookworm had strong and independent associations with serum ferritin concentrations, and no interaction between the two was seen.

CRP is probably the most commonly used acute phase protein for monitoring infection and inflammatory activity. In particular, it is useful in assessing bacterial and connective tissue infection (Young et al., 1991). However, even though we excluded those with elevated CRP levels (>10 µg/L), children reporting fever in the past week had a significantly higher serum ferritin than those not reporting fever, whereas haemoglobin concentrations were the same. In addition there was a non-significant trend (P=0.09) towards lower serum retinol values for those reporting fever compared to those not reporting it. CRP
concentrations may not remain abnormal long enough to detect patients in early convalescence (Young et al., 1991) and thus it may not be useful in nutritional studies of apparently healthy individuals.

6.2.3 Recommendations
Breast milk is an important source of vitamin A even in the second and third years of life in rural areas of Bangladesh and India (Greiner, 1999). Similarly, animal milk is likely to be an important source of vitamin A among preschoolers in certain areas of India (Persson et al., 1999; Granat, 2001). Thus, we recommend that breastmilk and animal milk are taken into account in rapid dietary assessments in settings where breastfeeding is commonly sustained longer than one year, and where animal milk is a normal part of young child diet.

This could be done by including breastmilk and animal milk in the category “animal food”. However, level of dilution of animal milk should be taken into account, since milk diluted to a large extent would contribute very little to children’s vitamin A intake. Another option might be to assume that children one to two years of age in Bangladesh and perhaps also India are largely protected from clinical VAD by breastfeeding and/or animal milk and to use HKI FFM on the two to six year old age group. Then breastmilk will have less overall importance as a source of vitamin A and can be more safely ignored. However, this younger age group is very vulnerable from a nutritional point of view. It may therefore be of importance to collect information on their feeding practices to help formulate potential interventions. However, the scales used in HKI FFM may need to be revalidated against serum retinol concentrations to determine the sensitivity and specificity in either case.

The sampling method recommended (Rosen et al., 1993) to randomly select households may not be appropriate in all countries. Firstly, it suggests to randomly select 15 communities from an area, and secondly, to randomly select 50 households with a child 1-6 years old from each community. However, in future studies in India, it would probably be more useful to stratify for caste rather than for village in settings where people of very different socio-economic status (i.e. caste group) live in the same villages but segregated. This may prevent interviewers from avoiding low-caste households.

More attention should be given to primary school children in Bangladesh in planning micronutrient interventions. Programs designed to reduce anaemia should also include efforts to improve vitamin A status (Bloem, 1995). Examples of programs which could be targeted to this age group are nutrition and hygiene education, school demonstration gardens, and school-based deworming programs. Albendazole might be the preferable anthelminthic drug as it has a better cure rate (57-100%) for hookworm than Mebendazole (22-30%) (WHO, 1996).
It may be better to use other acute phase proteins that remain elevated for a longer time than does CRP to detect children in whom infection has subsided but whose retinol concentrations may still be reduced. \(\alpha\)-acid glycoprotein (AGP) and \(\alpha\)-Antichymotrypsin (ACT) are two such proteins (Thompson et al., 1992). Reports from the Gambia (Northrop-Clewes et al., 1994), Bangladesh (Rousham et al., 1998) and Pakistan (Paracha et al., 2000) suggest that these indicators may be useful indicators of subclinical, chronic or recent disease. \(\alpha\)-acid glycoprotein has also been found to be useful as an indicator of malaria-induced hyporetinemia (Rosales et al., 2000). To correct for the increase in serum ferritin concentrations following infections, suggestions have been made to use a higher cut-off value for serum ferritin in populations where infections/inflammatory diseases are highly prevalent (Punnonen et al., 1997; Mast et al., 1998), but an exact cut-off level for serum ferritin has not been agreed upon. Suggestions have also been made to use transferrin receptor (TfR) as a complement to serum ferritin and CRP (Punnonen et al., 1997; Mast et al., 1998). TfR increases in iron deficiency and is thought to reliably reflect the degree of tissue iron supply (Cook et al., 1993). By using all three indicators it would be possible to find out whether anaemia is due to iron deficiency or due to other causes such as chronic disease.

6.3 Vitamin A improvement (paper 6)

6.3.1 The effect of DGLVs on vitamin A status in primary school children
We examined the effect of supplementary feeding on serum retinol and \(\beta\)-carotene concentrations in three groups of school children previously treated with Mebendazole, two of whom received \(\beta\)-carotene - either DGLV or sweet pumpkin. The major finding was that \(\beta\)-carotene concentrations increased significantly in all groups, with a statistically higher increase in the group receiving DGLV compared to the control group. However, the increase in serum retinol concentration was significant only in the DGLV group (P=0.04), and not different from the increases in the other groups. The small impact on serum retinol may have been due to the relatively high baseline serum retinol concentration and the relatively low cumulative dose of \(\beta\)-carotene provided, particularly to the pumpkin group.

de Pee and co-workers (1998) have shown that beta-carotene from YOFV is more bioavailable than beta-carotene from DGLV. In our study, we could not replicate their findings, since the dose of beta-carotene actually supplied to the sweet pumpkin group was eventually too low to make it an effective experimental group. However, the increases in serum levels of retinol and \(\beta\)-carotene were similar in the DGLV group and the sweet pumpkin group, although the cumulative dose of \(\beta\)-carotene given to the children in the sweet pumpkin group was only 34% of that given to the DGLV group. Thus, our
findings do not contradict de Pee and colleagues on the likely higher biavailability of YOFV.

6.3.2 Relation to other studies
Studies which have measured the impact of DGLV and/or carrot supplementation on vitamin A status, with or without deworming are listed in table 20. The criteria for inclusion in table 20 (apart from feeding the subjects vegetables) were: 1) a negative control group included, 2) both baseline and endpoint data available and 3) a sample size greater than 20. All but Takyi (1999) and Ncube and co-workers (2001) also provide data on serum β-carotene. The fat content per weight of the vegetable-stew was approximately the same in the studies, 8-10 gram/100 gram. However, the cumulative dose of β-carotene from vegetables varied, with the highest dose (60,000 RE) in Ncube and co-workers’ study on lactating women, and the lowest dose (25,700 RE) in ours. However, the amount of provitamin A carotenoids provided may differ more than table 20 suggests, due to differences in analytical methods used.

The role of intestinal helminths:
Perhaps one of the most important differences explaining the discrepancies in outcome between the studies may be the type of helminths and their intensity. The explanation may be reduced absorption of β-carotene in helminth-infected subjects, since a negative trend can be seen between *Ascaris lumbricoides* load and net increase in serum β-carotene, with the largest net increase taking place in Tang and co-workers (1999) study (0.27 µmol/L) and our study (0.23 µmol/L), both with *Ascaris*-free children.

Vitamin A status:
Another likely explanation for the discrepancies in the net changes in mean serum retinol concentration among the studies with subjects who were not dewormed prior to the feeding trial (de Pee et al., 1995; de Pee et al., 1998; Takyi, 1999; Ncube et al., 2001), could be differences in baseline serum retinol levels. One of the smallest increases (0.07) was seen among Tang and co-workers subjects who had the highest baseline vitamin A status. However, their study points out the importance of also taking into account the change in the control group (-0.26 µmol/L), leading to the net increase in serum retinol of 0.33 µmol/L presented in table 20. One of the largest increases (0.13 µmol/L) was seen among Takyi’s subjects, who also had the lowest baseline retinol. In our study, among participants with deficient/marginal serum retinol (<0.70 µmol/L) the serum concentration rose, 0.12 µmol/L in the DGLV group (N=10), 0.084 µmol/L in the sweet pumpkin group (N=7) and 0.063 µmol/L in the control group (N=6). With this small sample size, the improvement in the DGLV and control group did not differ from one another, and part of the seeming increase may be regression to the mean (data not shown).
Method of preparation:
The method of preparation of the vegetables differs among the studies and may be another reason for the different outcomes. In Takyi’s (1999) study the vegetables were pounded and homogenised in a blender before preparing the stews and thus the cells in the DGLV were well disrupted to liberate the β-carotene. In Ncube et al’s study, the carrots were grated before eating them. This could possibly make the β-carotene more bioavailable than in our study and the studies by de Pee and co-workers (1995), where the vegetables were simply stir-fried, the way they commonly are when prepared at home.

6.3.3 Lessons learned and recommendations

The role of intestinal helminths:
To further elucidate the role of helminths in the bioavailability of β-carotene from plant sources, a feeding trial with both DGLV and YOFV, would need to be done that randomises subjects for deworming. This could reveal whether or not the negative impact of Ascaris lumbricoides is greater on the bioavailability of provitamin A from vegetables compared to YOFV. However, there is already enough evidence to indicate a role of hookworm in iron deficiency anaemia (WHO, 1994). Other apparent consequences of intestinal parasite infection (hookworm, Ascaris lumbricoides and Trichuris trichiura) include reduced appetite, impaired absorption of other nutrients (WHO, 1994) and reduced growth (Gupta et al., 1977; Stephenson et al., 1993; Stoltzfus et al., 1997). Thus, periodic deworming should always be considered in areas where intestinal parasites are endemic. In addition, a positive control group, receiving the same amounts of beta-carotene either as purified β-carotene or retinol, would be useful, for example in case baseline serum retinol concentrations are too high to be improved by additional vitamin A.

Which source of vitamin A should be promoted?:
It is often said that plant sources are the only options for the poorer people in low-income countries. However, there are cases where increased consumption of animal foods was successfully promoted. In Indonesia, both egg and DGLV consumption increased following a social marketing campaign promoting an increased consumption of these foods (de Pee et al., 1998). Vietnam, a country which have been successful in eliminating xerophthalmia, included not only agriculture in their food-based approach to prevent micronutrient malnutrition in poor areas, but also intensive exploitation of water areas and husbandry activities, including raising poultry, pigs, cattle and even wild animals (Giay and Ngu, 2001).
Table 20  Summary of studies on the impact of vegetable feeding on serum retinol and β-carotene

<table>
<thead>
<tr>
<th>Study</th>
<th>Age (years) of subjects</th>
<th>Cumulative dose of B-C from DGLV (RE)</th>
<th>Baseline retinol</th>
<th>Net increase&lt;sup&gt;a&lt;/sup&gt; in serum-retinol</th>
<th>Net increase&lt;sup&gt;a&lt;/sup&gt; in serum β-carotene</th>
<th>De-worming</th>
<th>Ascaris load (epg)</th>
<th>Fat content of the suppl. meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Pee (1995)</td>
<td>lactating women</td>
<td>49,000</td>
<td>0.81-0.89</td>
<td>0.05</td>
<td>0.05</td>
<td>No</td>
<td>13,020</td>
<td>8g/port.&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>de Pee (1998)</td>
<td>7-11</td>
<td>36,900</td>
<td>0.69-0.73</td>
<td>0.07</td>
<td>0.11</td>
<td>No</td>
<td>4,720</td>
<td>c</td>
</tr>
<tr>
<td>Takyi (1999)</td>
<td>2.5-6</td>
<td>33,600</td>
<td>0.56-0.58</td>
<td>0.20/0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>no data</td>
<td>Yes/No</td>
<td>10g/100g</td>
<td></td>
</tr>
<tr>
<td>Persson (2001)</td>
<td>8-12</td>
<td>25,700</td>
<td>0.85-0.94</td>
<td>0.084</td>
<td>0.23</td>
<td>Yes</td>
<td>0</td>
<td>8g/100g</td>
</tr>
<tr>
<td>Tang (1999)</td>
<td>5-6</td>
<td>36,600</td>
<td>1.05-1.24</td>
<td>0.33</td>
<td>0.27</td>
<td>Yes</td>
<td>0</td>
<td>57g/day</td>
</tr>
<tr>
<td>Ncube (2001)</td>
<td>Lactating women</td>
<td>60,000</td>
<td>0.80-0.90</td>
<td>0.3</td>
<td>no data</td>
<td>no/No</td>
<td>no data</td>
<td>10g/port.</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control group change deducted  
<sup>b</sup> One portion was 100-150g  
<sup>c</sup> Total intake of fat during the intervention was 39 g/day (including both home consumption of fat and fat provided in the treatment group) 
<sup>d</sup> Net change for those also being dewormed / not being dewormed  
<sup>e</sup> 20% of the subjects had intestinal helminths and 3.5% of the positive cases had *Ascaris lumbricoides*
Also, it may be a better approach to promote dietary diversification, rather than promoting a single food. In particular, there are many constraints to increased consumption of the more bioavailable YOFVs. Firstly, seasonality. Mangoes in particular are only available during the summer, a season when vitamin A intakes usually rise (Zeitlin et al., 1992), while DGLVs usually have a longer season when they are abundant. Secondly, food taboos hinder women in parts of India from consuming papaya and mangoes during pregnancy and lactation (Johns et al., 1992; Persson, 1995). It is not known how readily nutrition education can overcome such taboos. Thus, most food consumption studies show that the contribution of vitamin A from YOFV is rather small or even negligible (Melse-Boonstra et al., 2000; Ncube et al., 2001), a trend observed also in our studies from India (Persson, 1997), Bangladesh (Persson et al., 1999) and Indonesia.

Similarly, the use of multiple strategies to combat VAD should be recommended, rather than to focus only on one. Most likely, supplementation with high doses of vitamin A is behind the fact that children in many countries in Asia now are virtually xerophthalmia-free. However, one important risk group, pregnant women, cannot be reached by occasional high doses of vitamin A, because of the risk for teratogenic effects. Thus, fortification is another important complementary food-based strategy, at least if a suitable food vehicle for fortification is chosen, and is in the planning phase in many low-income countries including India (Government of India, 1996) and Indonesia (MOH/WHO, 2000). Besides reaching all risk groups, fortification may be less vulnerable to economic fluctuations and can include multiple nutrients.

The scope of food processing and fat:
Processing of vegetables by mechanical homogenisation or heat treatment has the potential of increasing the bioavailability of carotenoids (van het Hof et al., 1998). However, whether or not people are willing to change their way of preparing vegetables needs to be explored. Addition of fat during preparation may also enhance bioavailability. The amount of fat required to meaningfully increase absorption is not clear, but may be only 3-5 g per serving (Jayaran et al., 1980; Jalal et al., 1998).

New technologies for field research on vitamin A improvement:
Serum retinol may not be the best indicator for measuring short-term changes in vitamin A status. A recent study by Ribaya-Mercado and colleagues (2000) using 3-d deuterated-retinol-dilution, suggests that improvement of vitamin A status after dietary intervention is influenced very little by serum retinol concentrations, but instead is strongly influenced by total body stores of vitamin A. By using the same isotope-dilution test as Ribaya-Mercado and colleagues, Tang and colleagues (1999) were able to confirm that total-body vitamin A stores were sustained in a group fed green-yellow vegetables, but
decreased in a group receiving vegetables low in carotenoids. In addition, van Lieshout and co-workers (2001) have developed an isotope dilution technique using $^{13}$C$_{10}$ β-carotene and found that the amount of β-carotene dissolved in oil required to form 1µg retinal was 2.4µg. This method has the advantage that it requires a limited number of blood samples to derive conversion factors. Hopefully, by using these new techniques, it will be possible to establish the appropriate conversion factor to apply to provitamin A compounds in predicting the formation of active vitamin A in humans. A new estimate has already been made for the US, where vitamin A activity is described in retinol activity equivalents; the conversion factor used for β-carotene coming from plant sources is now 12 instead of 6 (National Academy of Sciences, 2001). However, in developing countries helminth infestation and infection must be controlled for in any research aiming to establish new conversion factors.
7. CONCLUSIONS

- We found that the usual mean intake of several nutrients, including vitamin A, can be reliably measured with the 24-hour recall method during pregnancy in a developing country. However, our data on attenuation of simple regression coefficients suggest that many replicate days may be needed to establish associations between micronutrient intake, including vitamin A, and health outcomes.

- When using the 24-hour recall method, the nutrient of interest, the primary objectives of the study, and the methods of analysis to be used should all be taken into account when planning the sample size and number of replicates.

- The majority of pregnant women in Purworejo District, Central Java, consumed insufficient vitamin A in all three trimesters. Thus, strategies are needed to improve vitamin A intake in all pregnant women rather than targeting certain groups. Intakes of preformed vitamin A from animal and fortified sources as well as vitamin A density was strongly linked to education, with a higher intake among those with $\geq 10$ years of education.

- Breast milk, in agreement with earlier findings, is an important source of vitamin A even in the second and third years of life in rural areas of Bangladesh. Similarly, animal milk is likely to be an important source of vitamin A among preschoolers in certain areas of India. Therefore, the HKI FFM should be revalidated to make it a useful tool also in settings where breastfeeding is a common practice after the first year of life and/or animal milk is common in the diets of preschool children, even if somewhat diluted.

- A large proportion of the primary school children studied in rural Thakurgaon and Panchargar, Bangladesh, suffered from helminth infection, VAD and iron deficiency anaemia. Since no micronutrient interventions are specially aimed at primary school-children, more attention should be given to this group. Examples of such programs are nutrition education, school gardening, hygiene education and school-based deworming programs.

- Iron status was independently related both to hookworm and vitamin A status. Programs to improve iron status in Bangladesh and Indonesia should consider including both vitamin A prevention programs and deworming.

- Other acute phase proteins than C-reactive protein (such as $\alpha$-acid glycoprotein) should be used in the assessment of vitamin A and iron status in field studies where infections are prevalent.

- In children successfully treated for *Ascaris lumbricoides*, a substantial increase in serum $\beta$-carotene was seen after feeding with a moderately high
cumulative dose of DGLV for 6 weeks. A smaller impact was seen on serum retinol, perhaps due to the relatively high baseline serum retinol concentration and the relatively low cumulative dose of β-carotene provided.
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