

# Redistribution of vitamin A after iron supplementation in Indonesian infants<sup>1-3</sup>

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## ABSTRACT

**Background:** Deficiencies of iron and vitamin A are prevalent worldwide. Single-micronutrient supplementation is widely used to combat these deficiencies. However, micronutrient deficiencies often occur concurrently, and there are many interactions between micronutrients.

**Objective:** This study investigated interactions among 3 important micronutrients—iron, vitamin A, and zinc—when they are given as supplements.

**Design:** In a randomized, double-blind, placebo-controlled supplementation trial, 387 Indonesian infants aged 4 mo were supplemented 5 d/wk for 6 mo with 10 mg Fe, 10 mg Zn, 2.4 mg  $\beta$ -carotene, 10 mg each of Fe and Zn, 10 mg Zn + 2.4 mg  $\beta$ -carotene, or placebo. Complete data on micronutrient status, including hemoglobin, ferritin, retinol, zinc, and the modified relative dose response (a measure of liver retinol stores), were available from 256 infants at the end of the study.

**Results:** Iron-supplemented infants had significantly lower plasma retinol concentrations and a significantly higher prevalence of vitamin A deficiency, as defined by a plasma retinol concentration  $<0.70 \mu\text{mol/L}$ , than did the nonsupplemented infants. In contrast, the modified relative dose response of the iron-supplemented infants indicated greater liver stores of vitamin A. Iron supplementation improved iron status, and zinc supplementation improved zinc status, but  $\beta$ -carotene supplementation did not significantly improve vitamin A status.

**Conclusions:** In this study, iron supplementation in infants with marginal vitamin A status led to lower plasma vitamin A concentrations and simultaneously to greater vitamin A liver stores. This implies a redistribution of retinol after iron supplementation, which might induce vitamin A deficiency. Therefore, iron supplementation in infants should be accompanied by measures to improve vitamin A status. *Am J Clin Nutr* 2003;77:651-7.

**KEY WORDS** Retinol, iron, zinc, ferritin, modified relative dose response, interaction, deficiency, supplementation, anemia

## INTRODUCTION

Iron deficiency and its associated anemia, as well as vitamin A deficiency, are still very prevalent worldwide, with children and pregnant women being the most vulnerable groups. Anemia and iron deficiency in children can lead to delayed psychomotor development and impaired health (1). Vitamin A deficiency is associated with a markedly higher morbidity and mortality of infectious diseases, and severe deficiency leads to xerophthalmia and blindness (2). Zinc deficiency leads to growth impairment, and zinc supplementation has been shown to decrease the morbidity and mortality of infectious diseases in children (3-5). In

many countries, major efforts are being made by governments, supported by international organizations, to reduce micronutrient deficiencies. For example, high-dose vitamin A supplements are globally distributed to children under 5 y of age, and a joint committee of WHO/UNICEF recommends supplementation with iron in infants from age 6 mo upward in countries with a high prevalence of iron deficiency anemia (6).

Micronutrient deficiencies often occur together in populations, because the same dietary patterns and socioeconomic factors are associated with deficiency in numerous micronutrients. In many developing countries, diets are mostly cereal-based, low in animal products, and high in phytate, which leads to a high risk for micronutrient deficiencies. In Indonesia, deficiencies in iron, vitamin A, and zinc are prevalent and often concurrent (7).

Interactions between iron, vitamin A, and zinc nutrition are of great consequence, especially in the context of micronutrient supplementation. Iron supplementation is widely used to combat iron deficiency, but high iron intake is known to impair zinc uptake (8). Furthermore, vitamin A supplementation affects iron metabolism, has been shown to decrease anemia prevalence, and has a synergistic effect with iron supplementation in reducing anemia (9). Provitamin A carotenoids, especially  $\beta$ -carotene, are the most important sources of vitamin A in Indonesia, but absorption, especially from leafy green vegetables, is insufficient (10). Severe zinc deficiency can impair vitamin A status (11), and zinc supplementation may be able to improve the uptake of  $\beta$ -carotene, its subsequent bioconversion to retinol, and the mobilization of vitamin A from body stores in populations with marginal zinc status (12).

The present study investigated whether supplementation with micronutrients, alone or in combination, enhances or impairs a

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<sup>2</sup> Supported by the Dutch Foundation for the Advancement of Tropical Research, the Ter Meulen Fund (Royal Netherlands Academy of Arts and Sciences), and UNICEF-Jakarta.

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Received January 8, 2002.

Accepted for publication June 7, 2002.

person's status with respect to other micronutrients. Interactions between iron,  $\beta$ -carotene, and zinc and the effectiveness of supplementation in improving micronutrient status were analyzed. Special attention was given to iron and vitamin A, because these micronutrients are included in many nutrition intervention programs, but usually only as single micronutrients.

## SUBJECTS AND METHODS

### Study design, subjects, and procedures

The study was a randomized, double-blind, placebo-controlled supplementation trial in infants aged 4 mo at recruitment. The study was carried out in a rural area of the Bogor District, West Java, Indonesia. Supplementation was given 5 d/wk for 6 mo by trained village health volunteers. Six groups of infants were supplemented with a sweet, strawberry-flavored, clear syrup (2 mL/d) and neutral-tasting, bright orange corn oil (0.5 mL/d). Four syrup preparations contained ferrous sulfate, zinc sulfate, ferrous sulfate + zinc sulfate, or neither ferrous sulfate nor zinc sulfate. The oil contained either  $\beta$ -carotene or annatto (both from Quest International, Naarden, Netherlands), which is a permitted food additive with no provitamin A activity but the same color as  $\beta$ -carotene. The iron and zinc supplements were produced by a local pharmaceutical company (Kenrose Ltd, Jakarta, Indonesia) in cooperation with UNICEF-Jakarta. The infants received either 10 mg Fe/d, 10 mg Zn/d, 10 mg each of Fe and Zn/d, 10 mg Zn + 2.4 mg  $\beta$ -carotene, 2.4 mg  $\beta$ -carotene, or placebo. For masking purposes, all infants received 2 bottles of supplements, one containing syrup and one containing oil.

Sample sizes, calculated for the effects of supplementation on plasma zinc (minimal expected difference: 2.5  $\mu$ mol/L) and plasma retinol (minimal expected difference: 0.13  $\mu$ mol/L) concentrations, were 32–56 infants/group, with a confidence level of 0.05 and a power of 0.90. To allow for dropouts, a target of 60 infants/group was set.

Eligible infants were identified by village health volunteers, and the mothers were invited to participate in the study. Mothers were informed of the procedures and purpose of the study. After written informed consent was given by the mother, infants were assessed anthropometrically, and a short history of health, diet, lactation, and socioeconomic status was taken. Infants were assigned to 1 of the 6 groups on the basis of individual randomization, with the use of a block-randomized group allocation list, which was generated by computer before the study began. At recruitment, each subject was allocated 2 bottles with 2 dosing syringes, which were labeled with the subject's name, subject number, and date. The bottles were kept by the health volunteer to prevent accidental overdosing. Bottles were weighed before allocation, replaced every month with 2 new bottles, and weighed again after return to estimate the dose given to the infant as a measure of compliance.

Supplementation was double-blind, and the supplements were coded with a letter at production. The allocation code was kept confidential at the Wageningen University until the study was finished.

At each monthly follow-up, the infant was assessed anthropometrically, and a short history of health, diet, lactation, and possible adverse effects of the supplements was taken. After 6 mo of supplementation, a blood sample was taken from the infants for

biochemical assessment of nutritional status. Five hours before the blood sampling, the infants received a dose of 3,4-didehydroretinol in corn oil (1.5 mg in 700  $\mu$ L oil) for the modified relative dose response (MRDR) test. The MRDR test uses the ratio of 3,4-didehydroretinol to retinol as an indicator of liver stores of vitamin A (13). When vitamin A liver stores are low, more 3,4-didehydroretinol appears in the blood relative to retinol concentrations. A ratio of  $>0.06$  of the concentrations in plasma is considered indicative of insufficient stores of vitamin A in the liver (13). All infants with a hemoglobin concentration of  $<110$  g/L were given iron-supplementation treatment on completion of the trial. Although the Indonesian government had endorsed vitamin A capsule distribution to infants at age 6 mo, this step was not implemented in the Bogor District at the time of the study. Infants received a vitamin A capsule at 12 mo of age via the customary primary health care services. The protocol was approved by the ethics committees of the National Health Research and Development Institute of Indonesia and of the Royal Netherlands Academy of Arts and Sciences.

### Measurements and analysis

Nonfasting venous blood samples (5 mL) were collected in heparinized evacuated tubes (Becton Dickinson, Leiden, Netherlands). Blood samples were immediately stored at 4 °C to prevent microhemolysis and were separated within 5 h. Plasma samples were aliquoted and stored at  $-30$  °C until they were analyzed.

Hemoglobin concentrations were measured the same day by a standard cyanoblu method (Humalyzer, Tanusstein, Germany; 14). Plasma zinc concentrations were analyzed with flame atomic absorption spectrophotometry (Varian, Clayton South, Australia) with the use of trace element-free procedures. The CV (10% duplicate analysis and pooled control samples) for zinc analyses was  $<5\%$ . Plasma retinol, carotenoid, and 3,4-didehydroxyretinol concentrations were measured by HPLC (Millipore Waters, Harrow, United Kingdom; 15). The 3,4-didehydroxyretinol and retinol were measured in the same extract as the carotenoids but with the use of a different mobile phase [methanol:water (90:10, by vol)]. The CV (10% duplicate analysis and pooled control samples) for the retinol and carotenoid analyses was  $<10\%$ . Ferritin concentrations were measured with the use of commercial enzyme-linked immunosorbent assay kits (IBL, Hamburg, Germany) according to the guidelines of the manufacturer. C-reactive protein (CRP) and  $\alpha_1$ -acid glycoprotein (AGP) were measured by immunoturbidimetric techniques (Cobas Fara Analyzer; Roche Products, Welwyn, United Kingdom). The CV for the ferritin, CRP, and AGP assays was  $<10\%$ . Plasma CRP and AGP concentrations were analyzed to assess the acute phase reaction, which decreases the plasma concentrations of zinc and retinol and increases those of ferritin (16). Plasma CRP concentrations increase early in the acute phase response but generally normalize within 2 wk. Plasma AGP concentrations increase more slowly than those of CRP, but they stay elevated for  $\leq 2$  mo after the initial acute phase response (17).

### Statistical analysis

Data were checked for normal distribution by use of the Kolmogorov-Smirnov test of normality. Plasma concentrations of ferritin and zinc were transformed to logarithms before statistical analysis. Infants with an elevated CRP (plasma CRP concentration  $>10$  mg/L) were excluded from calculations of the prevalence

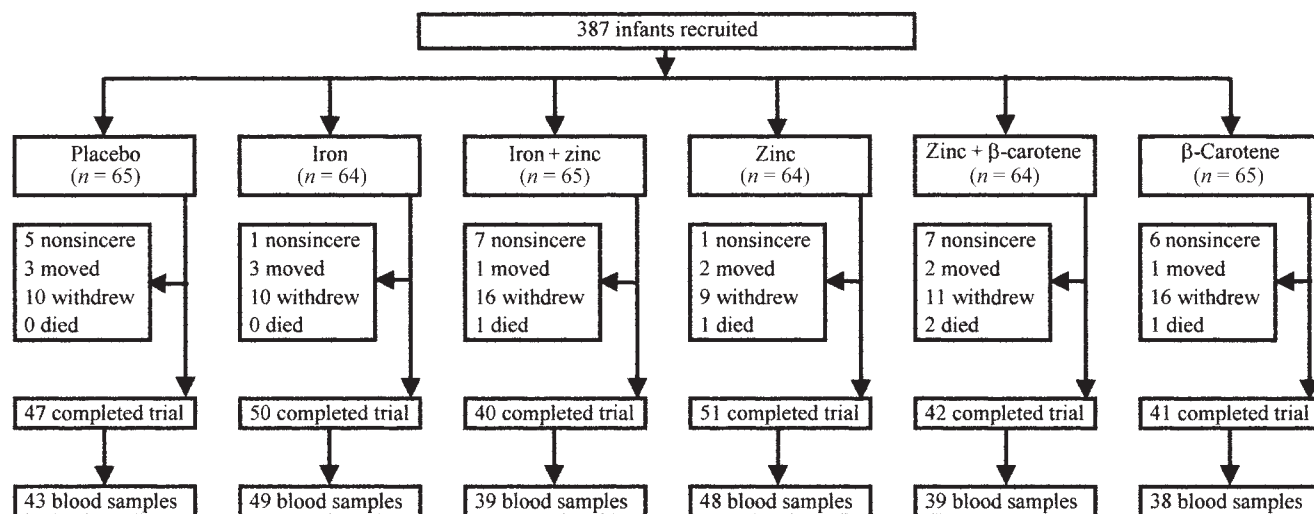


FIGURE 1. Trial profile. Infants labeled "nonsincere" were those who never returned for follow-up.

of low plasma retinol, iron deficiency anemia, and zinc deficiency, because those indicators are affected by the acute phase reaction, which makes the cutoff for deficiency less reliable. Because the number of infants with an elevated CRP was small, the mean or median concentrations of these indicators were hardly affected. Therefore, these infants were not excluded from the calculation of the mean or median concentrations, but CRP was included in the subsequent analysis as a covariate. Differences in prevalence were tested with Pearson's chi-square test, and differences between the infants who dropped out and the infants who completed the study were tested with Student's *t* test. Differences in compliance and plasma  $\beta$ -carotene concentrations were tested with the use of the nonparametric Kruskal-Wallis test. Differences in age, biochemical indicators, and anthropometry were evaluated with analysis of variance (ANOVA) or analysis of covariance (ANCOVA). To control for the effects of the acute phase response, plasma CRP and AGP concentrations were used as covariates in the analysis of plasma concentrations of retinol, ferritin, and zinc. Hemoglobin concentrations and the MRDR were not affected by the acute phase response. When the overall *F* test was significant, differences between the groups were further explored with post-hoc multiple comparisons (Dunnett's test) for ANOVA and in a general linear model (GLM) for ANCOVA. Plasma retinol concentrations were transformed to logarithms as a variance-stabilizing measure for GLM analyses when necessary.

Statistical analysis was carried out with SPSS software, version 7.5.2 (SPSS Inc, Chicago), and anthropometric *z* scores were calculated with EPI-INFO, version 6.04b (CDC, Atlanta).

## RESULTS

Between November 1997 and July 1998, 387 infants were recruited from 6 adjacent villages in the Bogor District. Of these infants, 271 completed the supplementation trial and 256 gave a complete blood sample (Figure 1). Of the recruited infants, 27 (7%) never returned for follow-up, and they were considered nonsincere recruits. An additional 89 infants (23%) dropped out of the study for various reasons [noncooperation, 18%; moving, 3%; and death, 1% ( $n = 5$ )]. The infants who dropped out and the infants who completed the trial did not differ significantly in any of the

characteristics at recruitment. The dropout rate among the groups ranged from 20% in the groups receiving iron or zinc to 39% in the group receiving the combination of iron and zinc (iron + zinc;  $P = 0.06$ , chi-square). At recruitment, the mean ( $\pm$ SD) age of the infants was  $4.2 \pm 0.4$  mo, and the male:female ratio was 206:181. Anthropometry was expressed as *z* scores, and the mean weight-for-age, height-for-age, and weight-for-height were  $-0.02 \pm 0.85$ ,  $-0.88 \pm 0.77$ , and  $0.82 \pm 0.82$ , respectively. There were no differences among the groups in any of the baseline characteristics. During the study, there was no significant difference in growth, either length or ponderal, among the supplementation groups. The median overall compliance was 91% (interquartile range: 76–98%) of the total intended dose, and it did not differ significantly between the groups.

After 6 mo of supplementation, micronutrient status was assessed (Table 1). The mean age of the infants was  $10.2 \pm 0.5$  mo. The infants receiving iron or iron + zinc had significantly lower plasma retinol concentrations than did the infants receiving placebo ( $P < 0.05$  and  $P < 0.001$ , respectively, ANCOVA with control for CRP). Hemoglobin concentrations were significantly higher in the infants receiving iron ( $P < 0.01$ , ANOVA), but the increase for the infants receiving iron + zinc was not statistically significant ( $P = 0.052$ , ANOVA). Plasma ferritin concentrations were highest in the group receiving iron and were significantly higher in the iron + zinc-supplemented group than in the placebo group ( $P < 0.001$  for both, ANCOVA with control for CRP). Plasma zinc concentrations were significantly higher in the groups receiving zinc and zinc +  $\beta$ -carotene ( $P < 0.001$ , ANCOVA controlling for CRP) and were significantly higher, but to a lesser extent, in the group receiving iron + zinc ( $P < 0.05$ ). The infants who received  $\beta$ -carotene supplementation, either alone or in combination with zinc, had significantly higher plasma  $\beta$ -carotene concentrations ( $P < 0.001$ , Kruskal-Wallis) than the other infants had, but their plasma retinol concentrations were not significantly higher.

The effect of the 3 supplemented micronutrients on plasma retinol concentrations was further investigated with the use of a GLM, with CRP included as a covariate. Supplementation with iron was associated with lower plasma retinol concentrations of  $0.15 \mu\text{mol/L}$  ( $P < 0.01$ ), but neither zinc nor  $\beta$ -carotene supplementation contributed significantly to changes in plasma retinol concentration.

**TABLE 1**

Biochemical indicators of the micronutrient status of infants after being supplemented 5 d/wk for 6 mo with 10 mg Fe/d, 10 mg Zn/d, 2.4 mg  $\beta$ -carotene/d, 10 mg each of Fe and Zn/d, 10 mg Zn/d and 2.4 mg  $\beta$ -carotene/d, or placebo<sup>1</sup>

	Supplementation group					
	Placebo (n = 43)	Iron (n = 49)	Iron + zinc (n = 39)	Zinc (n = 48)	Zinc + $\beta$ -carotene (n = 39)	$\beta$ -Carotene (n = 38)
Subjects with elevated CRP (n) <sup>2</sup>	5	6	7	6	3	5
Plasma retinol ( $\mu$ mol/L)	0.74 $\pm$ 0.26 <sup>3</sup>	0.62 $\pm$ 0.17 <sup>4</sup>	0.56 $\pm$ 0.17 <sup>5</sup>	0.71 $\pm$ 0.25	0.72 $\pm$ 0.23	0.77 $\pm$ 0.23
Plasma $\beta$ -carotene ( $\mu$ mol/L)	0.03 (0.02–0.08) <sup>6</sup>	0.03 (0.02–0.06)	0.03 (0.02–0.06)	0.04 (0.02–0.08)	0.11 (0.05–0.40) <sup>5</sup>	0.16 (0.07–0.35) <sup>5</sup>
Hemoglobin (g/L)	110 $\pm$ 11	118 $\pm$ 10 <sup>7</sup>	115 $\pm$ 9 <sup>8</sup>	110 $\pm$ 10	107 $\pm$ 10	112 $\pm$ 9
Plasma ferritin ( $\mu$ g/L)	15.2 (8.2–28.1)	38.2 (18.0–68.1) <sup>5</sup>	31.7 (17.4–41.6) <sup>5</sup>	10.9 (5.4–18.5)	9.6 (5.6–22.0) <sup>9</sup>	9.1 (5.5–22.3)
Plasma zinc ( $\mu$ mol/L)	14.2 (11.9–15.9)	13.5 (11.4–15.1)	15.5 (13.8–19.0) <sup>4</sup>	16.5 (13.7–21.0) <sup>5</sup>	16.5 (14.2–21.0) <sup>5</sup>	14.0 (12.0–15.5)

<sup>1</sup>CRP, C-reactive protein.

<sup>2</sup>Elevated CRP was defined as a plasma CRP concentration > 10 mg/L.

<sup>3</sup> $\bar{x} \pm$  SD.

<sup>4,5,7–9</sup>Significantly different from placebo group [ANOVA with Dunnett's post hoc test (hemoglobin), analysis of covariance (ferritin, zinc, and retinol) with control for CRP, or Kruskal-Wallis ( $\beta$ -carotene)]; <sup>4</sup> $P < 0.05$ , <sup>5</sup> $P < 0.001$ , <sup>7</sup> $P < 0.01$ , <sup>8</sup> $P = 0.052$ , <sup>9</sup> $P = 0.059$ .

<sup>6</sup>Median; interquartile range in parentheses.

In the placebo group, the prevalence of micronutrient deficiencies was high (**Figure 2**): 53% had marginal vitamin A deficiency as defined by a plasma retinol concentration < 0.70  $\mu$ mol/L, 49% were anemic (hemoglobin concentration < 110 g/L), 29% had iron deficiency anemia (anemia and plasma ferritin concentration < 12  $\mu$ g/L), and 11% had zinc deficiency (plasma zinc concentration < 10.7  $\mu$ mol/L). However, the prevalence of low plasma retinol concentrations was significantly higher in the infants receiving iron (70%) or iron + zinc (75%) supplementation than in the placebo group ( $P < 0.05$ , chi-square). In contrast, the prevalence of insufficient vitamin A liver stores, as indicated by an MRDR > 0.06, was lowest in the iron and iron + zinc groups ( $P < 0.001$ , chi-square; 51% and 49%, respectively, compared with 81% in the placebo group). As expected, in the groups receiving iron, either alone or in combination with zinc, the prevalences of anemia and iron deficiency anemia were lowest ( $P < 0.001$  for anemia and iron deficiency anemia in the iron group and  $P < 0.05$  for anemia and  $P < 0.01$  for iron deficiency anemia in the iron + zinc group; chi-square). The prevalence of vitamin A deficiency was 14% lower in the groups receiving  $\beta$ -carotene or zinc +  $\beta$ -carotene than in the placebo group, but the difference failed to reach statistical significance ( $P < 0.1$ , chi-square). Even though there was an effect of zinc supplementation on mean plasma zinc concentrations, the prevalence of zinc deficiency (plasma zinc concentration: < 10.7  $\mu$ mol/L) did not differ significantly among the supplementation groups.

Furthermore, there was a tendency for zinc supplementation to negatively affect iron status, especially in the infants receiving zinc +  $\beta$ -carotene. The prevalence of anemia was significantly higher in this group ( $P < 0.05$ ), and the prevalence of iron deficiency anemia was higher in the zinc and zinc +  $\beta$ -carotene groups, but the difference was not statistically significant.

The effect of iron supplementation on vitamin A status is clearly shown by the relation between plasma retinol concentration and MRDR (**Figure 3**). There is a strong linear relation between plasma retinol concentrations and the MRDR for the groups not receiving iron ( $r = -0.69$ ; 95% CI:  $-0.80, -0.57$ ;  $P < 0.001$ ), in that the infants with low plasma retinol concentrations had high MRDR values, which is indicative of depleted vitamin A liver stores. However, the groups receiving iron and iron + zinc supplements clearly form a separate subgroup ( $P < 0.001$ ,

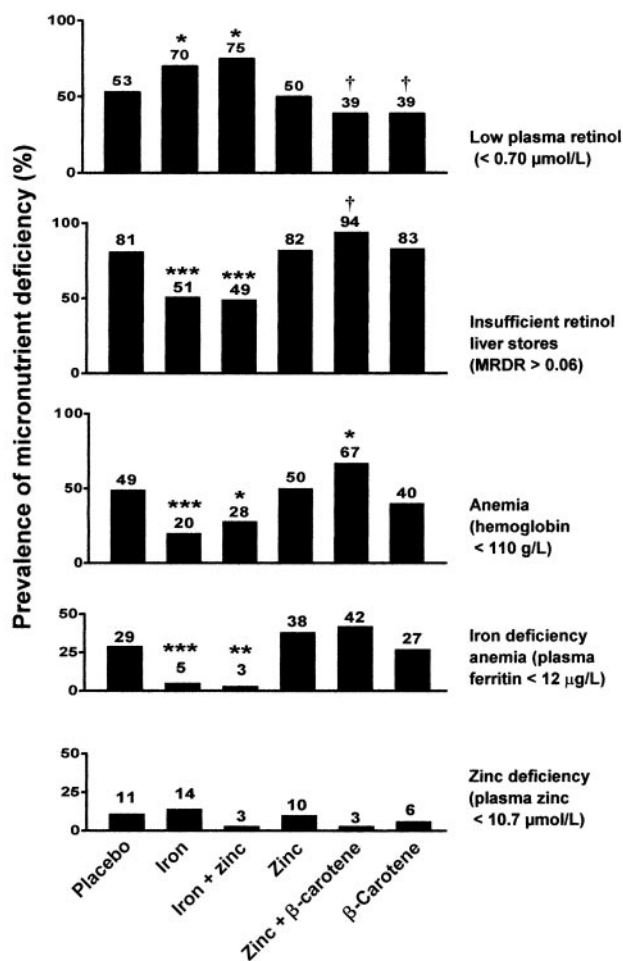
chi-square), placed outside this relation ( $r = 0.08$ ; 95% CI:  $-0.13, 0.30$ ;  $P > 0.1$ ). These infants, in contrast to those not receiving iron or iron + zinc (95% CI:  $-0.80, -0.57$  compared with  $-0.13, 0.30$ ;  $P < 0.01$ ), have low plasma retinol concentrations in combination with low MRDR values, which is indicative of replete vitamin A liver stores.

The relative effects of plasma retinol concentration and micronutrient supplementation on the MRDR were further investigated in a GLM. Both plasma retinol concentration and iron supplementation contributed significantly ( $P < 0.01$ ) to changes in the MRDR, whereas the other supplemented micronutrients did not. Furthermore, there was a significant interaction between plasma retinol concentration and iron supplementation in this model ( $P < 0.01$ ).

## DISCUSSION

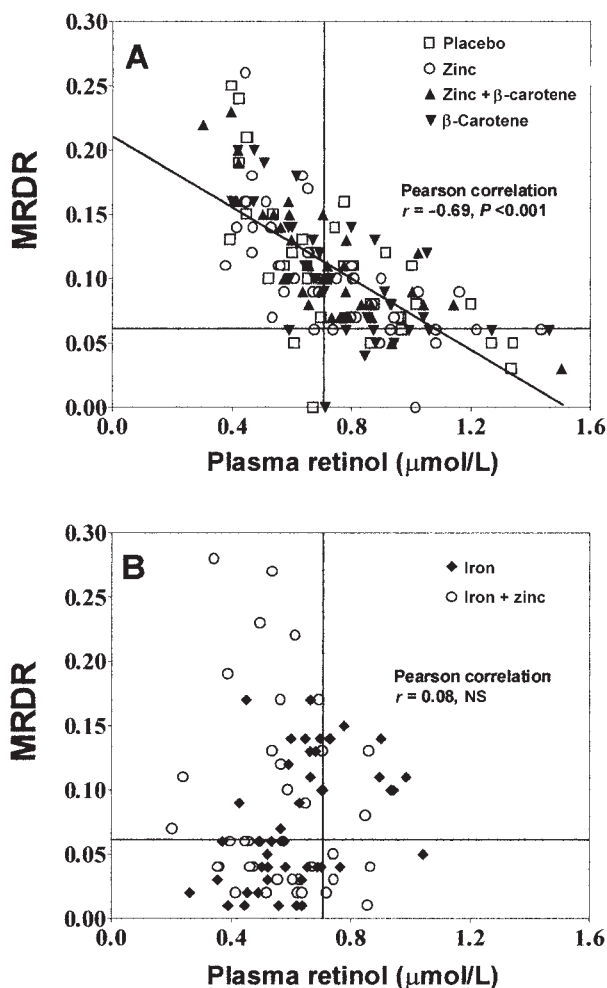
We found that in Indonesian infants a daily supplement of 10 mg Fe, which does not differ greatly from the recommended dietary allowance, led to markedly lower plasma retinol concentrations and simultaneously to higher liver vitamin A concentrations. Hence, we surmise that iron supplementation leads to a redistribution of retinol from the plasma to the liver. The prevalence of marginal vitamin A deficiency as defined by a plasma retinol concentration < 0.70  $\mu$ mol/L was > 20% higher in the infants receiving iron supplementation. We believe that this is a cause for concern, because plasma retinol concentrations are directly related to such consequences of vitamin A deficiency as xerophthalmia (18). In general, plasma retinol concentrations reflect improved vitamin A status after interventions (19). Moreover, Christian et al (20) reported that hyporetinolemia caused by a redistribution of retinol during the acute phase response is related to night blindness, a functional sign of vitamin A deficiency. A redistribution of retinol from the plasma to the liver reduces the amount of circulating retinol, which could reduce the availability of retinol to target cells and thus induce a state of functional vitamin A deficiency. This effect of iron supplementation would be especially important in populations whose vitamin A status is already marginal.

The effect of iron supplementation on vitamin A distribution has not been reported previously. Earlier, our group reported



**FIGURE 2.** Prevalence of micronutrient deficiency or depletion in infants supplemented with various combinations of micronutrients. For the graphs depicting the prevalence of insufficient liver stores [as determined by the modified relative dose response (MRDR) test] and anemia, all infants are included ( $n = 256$ ). For all other graphs, infants with plasma C-reactive protein concentrations > 10 mg/L are excluded ( $n = 224$ ). The total number of infants and the number of infants with plasma C-reactive protein concentrations > 10 mg/L are specified per group in Table 1. Significantly different from the placebo group (chi-square): \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , † $P < 0.1$ .

that iron supplementation had no effect on plasma retinol concentrations in pregnant women (9). However, only 10% of the pregnant women were vitamin A deficient, and they received supplementation for only 8 wk. No effect of either iron or zinc supplementation on plasma retinol concentrations was found in children in Mexico, although plasma retinol concentrations were found to be higher in all supplemented groups than in the other groups 6 mo after supplementation had stopped (21). Animal studies have shown a relation between iron status and vitamin A distribution, but results have been inconsistent. Rosales et al (22) showed that the distribution of retinol through various body compartments is dependent on iron status, with higher concentrations of vitamin A in the livers of food-restricted control rats than in those of iron-deficient rats. In contrast, Jang et al (23) showed that iron deficiency inhibits the mobilization of retinol stores in rats and may impair the absorption and utilization of vitamin A. A possible explanation could



**FIGURE 3.** Relations between the modified relative dose response (MRDR) and plasma retinol concentrations in infants supplemented with various combinations of micronutrients: A) all infants not supplemented with iron and B) infants supplemented with either iron or iron + zinc. Reference lines indicate cutoffs for marginal vitamin A deficiency (plasma retinol < 0.70  $\mu$ mol/L) and insufficient vitamin A liver stores (MRDR > 0.06).

be that deficiency and supplementation of iron have different effects on the complicated intrahepatic balance between cellular uptake and mobilization of vitamin A (24).


Observations of the interaction between vitamin A and iron metabolism can be obscured by the acute phase response, which leads to increased plasma ferritin concentrations and to decreased plasma retinol concentrations. In the present study, CRP was included in the analyses to control for these effects. The proportion of infants with plasma CRP concentrations > 10 mg/L did not differ significantly among the supplementation groups. Furthermore, the exclusion of the 32 infants with a CRP concentration > 10 mg/L did not change the findings. The use of a different acute phase protein (ie, AGP) as a covariate or an exclusion criterion (plasma AGP concentration > 1.2 mg/L; excluded:  $n = 56$ ) also gave identical results. Hence, the decrease in plasma retinol concentrations in the iron-supplemented groups cannot be attributed to differences in the acute phase response.

An alternative explanation for the lower plasma retinol concentrations after iron supplementation may be that vitamin A

requirements are increased because of accelerated erythropoiesis. However, such an explanation would not be consistent with the reduced MRDR, which signifies increased retinol liver stores.

The MRDR method may have certain limitations. The precision is less than that of the measurement of plasma retinol, because the MRDR method is derived from the ratio of the concentration of 3,4-dihydroxyretinol to that of retinol. In addition, the MRDR is less sensitive in protein-energy malnutrition, because the low concentration of retinol-binding protein in protein-energy malnutrition distorts the competitive binding between 3,4-dihydroxyretinol and retinol (25). However, because the effect of iron supplementation on the MRDR was highly significant and because the infants were not malnourished, it may be assumed that the MRDR in the present study adequately reflects retinol liver stores.

Iron and zinc are known to mutually inhibit the other's uptake from the intestinal lumen via competitive inhibition (8, 26). This phenomenon is also reflected in this study by the lower efficacy of iron + zinc supplementation to improve iron status and by the higher prevalence of anemia and iron deficiency anemia in the zinc-supplemented groups not receiving iron. It is interesting that  $\beta$ -carotene appears to exacerbate the negative effect of zinc supplementation on iron status, possibly by increasing the utilization of iron for erythropoiesis (9). In the present study, no synergistic effect of zinc and  $\beta$ -carotene supplementation on vitamin A status was seen. The lack of effect of zinc supplementation on growth in Indonesian infants was described in an earlier report (27).

Deficiencies of both vitamin A and iron are prevalent worldwide and often occur together. However, most efforts to combat these deficiencies are directed toward only one of these micronutrients. In view of the possible adverse effect of iron supplementation on vitamin A status found in this study, supplementation with iron alone in infants may compromise vitamin A status. Whether redistribution of retinol after iron supplementation occurs only in populations with a high prevalence of both iron deficiency and vitamin A deficiency remains to be investigated. However, this question is of great importance because these are precisely the populations targeted by micronutrient supplementation programs. 

We thank all the mothers and infants and the health volunteers who participated in this study, and we are grateful for the enthusiastic support we received from Dr Hendra, Dr Anni, and their staff at the Situ Udik Health Centre, Bogor Sistrir. Furthermore, we thank the field team of Puslitbang Gizi and the staff from the Bogor laboratory for their untiring efforts. We thank Jen-Shiu Chiang-Chiau, C Northrop-Clewes, and J Coulter from NICHE, the University of Ulster, for their help with the analysis of retinol, 3,4-didehydroretinol, carotenoids, CRP, and AGP; S Tanumihardjo from the Department of Nutrition Sciences, University of Wisconsin, for providing the 3,4-didehydroretinol and sharing her experience with MRDR testing; Quest International, Naarden, The Netherlands, for supplying  $\beta$ -carotene and annatto; and M Van der Heuvel for commenting on earlier drafts of the manuscript.

FTW and MAD were involved in the study design, data collection, data analysis, and writing of the manuscript. CEW was involved in the study design, data analysis, and writing of the manuscript; DIT was involved in the data analysis and writing of the manuscript; M was involved in the study design, data collection, and data analysis; and JWMVdM was involved in the study design and data analysis. All authors contributed to the final version of the manuscript. None of the authors had any financial or personal interest in any company or organization sponsoring the research, including advisory board affiliations.

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