Daily supplements of vitamin A (8.4 μmol, 8000 IU) improve the vitamin A status of lactating Indonesian women¹–⁴

Sherry A Tanumihardjo, Muherdisyantiningsih, Dewi Permaesih, Komala, Muhlal, Darwin Karyadi, and James A Olson

ABSTRACT  The vitamin A statuses of lactating Indonesian women \( n = 23 \) starting at 1–3 mo after delivery were determined at three monthly intervals (times 1, 2, and 3) during lactation and then again (time 4) after they had ingested vitamin A capsules (8.4 μmol, 8000 IU) daily for 35 d. Vitamin A status was determined by using the modified-relative-dose-response (MRDR) test and serum retinol concentrations. The mean MRDR ratio in these women rose from 0.084 ± 0.047 (time 1) to 0.099 ± 0.045 (time 2) and then to 0.100 ± 0.054 (time 3). After supplementation the mean MRDR ratio fell to 0.040 ± 0.021 (time 4) \( (P < 0.0001) \). Mean serum retinol concentrations at the first three times were 0.94 ± 0.23, 0.87 ± 0.20, and 0.80 ± 0.20 μmol/L, but then rose to 1.10 ± 0.31 μmol/L at time 4 \( (P < 0.04) \). After supplementation mean MRDR values and mean serum retinol concentrations were 60% lower and 38% higher, respectively, than those just before supplementation. MRDR values better distinguished the vitamin A statuses of the women than did serum retinol concentrations. Mean hemoglobin values increased during the study from \( 118 ± 9 \) g/L at time 1 to 122 ± 6 g/L at time 4 \( (P = 0.0187) \). The mean hematocrit also rose from 0.35 ± 0.03 at time 1 to 0.361 ± 0.17 at time 4 \( (P = 0.0478) \). Thus, iron status may also have improved marginally from time 1 to time 4, but most of the increase appeared before the vitamin A intervention. Am J Clin Nutr 1996;63:32–35.

KEY WORDS  Vitamin A, lactation, women, vitamin A assessment, modified relative dose response, dehydroretinol

INTRODUCTION  Marginal vitamin A status, common among children in developing countries, also affects women of childbearing age (1–3). As a consequence, the World Health Organization has suggested that 210 μmol (200 000 IU) vitamin A be given to lactating women in high risk areas, but only within 1 mo of delivery (4), largely to avoid possible adverse effects of large doses of vitamin A on fetal development early in a subsequent pregnancy. Indeed, lactating Indonesian women have shown significant improvement in serum retinol concentrations after high-dose (315 μmol, 300 000 IU) supplementation soon after delivery (2).

Vitamin A supplementation has also been shown to increase hemoglobin concentrations in both children (5–7) and pregnant women (8, 9). Whereas the mechanism by which this occurs has not been fully elucidated, Thornham (10) suggested that vitamin A may act by down-regulating the acute-phase response, which ultimately should lead to increased transferrin synthesis, augmented iron transport from liver to bone marrow, and enhanced hemoglobin formation.

Our current study was designed with two purposes: 1) to evaluate the effectiveness of daily supplements (8.4 μmol) of vitamin A for 35 d on the vitamin A statuses of lactating women, and 2) to determine whether this treatment also affected the hemoglobin concentration and hematocrit values of these same women.

Vitamin A status was assessed by using the modified-relative-dose-response (MRDR) test (3, 11–14) and by concomitantly measuring serum retinol concentrations. Hemoglobin and hematocrit values were monitored by conventional procedures in the same blood samples. The molar ratio of dehydroretinol to retinol (DR:R, vitamin A-2:vitamin A-1) measured 4–6 h after dosing with 3,4-dehydroretinyl acetate that is used to define marginal vitamin A status is ≥ 0.060 in developing countries and ≥ 0.030 in developed countries (3, 14). The intermediate range of ratios from 0.030 to 0.060 represents a “gray” area of uncertain vitamin A status (3, 14). Serum retinol concentrations < 0.7 μmol/L are considered inadequate in Indonesian women (1, 3). Hemoglobin concentrations < 110 g/L are considered low (1). Significantly increased hemoglobin at time 4 compared to time 3: (1) women aged and parity, (2) hemoglobin concentrations at time 1, (3) hemoglobin concentrations at time 3.

SUBJECTS AND METHODS

Subjects  Subjects were recruited from a suburban village near Bogor in West Java, Indonesia, by working in cooperation with the village health posts (pos yandu). The sample consisted of 25 lactating mothers aged 15–40 y, each with 1–8 children. Women were chosen based on the age of their breast-fed infants; the age of the infants at the time of enrollment was

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30–89 d. Because the vitamin A statuses of lactating mothers in this region are generally unsatisfactory, each of their breast-fed infants was given a single dose of 52.4 μmol (50 000 IU) vitamin A during the first month of the study at the local pos yandu. Informed consent procedures were used that were in full accord with the guidelines established by the Human Use Committee of Iowa State University and by the Indonesian Ministry of Health.

MRDR test and iron-status assessment

3,4-Didehydroretinyl acetate, commonly called vitamin A-2, was synthesized from retinoic acid (15, 16). The purified product was then dissolved in corn oil by sonication. On the day of the study each mother was given a dose of 8.8 μmol (2.5 mg) DRA in 200–250 μL corn oil in the morning either at her home or at the home of a village volunteer. The dose was followed by a locally purchased snack that was high in fat but low in vitamin A, eg, deep-fried white banana, potato, or tofu (bean curd).

Later in the morning, the mothers were brought to a volunteer’s home. Height and weight were measured by using portable clinical equipment and questions were asked regarding the number of children in the family, their own ages, and any recent or current illnesses. At this time, 5 h after dosing, nonfasting venous blood samples were drawn from an antecubital vein. Of the 25 women initially enrolled in the study, only two dropped out. Three MRDR tests were performed at monthly intervals (times 1, 2, and 3). Thereafter, the women were supplied with 35 8.4-μmol (8000 IU) vitamin A capsules (Pharmavite Corp, Mission Hills, CA) and instructed to take one per day. Compliance was monitored by counting the number of pills remaining in the bottle. A fourth MRDR test (time 4) was performed after the supplementation period.

Blood samples, after aliquots were removed for measuring hematocrit and hemoglobin, were placed on ice inside a cooler while in the field, carried to the laboratory, and immediately centrifuged at 500 × g for 15 min at room temperature. Serum was stored frozen at −20 °C until analyzed. Hematocrit and hemoglobin determinations were performed on fresh blood. Capillary tubes, which were filled with fresh blood and stoppered in the field, were carried to the laboratory and centrifuged. Hemoglobin was determined in 20 μL fresh blood by using the cyanmethemoglobin method.

Determination of the molar ratios of dehydroretinol to retinol

In a dimly lit room, the serum was thawed and 500-μL aliquots were treated with an equal volume of ethanol to precipitate proteins and then were extracted twice with hexanes. Retinyl acetate dissolved in ethanol was used as an internal standard to determine extraction efficiencies. The hexane layers were pooled and evaporated to dryness with nitrogen. The sample was redissolved in 50 μL methanol:methylene dichloride (3:1, by vol); then 40 μL was injected onto the HPLC system via a Rheodyne manual injector (model 7125; Cotati, CA). A Shimadzu SPD-UV-VIS absorbance detector (Kyoto, Japan) monitored the wavelength at 350 nm, which optimizes detection of 3,4-didehydroretinol (DR). A Waters pump (model 501; Milford MA) delivered 90:10 methanol: water at a flow rate of 1 mL/min to the 5-μm Phenomenex Resolve 15-cm reversed-phase column (Torrance, CA). A Shimadzu CR601 Chromatopac integrator calculated peak areas. DR and R were quantitated against authentic external standards and DR:Rs were calculated. This method gives an average extraction efficiency of 86%, a CV of 4% for repeated serum retinol values, and a CV of 1% for repeated DR:Rs on the same person on the same day.

Statistical methods

Mean (± SD) serum retinol, hemoglobin, and hematocrit values and DR:Rs were calculated for each of the four time points. Comparisons were made for the difference at each time point from point 4 by using the paired t test for difference to determine significance. SAS statistical software (version 6.07; SAS Institute Inc, Cary, NC) was used to determine P values. Because data were collected before treatment at each of three time points and were separately compared with the data collected after treatment, Bonferroni’s global test of significance was applied (17). That is, the difference was considered to be significant if P ≤ 0.05/3 (or 0.017) and highly significant if P ≤ 0.01/3 (or 0.0033). Mean values, SDs, and CVs were calculated for hemoglobin and hematocrit determinations.

RESULTS

Subject characteristics and compliance

The mean number of pregnancies of the subjects was 2.8 ± 2.2, whereas the mean number of live children was 2.5 ± 1.8. The mean age of the women was 24.7 ± 6.3 y. The mean body weight of the lactating women remained fairly constant throughout the study, ranging from 47.4 ± 6.9 kg (time 1) to 46.9 ± 6.6 kg (time 4) (P > 0.5). Only 4 of the 23 women lost a significant amount of weight (≥ 3 kg) during the observed lactation period. Formal education ranged from 0 to 12 y for the enrolled women and from 0 to 16 y for their spouses.

The growth of the babies was monitored monthly while the mothers were enrolled. All of the babies continued to grow normally during the study. The mean weight of the infants increased from 4.9 ± 1.2 kg (time 1) to 6.8 ± 1.0 kg (time 4).

Most of the women took all, or nearly all, of the 35 capsules provided. Only 3 of the 23 women had five or more capsules remaining. Thus, if ≥ 30 capsules were ingested, 20 of the women would have received ≥ 252 μmol (240 000 IU) vitamin A between times 3 and 4.

Molar ratios of dehydroretinol to retinol before and after supplementation

The mean DR:R increased slowly from time 1 to time 3 (Table 1). At each time, the percentage of the women tested who showed DR:Rs above the provisional cutoff value of 0.060 was 70–83% before supplementation but was only 13% after supplementation. Although the DR:R did become more positive with the length of lactation in many of the women, the mean ratios at times 1, 2, and 3 were not significantly different from each other. After supplementation, the mean ratio (time 4) decreased to 0.040 ± 0.021, which was significantly different from all presupplementation mean values when the paired t test was used (Table 1). The mean DR:Rs for three women were ≥ 0.060 after supplementation. Nonetheless, their initial mean ratio of 0.113 ± 0.061 fell to 0.078 ± 0.015 after supplemen-
TABLE 1
Assessments of vitamin A status by using the modified-relative-dose-response (MRDR) test and serum retinol concentrations in lactating Indonesian women before (times 1, 2, and 3) and after (time 4) daily supplementation with 8.4 μmol vitamin A for 35 d

<table>
<thead>
<tr>
<th>Time</th>
<th>DR:R</th>
<th>≥ 0.060</th>
<th>Value</th>
<th>&lt; 0.70 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.084 ± 0.047</td>
<td>70</td>
<td>0.94 ± 0.23</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>0.099 ± 0.045</td>
<td>83</td>
<td>0.87 ± 0.20</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>0.100 ± 0.054</td>
<td>74</td>
<td>0.80 ± 0.20</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>0.040 ± 0.021</td>
<td>13</td>
<td>1.10 ± 0.31</td>
<td>9</td>
</tr>
</tbody>
</table>

1 Molar ratio of dehydroretinol to retinol in serum after dosing with 3.4-dehydroretinol acetate.
2 ± SD; n = 23.
3 Significantly different from time 4, *P = 0.0001. *P = 0.00001.
4 P = 0.0034.

Serum retinol concentrations

Serum retinol concentrations were 0.45–1.54 μmol/L before supplementation and 0.69–2.03 μmol/L after supplementation. The mean serum retinol concentration decreased slightly with time until after supplementation, when it increased by 38% (Table 1). Eight of the 23 women still showed net decreases in serum retinol concentrations from time 1 to time 4. As a result, the changes in serum retinol values between time 1 and 4 were not significant (Table 1). The differences between times 2 and 4 and times 3 and 4, however, were significant.

Figure 1 illustrates the relation between the DR:R in serum and serum retinol concentrations before and after supplementation. During the presupplementation period serum retinol concentrations < 0.70 μmol/L were always associated with DR:Rs ≥ 0.060, whereas serum retinol concentrations ≥ 0.70 μmol/L were associated with ratios both ≥ 0.060 (78%) and < 0.060 (22%). After supplementation, serum retinol concentrations ranged from 0.68 to 2.02 μmol/L and 13% of the women had DR:Rs ≥ 0.060.

Iron-status assessment

There was a significant increase in the hemoglobin concentration from time 2 to time 4 (Table 2). The mean CV of the hemoglobin determination within individuals was 4 ± 2%. The lowest hemoglobin values at the beginning and at the end of the study were 101 and 109 g/L, respectively. Hematocrit values increased with time but this trend was not significant (Table 2). The mean CV was 4 ± 1% for hematocrit values within individuals.

DISCUSSION

Marginal vitamin A status is common among lactating and pregnant Indonesian women (1–3). Our goal in this study was to determine whether a low-dose vitamin A supplement made available to a group of women would improve their vitamin A status. Even though the length of the intervention was only 35 d, the statuses of most of the women improved significantly. In fact, 10 of the 23 women studied had DR:Rs < 0.030 after 35 d of supplementation, which is the value found in healthy American adults and children (11, 13). Although the vitamin A statuses from times 1–3 to time 4 clearly responded to daily vitamin A administration, the magnitude of the change directly attributable to the intervention could not be determined in the absence of a concurrent control group.

The iron status of these women also improved with time. Because the increases in hemoglobin concentration and hematocrit value were continuous from time 1 to time 4, the improvement was most likely due to recovery from pregnancy rather than to a response to the supplement. Although an improvement in iron-status variables with vitamin A supplementation has been reported (5–9), the women in this study were not seriously anemic. Indeed, only 13% of the women entering the study had hemoglobin concentrations < 110 g/L; this percentage fell to 4% by time 4 (Table 2). In other developing countries, a significant difference in hemoglobin concentration was observed between lactating and pregnant

TABLE 2
Iron-status assessment in lactating Indonesian women during a 3-mo period

<table>
<thead>
<tr>
<th>Time</th>
<th>Hemoglobin &lt; 110 g/L</th>
<th>Value &lt; 0.33</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/L</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>118 ± 9</td>
<td>13±</td>
</tr>
<tr>
<td>2</td>
<td>118 ± 8</td>
<td>17±</td>
</tr>
<tr>
<td>3</td>
<td>120 ± 8</td>
<td>9±</td>
</tr>
<tr>
<td>4</td>
<td>122 ± 6</td>
<td>4±</td>
</tr>
</tbody>
</table>

1 ± SD; n = 23.
2 Significantly different from time 4; P < 0.017.
women studied cross-sectionally in Nigeria (18), but not in Zaire (19).

The effects of vitamin A supplementation in this study were similar to those found earlier in groups of lactating and nonlactating, nonpregnant Indonesian women (3). That is, serum retinol concentrations < 0.70 μmol/L were almost invariably associated with positive (≥ 0.060) DR:Rs. Serum retinol concentrations > 0.70 μmol/L, however, were not a good indicator of vitamin A status. Indeed, even after supplementation, 52% of these women still had serum retinol concentrations < 1.05 μmol/L, which is the cutoff for inadequate vitamin A status used in industrialized countries (20). Factors that reduce serum retinol concentrations, but not necessarily vitamin A status, include infections and other micronutrient deficiencies, ie, of iron, zinc, and vitamin E.

All of the infants grew normally during the course of the study. In groups of women at risk for vitamin A deficiency, infant growth is typically maintained, but infant liver reserves of vitamin A are often inadequate (2). By providing low-dose vitamin A supplements to lactating mothers, which avoids the possible adverse effects of single high doses, the vitamin A status of both the mother and infant were improved.

In this study both serum retinol concentrations (P < 0.04) and DR:Rs (P < 0.0001) showed a response to the supplementation. However, because the distributions of values found before and after supplementation were better separated by use of the MRDR test than by analysis of serum retinol concentrations, more statistical power can be realized by using the MRDR test than by analyzing serum retinol concentrations. Both the MRDR test and serum retinol concentration determinations require a single blood sample; however, there is an additional cost associated with the MRDR test for the preparation of the 3,4-didehydrotetrinyl acetate, which could be more than offset by the lower sample size requirements.

In food intervention trials, in which the amount of dietary vitamin A is not as predictable as in studies in which preformed vitamin A is supplemented, this added statistical power of the MRDR test could aid in reducing the required sample size. Furthermore, serum retinol concentrations can remain unchanged after supplementation or dietary modification because of the prevalence of infections or the maintenance of homeostatic control. Thus, the DR:R invariably responds to vitamin A treatment in populations with inadequate vitamin A status, whereas serum retinol concentrations may not.

Whereas this study showed that low-dose supplements improved the vitamin A status of lactating women, the question remains whether supplements are a sustainable intervention. Depending on local conditions, fortification of local core or secondary foods may be a more realistic approach; however, effective fortification programs and dietary modification programs present many logistical problems, which can take years to overcome. Thus, giving the 200,000-IU dose at birth for mothers (4) and making the low-dose capsules available through the health posts for women of childbearing years remain as short-term solutions to the vitamin A inadequacy problem that exists today.

REFERENCES