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Vitamin A and Iron Status Are Improved by Vitamin A and Iron Supplementation in Pregnant Indonesian Women^{1,2}

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ABSTRACT In Indonesia, deficiencies of vitamin A and iron are of public health concern during pregnancy. We sought to determine the effects of vitamin A and iron supplementation on the vitamin A and iron status of pregnant Indonesian women. The women ($n = 27$) were randomly assigned to four groups. The modified relative dose response (MRDR) test for vitamin A status and hemoglobin, hematocrit and ferritin values were determined at baseline. Thereafter, daily supplements were administered: placebo [PI] ($n = 7$), 8.4 μmol vitamin A [A] ($n = 7$), 1.07 mmol iron [Fe] ($n = 5$), and 8.4 μmol vitamin A plus 1.07 mmol iron [A + Fe] ($n = 8$). Post-treatment tests were performed after 8 wk. The MRDR value was reduced, i.e., vitamin A status improved, more markedly by the combination of vitamin A and iron than by either nutrient alone ($P = 0.034$). The decrease in the MRDR relative to baseline was significant in the A + Fe group ($P = 0.008$). Iron status was also significantly improved in these women ($P < 0.05$) with both iron and vitamin A supplementation. The mechanism of the enhancing effect of iron on the vitamin A-induced reduction in the MRDR is not known. *J. Nutr.* 132: 1909–1912, 2002.

KEY WORDS: • vitamin A status assessment • modified relative dose response • pregnant women • iron status • Indonesia

Micronutrient deficiencies during pregnancy are common and can potentially cause irreversible abnormalities in the fetus, thus affecting children throughout their lives. Vitamin A and iron depletion have been documented in many countries and regions (1), including Nepal (2), Malawi (3), the Middle East (4), India (5) and even the United States (6,7). Vitamin A deficiency during pregnancy affects both the prospective mother and the fetus. Although the manifestations of vitamin A deficiency during pregnancy have been clearly shown in animals, evidence for an adverse effect in humans is indirect (8).

Several studies using various vitamin A status indicators have shown that indeed Indonesian pregnant and lactating women do not have satisfactory vitamin A status (9–12). For example, based on serum retinol $<0.70 \mu\text{mol/L}$, 34% of a sample ($n = 318$) of Indonesian women had marginal vitamin A status (9). By applying the relative dose response test (13) to a subgroup of these women ($n = 45$), 9% had positive (abnormal) responses. Serum retinol alone is not a good indicator of status because it is homeostatically controlled. Moreover, hemodilution during pregnancy may also falsely put

women into the marginal category. In a longitudinal study (14), a 30% reduction was observed in serum retinol concentration from the 1st to the 3rd trimester of pregnancy. Because the plasma volume expands ~50% during pregnancy, the concentrations of both vitamin A and its carrier protein are reduced correspondingly (15).

Although not well understood, the link between vitamin A and iron deficiency anemia continues to be studied and improvement is repeatedly seen when vitamin A and iron are given together (10,16,17). However, this improvement is not always maintained after delivery when supplementation is discontinued (18).

Before appropriate public health measures can be applied to a community, nutrient needs must be assessed. The modified relative dose response (MRDR)⁴ has been used in low income, pregnant American (6) and Indonesian women (12) to assess vitamin A status. The MRDR test offers more sensitivity to changes in vitamin A status than serum retinol concentrations alone (19,20) and thus smaller sample sizes can be used to assess response to interventions targeted to improve vitamin A status. Our objective in this study was to determine the change in vitamin A and iron status in pregnant Indonesian women in response to vitamin A and iron supplementation given either separately or combined.

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⁴ Abbreviations used: DR, dehydroretinol; DRA, 3,4-didehydroretinyl acetate; DR:R, dehydroretinol to retinol ratio; MRDR, modified relative dose response; R, retinol.

SUBJECTS AND METHODS

Subjects. Pregnant women ($n = 27$), in the second or early third trimester (17.6 ± 3.9 wk gestation), were recruited from the suburban areas of Bogor in West Java, Indonesia. Ages ranged from 18 to 37 y and parity from 0 to 4 children. The study was done in conjunction with the local health posts ("pos yandu"). Approximately 70% of the pregnant women used the Mother and Child Health Services available in the villages (21). Anthropometric measurements included weight and height. Information on age and parity (both live children and miscarriages) was obtained. Gynecological exams were performed by a medical doctor. Baseline characteristics are summarized in Table 1. Informed consent guidelines were used as established by the University Committee on the Use of Human Subjects in Research of Iowa State University and by the Indonesian Ministry of Health.

MRDR test. The 3,4-didehydroretinyl acetate (DRA) was synthesized from retinoic acid (22,23), purified and dissolved directly into corn oil using sonication. All of the women received a single dose of $8.8 \mu\text{mol}$ DRA dissolved in corn oil in the morning. Women were dosed either in their homes, at the health post or at the clinic. The dose was followed by high fat, low vitamin A snacks. Five hours after dosing, a single blood sample was drawn from an antecubital vein. The blood was stored on ice in a light-protected cooler until transported to the laboratory. Clotted blood was centrifuged, and the serum was stored at -20°C until analysis. Abnormal responses for the MRDR test were defined as a dehydroretinol to retinol ratio (DR:R) of ≥ 0.060 , the range of 0.030 to 0.060 indicated an uncertain vitamin A status and <0.030 was defined as normal (12).

Supplementation trial. After the initial MRDR test determination, the women were randomly assigned to the following four supplementation groups: placebo (PI), $8.4 \mu\text{mol}$ (8000 IU) vitamin A as retinyl palmitate with an iron placebo (A), 1.07 mmol (60 mg) ferrous sulfate with a vitamin A placebo (Fe) and vitamin A plus iron (A + Fe). Subjects and village volunteers (cadres) were unaware of group assignment. The daily supplementation was monitored using a control card and check list by the volunteers who were responsible for administration of the doses. This was our only measure of compliance. After 8 wk of supplementation, the MRDR test was repeated.

Sample analysis. Serum was thawed and $500\text{-}\mu\text{L}$ aliquots were extracted and analyzed for DR and retinol R by using HPLC. All extractions were done in a dimly lit room. HPLC-purified retinyl acetate dissolved in ethanol was used as an internal standard to determine extraction efficiencies. The serum was treated with ethanol and then extracted twice with hexane. The hexane layers were pooled and dried under nitrogen. The samples were redissolved in $50 \mu\text{L}$ of 3:1 methanol/methylene dichloride, and $40 \mu\text{L}$ was injected onto a $5\text{-}\mu\text{m}$ Waters "Resolve" 15-cm reversed-phase column (Milford, MA) via a Rheodyne 7125 injector (Cotati, CA). The wavelength of detection was set at 350 nm on a Shimadzu SPD-6AV UV-VIS absorbance detector (Kyoto, Japan) to optimize for DR. The flow rate on a 501 Waters pump (Milford, MA) was 1 mL/min of 90:10 methanol/water. HPLC-purified standards were used to quantify the DR and R. Peak areas of standards and samples were calculated with a Shimadzu CR601 Chromatopac integrator (Kyoto, Japan).

TABLE 1

The characteristics of pregnant Indonesian women at time of enrollment into the study¹

Body weight, kg	46.8 ± 7.2
Age, y	25.9 ± 6.0
Children, n	2.0 ± 1.5
Gestation, wk	17.6 ± 3.9
DR:R, ² mol/mol	0.038 ± 0.019
Serum retinol, $\mu\text{mol/L}$	0.843 ± 0.216
Hematocrit, vol/vol	0.327 ± 0.027
Hemoglobin, g/L	107.4 ± 8.8
Ferritin, $\mu\text{g/L}$	30.6 ± 18.0

¹ Values are means \pm SD, $n = 27$.

² The dehydroretinol to retinol (DR:R) was determined 5 h after dosing with 3,4-didehydroretinyl acetate.

Iron status. Hematocrit and hemoglobin concentrations were determined 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 $20 \mu\text{L}$ fresh blood by using the cyanmethemoglobin method. Serum was stored at -20°C until analysis of ferritin by an ELISA (Boehringer Mannheim, Mannheim, Germany).

Statistical analysis. ANOVA (two-way) with the general linear model using SAS software (SAS version 8, SAS Institute, Cary, NC) was used to determine the overall effect of vitamin A, iron and the interaction of vitamin A and iron on all variables of interest: the DR:R ratio, serum retinol concentration, hematocrit, hemoglobin and ferritin. The P -values reported are those from the Type III sums of squares. Least significant difference testing was performed after the ANOVA to determine which groups differed (Table 2). Difference t testing was used within a group to compare means before and after treatment (Table 3). The changes and relationships were considered significant when $P < 0.05$. Values are means \pm SD.

RESULTS

Anthropometric characteristics (Table 1). The body weights of the women at the time of the MRDR test ranged from 31.0 to 69.0 kg with a mean of 46.8 ± 7.2 kg. The number of children was 2.0 ± 1.5 with a range of 0–4 and the age of the women was 25.9 ± 6.0 y. Other relevant data are reported in Table 1.

ANOVA using the GLM procedure. Using ANOVA to examine vitamin A, iron and the interaction of vitamin A and iron separately in all 27 women, the decrease observed in the DR:R in response to the vitamin A supplementation alone approached significance ($P = 0.051$). Moreover, the interaction of the vitamin A and iron supplementation significantly reduced DR:R ($P = 0.034$). None of the supplementations affected serum retinol concentrations ($P > 0.43$). Vitamin A and iron alone each affected hematocrits ($P = 0.0003$ and <0.0001 , respectively), but the interaction of vitamin A and iron was not significant ($P = 0.134$). Similarly, vitamin A and iron affected hemoglobin concentrations ($P = 0.0004$ and 0.0002 , respectively), but the interaction of vitamin A and iron was not significant ($P = 0.115$). Iron supplementation improved ferritin concentrations ($P = 0.002$). The interaction of vitamin A and iron on ferritin concentrations approached significance ($P = 0.0501$).

Vitamin A status after the supplementation. Of the 27 women, two (7.4%) had baseline DR:R ≥ 0.060 , indicative of subclinical vitamin A deficiency and 12 (44%) were in the range of 0.030–0.060. The DR:R of many of the women decreased after supplementation with vitamin A. Table 3 summarizes the changes in response to treatment of the variables of interest within each treatment group. In the placebo (PI) group ($n = 7$), the mean DR:R did not change after the intervention. In the vitamin A (A) treated group ($n = 7$), although the DR:R fell in 5 cases, it increased in 2 subjects, thus giving no overall improvement in the mean. In one of these women, the DR:R increased from 0.113 to 0.138 and serum retinol decreased from 0.53 to $0.34 \mu\text{mol/L}$ after supplementation. In the iron (Fe) supplemented group ($n = 5$), the DR:R decreased in 2 subjects and increased in 3 others.

The most dramatic changes were observed in the vitamin A plus iron (A + Fe) supplemented group ($n = 8$). In 6 subjects, the ratios decreased drastically and in the other 2 there was no appreciable change after supplementation. Using LSD testing, the A + Fe treatment response was significantly different from all of the other treatment groups. All of the other comparisons for DR:R between intervention groups were not different from one another (Table 2).

Changes in iron status within and between treatment groups. When comparing the iron status of the groups using

TABLE 2

Vitamin A and iron status indicators in Indonesian women who were either supplemented with placebo, vitamin A (8.4 μmol retinyl palmitate), iron (1.07 mmol ferrous sulfate), or vitamin A and iron for 8 wk¹

Indicator	Placebo (n = 7)		Vitamin A (n = 7)		Iron (n = 5)		Vitamin A and iron (n = 8)	
	Before	After	Before	After	Before	After	Before	After
DR:R, ² mol/mol	0.032 \pm 0.008	0.031 \pm 0.011 ^a	0.043 \pm 0.034	0.043 \pm 0.044 ^a	0.032 \pm 0.009	0.037 \pm 0.007 ^a	0.042 \pm 0.013	0.021 \pm 0.015 ^b
Retinol, $\mu\text{mol/L}$	0.96 \pm 0.16	0.89 \pm 0.32	0.71 \pm 0.23	0.72 \pm 0.25	0.91 \pm 0.28	0.94 \pm 0.40	0.82 \pm 0.16	0.91 \pm 0.31
Hematocrit, vol/vol	0.347 \pm 0.033	0.313 \pm 0.028 ^c	0.309 \pm 0.011	0.333 \pm 0.018 ^{a,b}	0.340 \pm 0.027	0.358 \pm 0.018 ^b	0.316 \pm 0.015	0.365 \pm 0.012 ^a
Hemoglobin, g/L	113 \pm 11.8	104 \pm 11.0 ^b	102 \pm 4.1	109 \pm 6.0 ^a	112 \pm 9.9	118 \pm 6.7 ^a	105 \pm 4.7	119 \pm 3.2 ^a
Ferritin, $\mu\text{g/L}$	41.4 \pm 5.9	27.6 \pm 8.6 ^b	22.6 \pm 24.8	27.3 \pm 17.8 ^a	28.8 \pm 9.4	43.8 \pm 18.3 ^a	22.6 \pm 8.6	34.8 \pm 9.3 ^a

¹ Values are means \pm SD. After treatment means in a row not sharing a letter are different, $P < 0.05$.

² The dehydroretinol to retinol (DR:R) was determined 5 h after dosing with 3,4-didehydroretinyl acetate.

LSD testing, all of the supplementations (i.e., A, Fe and A + Fe) had a positive effect on iron status (Table 2). In group Pl, hematocrit, hemoglobin and ferritin concentrations were significantly reduced after the intervention period (Table 3). Although the hematocrit and hemoglobin concentrations were improved in groups A and A + Fe from baseline, ferritin was significantly improved only in group A + Fe (Table 3).

DISCUSSION

The vitamin A and iron status of pregnant Indonesian women from lower and middle socioeconomic groups were evaluated using the MRDR test for vitamin A and hematocrit,

hemoglobin, and ferritin concentrations before and after an intervention with vitamin A and iron. At baseline, 7% of the women had marginal vitamin A status with a DR:R \geq 0.060. Another 44% of the women tested in the intermediate zone of 0.030–0.060. Interestingly, this is comparable to the status of low income, pregnant American women (6); in that group, 9% tested \geq 0.060 and 17.5% tested between 0.030 and 0.060.

The hemodilution of vitamin A during pregnancy is a major confounding factor in the interpretation of serum retinol concentrations. A 30% decrease in serum retinol from the 1st to the 3rd trimester has been noted (14). Serum retinol concentrations $<0.53 \mu\text{mol/L}$ in pregnant Indonesian women are associated with a positive DR:R (12). Positive DR:R values (≥ 0.060) are almost invariably associated with serum retinol concentrations $<0.7 \mu\text{mol/L}$ in Indonesian lactating women, for whom hemodilution would not be a factor (19,24). Thus, the cut-off value for serum retinol in pregnant Indonesian women is 25% lower than in lactating women, which concurs with the expected hemodilution. Moreover, when using only serum retinol concentrations to assess vitamin A status, the degree of infection in the population should also be assessed because infections will cause serum retinol concentrations to be decreased transiently due to the acute-phase response (25).

The DR:R is less affected by hemodilution (6,12) and infections and is more dependent on vitamin A status (26,27). Even when supplemented, 17% of American, low income pregnant women who were taking prenatal vitamin supplements had serum retinol concentrations $<0.7 \mu\text{mol/L}$ (28). Therefore, a certain percentage of vitamin A-sufficient women might well have a serum retinol concentration $<0.7 \mu\text{mol/L}$ during pregnancy. By using the MRDR test, the severity of the vitamin A problem can be more easily assessed than with serum retinol concentrations alone.

Of the 15 pregnant women in the present study who were in either the vitamin A or vitamin A plus iron-supplemented groups, 11 (73%) had a net decrease in DR:R. The 4 women who had either net increases in DR:R (≥ 0.025) or no change (≤ 0.005), also showed an average decrease in serum retinol concentration of 32% between testing points. Although the decrease in serum retinol concentrations may have been due to hemodilution, the increases or no change in DR:R indicates that they were probably not taking the supplements provided to them or that the supplement was not able to meet the extra vitamin A demands of pregnancy and provide extra vitamin A for liver storage. For example, one of these latter women was clearly still vitamin A depleted because her post-treatment

TABLE 3

Changes in vitamin A and iron status indicators in the serum after vitamin A and iron supplementation and the significance of the difference from baseline within each treatment group of pregnant Indonesian women¹

Group	n	Indicator	Average change	P-values ²
Placebo	7	DR:R, mol/mol ³	-0.001 \pm 0.011	NS
		Retinol, $\mu\text{mol/L}$	-0.067 \pm 0.26	NS
		Hematocrit, vol/vol	-0.034 \pm 0.029	0.020
		Hemoglobin, g/L	-9.0 \pm 9.0	0.039
		Ferritin, $\mu\text{g/L}$	-13.8 \pm 7.8	0.016
Vitamin A	7	DR:R, mol/mol	0.0001 \pm 0.018	NS
		Retinol, $\mu\text{mol/L}$	0.017 \pm 0.15	NS
		Hematocrit, vol/vol	0.0364 \pm 0.016	0.007
		Hemoglobin, g/L	7.1 \pm 5.8	0.018
		Ferritin, $\mu\text{g/L}$	4.7 \pm 13.0	NS
Iron	5	DR:R, mol/mol	0.005 \pm 0.009	NS
		Retinol, $\mu\text{mol/L}$	0.024 \pm 0.27	NS
		Hematocrit, vol/vol	0.018 \pm 0.019	NS
		Hemoglobin, g/L	6.6 \pm 5.7	NS
		Ferritin, $\mu\text{g/L}$	15 \pm 16	NS
Vitamin A + Iron	8	DR:R, mol/mol	-0.021 \pm 0.016	0.008
		Retinol, $\mu\text{mol/L}$	0.095 \pm 0.29	NS
		Hematocrit, vol/vol	0.049 \pm 0.018	0.0001
		Hemoglobin, g/L	3.9 \pm 4.0	0.00003
		Ferritin, $\mu\text{g/L}$	12 \pm 7.1	0.002

¹ Values are means \pm SD.

² The P-values are from paired *t* tests of average difference; NS, nonsignificant, $P > 0.05$.

³ The dehydroretinol to retinol (DR:R) was determined 5 h after dosing with 3,4-didehydroretinyl acetate.

DR:R was 0.138, which is quite elevated. In an evaluation of the iron supplementation program in Jakarta, Indonesia (29), 64% of the women given supplements claimed that they took the pills. However, only 36% of the women showed a positive stool test for iron. In the present study, we relied upon the village volunteers to administer the supplements and to monitor the project. It is likely that compliance was not universal; however, we chose not to omit data points from the analysis.

Vitamin A and iron interact in ways that are not fully understood. Results in rats suggest that the nutritional status of vitamin A and iron may affect the other's metabolism (30,31). Vitamin A seems to be sequestered in the liver during iron deficiency, resulting in plasma retinol concentrations that were only 40% of control animals (31). Larger survey intervention studies with stable isotopes and experimental studies with cell cultures may help to better define this interaction. Moreover, although the recommended dietary allowance for vitamin A during pregnancy (770 μg) is currently 10% above that for nonpregnant women (32), human studies to assess vitamin A needs during pregnancy have not been systematically performed. Stable isotope studies (33–35) with mathematical modeling (36) offer exciting opportunities to assess vitamin A requirements during the life cycle. Although we may have expected an improvement in vitamin A status in all of the women who received 8 wk of supplementation with vitamin A or vitamin A plus iron (20), vitamin A requirements during pregnancy may be enhanced in countries in which various infections and vitamin A depletion are endemic.

Pregnant and lactating women from lower socioeconomic classes in both developing countries and the United States do not have optimal vitamin A status. Encouraging supplementation programs and food-based interventions are important factors in potentially improving birth outcome and subsequent infant vitamin A status.

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