Influence of iron on vitamin A nutritional status

Julicristie M Oliveira, Fernanda B Michelazzo, Juliana Stefanello, and Patrícia HC Rondó

Iron deficiency seems to deteriorate vitamin A metabolism leading to a reduction in serum retinol and an increase in hepatic retinol and retinyl ester. These alterations probably result from an increase in retinol sequestration to the liver and/or impairment in the activity of hepatic retinyl ester hydrolases decreasing vitamin A mobilization.

INTRODUCTION

Vitamin A plays an important role in normal vision, gene expression, growth and physical development, maintenance and proliferation of epithelial cells, and immune function. Iron plays an essential role in several metabolic processes including oxygen transport, oxidative metabolism, and cellular growth. Iron deficiency and vitamin A deficiency (VAD) are the most prevalent micronutrient deficiencies in developing countries; they particularly affect women of reproductive age and preschool children. These deficiencies often occur together as a consequence of dietary patterns and associated social factors.

VAD is associated with increased risks of infant mortality and morbidity from measles and diarrheal infections, blindness, and anemia. Among women, this deficiency is also associated with high mortality related to pregnancy. Iron deficiency and iron deficiency anemia in children can lead to impaired psychomotor development and may have long-term consequences. In women, iron deficiency anemia is associated with premature birth, low birth weight and increased risk of maternal mortality.

The influence of vitamin A on iron status is well described in the literature. Evidence indicates that VAD is associated with reduced incorporation of iron into erythrocytes, and low hemoglobin (Hb) concentration. The studies also reported associations of VAD with reduced serum iron, low total iron-binding capacity (TIBC) and transferrin saturation, and increases in serum ferritin, with a higher deposition of iron in the liver and spleen, but there is no consensus about the effect of VAD on iron absorption.

Several studies showed a positive effect of vitamin A on iron status in humans with anemia and in animals with VAD but without anemia. Supplementation trials that combine these two micronutrients have shown a higher impact on the reduction of iron deficiency anemia than the administration of either iron or vitamin A alone. In spite of this evidence, most efforts to combat these deficiencies have been directed to the use of a single micronutrient.

Few studies have investigated the effects of iron on vitamin A metabolism and nutritional status. Studies on rats have shown that iron deficiency is associated with low plasma retinol concentration and increased hepatic vitamin A, a higher molar ratio of hepatic retinyl ester to retinol (increased vitamin A deposition), which has been associated with lower blood Hb level. The results of these experiments suggest that liver vitamin A metabolism is altered and the mobilization of this vitamin might be impaired in the presence of iron deficiency.

EXPERIMENTAL STUDIES IN ANIMALS

The following investigations were carried out to determine the molecular mechanisms whereby iron and vitamin A interact. In a study involving rats, Rosales et al. investigated in which situations iron deficiency alters the retinol concentration in plasma or liver. The animals were divided into four groups (n = 10 per group), each of which received controlled amounts of a diet.
containing one of the following quantities of iron per kg ration for 5.5 weeks: 3 mg (low iron), 15 mg (marginal iron), 35 mg (control diet with restricted food intake), and 35 mg (control diet ad libitum) (Table 1). The authors observed a significantly lower plasma retinol concentration in the groups receiving 3 mg and 35 mg-restricted food intake compared to the groups fed 15 mg and 35 mg-ad libitum. Paradoxically, the liver vitamin A concentration was higher in the group receiving 35 mg-restricted food intake with more hepatic accumulation of retinol and retinyl ester compared to the other groups. The statistical analysis was adjusted by differences in plasma volume, liver, and body weights. Thus, the effect of growth retardation secondary to iron deficiency was controlled. The molar ratio of hepatic retinyl ester to retinol is reduced in iron-deficient rats as a result of increased esterification of hepatic retinol.

Strube et al.17 investigated the effects of iron and vitamin A deficiencies alone and in combination with hematological, biochemical, and molecular indices of these micronutrients. Male rats aged 3 to 8 weeks were fed on diets that had adequate or restricted iron and vitamin A: low iron (3.34 mg/kg) + normal vitamin A (405 µg/kg), low iron (3.03 mg/kg) + low vitamin A (55 µg/kg), normal iron (22.20 mg/kg) + low vitamin A (51 µg/kg), and normal iron (20.30 mg/kg) + normal vitamin A (367 µg/kg), with the latter group serving as control (Table 1). Iron restriction reduced weight gain, feed efficiency, Hb, hematocrit (Ht), serum iron, and transferrin saturation. The authors detected that dietary iron affected plasma and hepatic retinol concentration. Plasma retinol decreased progressively in the following groups: normal iron and normal vitamin A > normal iron and low vitamin A > low iron and normal vitamin A > low iron and low vitamin A (p < 0.05). On the other hand, the hepatic retinol concentrations were higher (p < 0.05) in the low iron and low vitamin A group than in the normal iron and low vitamin A group. Similarly, the hepatic retinol concentrations were higher in the low iron and normal vitamin A group compared to the normal iron and normal vitamin A group. Hepatic transferrin mRNA and transferrin receptor mRNA levels were elevated in the groups subjected to iron restriction, as was serum ferritin light-chain protein and mRNA. In contrast, ferritin heavy-chain mRNA, hemopexin, ceruloplasmin, and cellular retinol-binding protein mRNA were not affected by iron and/or vitamin A restriction. Marginal vitamin A deficiency did not exacerbate the poor iron parameters during iron deficiency, but iron deficiency was associated with low plasma retinol and increased hepatic vitamin A concentrations, indicating impaired mobilization of liver vitamin A stores during iron deficiency.

In the study by Jang et al.18 16 male rats were divided into two groups (eight per group), which received either a free-access diet containing low iron (4 mg/kg) + vitamin A (716 µg/kg) or a control diet, with access to food restricted, containing adequate iron (45 mg/kg) + vitamin A (716 µg/kg) for 15 weeks (Table 1). The authors used a model-based compartmental analysis to assess the effects of iron deficiency on vitamin A dynamics, confirming the previously proposed hypothesis that plasma retinol is reduced in iron-deficient rats as a result of reduced vitamin A mobilization from the liver to plasma and/or of increased vitamin A deposition in the liver. After 15 weeks of dietary treatment, plasma retinol concentration in rats receiving the low-iron diet was significantly lower than in rats receiving the control diet (1.34 ± 0.11 vs 0.53 ± 0.11 µmol/L, p < 0.001). In contrast, liver vitamin A status in rats receiving reduced iron was greater than in rats receiving the control diet (808.5 ± 94.0 vs 112.2 ± 23.5 nmol/L, p < 0.050). The authors indicated that this situation might result from a reduction in the activity of one or more retinyl ester hydrolases, with a consequent decrease in vitamin A mobilization even in the presence of sufficient or increased quantities of this vitamin.

STUDIES IN HUMANS

In a longitudinal, double-blind, placebo-controlled trial, 219 Mexican children aged 1.5 to 3 years were divided into four groups, which received zinc (20 mg/day), iron (20 mg/day), iron (20 mg/day) + zinc (20 mg/day), or placebo, for 6 months (Table 2). At baseline, there were no differences between the nutritional status of the groups assessed by the mean values of weight-for-height z-score and height-for-age z-score. The prevalence of anemia, low plasma concentrations of ferritin, zinc, and retinol were, respectively, 73%, 51%, 25%, and 29%, but there were no statistically significant differences among the groups. The authors observed that a single supplementation with zinc resulted in a significant increase (p < 0.05) of serum retinol (0.08 µmol/L) and transthyretin-TTR (42 mg/L), and a non-significant increase of retinol-binding protein (RBP) (1.9 mg/L). Combined supplementation (zinc + iron) had a beneficial effect on serum retinol (increase of 0.08 µmol/L), when compared to the placebo group (p < 0.05). Supplementation with iron was associated with a significant increase in serum retinol (0.27 µmol/L), RBP (5.4 mg/L), and TTR (33 mg/L) compared to the placebo group (p < 0.05). Therefore, iron supplementation alone

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### Table 1: Experimental studies in animals to evaluate the influence of iron on vitamin A status.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Design</th>
<th>Animals</th>
<th>Intervention (groups)</th>
<th>Time (weeks)</th>
<th>Results</th>
<th>Conclusions</th>
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</thead>
<tbody>
<tr>
<td>Jang et al. (2000)16</td>
<td>Model-based compartmental analysis</td>
<td>16 22-day-old male Sprague-Dawley rats</td>
<td>Low iron diet = AIN93G diet with 716 μg of VA*/kg + 4 mg of iron/kg – free access to diet</td>
<td>15</td>
<td>After 15 weeks Ht = 0.22 ± 0.01, Hb = 37.20 ± 3.40, liver Fe = 4.10 ± 0.47, serum retinol = 0.52 ± 0.11, liver retinol = 808.50 ± 94.00, VA balance = 6.80</td>
<td>Significant differences between the groups (p &lt; 0.05)</td>
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<td>Adequate iron diet (control) = AIN93G diet with 716 μg of VA*/kg + 45 mg iron/kg – food restricted*</td>
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<tr>
<td>Rosales et al. (1999)16</td>
<td>Animals randomly assigned to 4 different dietary groups</td>
<td>40 21-day-old male Sprague-Dawley rats</td>
<td>Low iron diet – ad libitum = AIN93G diet with 3 mg of iron/kg</td>
<td>5.5</td>
<td>After 5.5 weeks Molar ratio = 20.1 ± 1.4</td>
<td>Significant differences between low iron diet group and the other groups (p = 0.02)</td>
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<td>Marginal iron diet – ad libitum = AIN93G diet with 15 mg of iron/kg</td>
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<td>Control – ad libitum = AIN93G diet with 35 mg of iron/kg</td>
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<td>Control – restricted## = AIN93G diet with 35 mg of iron/kg</td>
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<tr>
<td>Strube et al. (2002)17</td>
<td>2×2 randomized block study</td>
<td>4 lactating§ Sprague-Dawley rats with 10 male pups each</td>
<td>When the pups were 21 days old they were randomly assigned to one of the following groups:</td>
<td>5</td>
<td>After 5 weeks Body weight = 270.7 ± 10.4 g, liver weight = 9.45 ± 0.30 g, relative liver weight** = 3.49 ± 0.08</td>
<td>Significant differences among groups for body weight and liver weight (p &lt; 0.05). No differences for relative liver weight.</td>
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<td>Normal iron and normal VA diet – ad libitum = AIN93 diet with 367 μg of VA*/kg + 20.30 mg of iron/kg</td>
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<td>Low iron and normal VA diet – ad libitum = AIN93 diet with 405 μg of VA*/kg + 3.34 mg of iron/kg</td>
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<td></td>
<td>Normal iron and low VA diet – ad libitum = AIN93 diet with 51 μg of VA*/kg + 22.20 mg of iron/kg</td>
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<td>Low iron and low VA diet – ad libitum = AIN93 diet with 55 μg of VA*/kg + 3.03 mg of iron/kg</td>
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</table>

*Vitamin A (retinyl palmitate).
† Ferrous sulfate.
‡ Restricted to an amount of diet equal to the average consumed by the low iron diet group.
§ The dams were fed with VA-free diet upon arrival.
# Ferric citrate.
** Relative liver weight = liver weight/body weight (g/100g).

Abbreviations and units of measure: Fe, iron; serum or plasma retinol (μmol/L); Hb, hemoglobin (g/L); Ht, hematocrit; VA balance, liver retinol after 15 weeks – liver retinol after 8 weeks/48 days.
### Table 2  Controlled clinical trials in humans investigating the impact of iron supplementation alone or in combination with other micronutrients on indicators of iron and vitamin A status.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country and population (age)</th>
<th>No. of subjects</th>
<th>Intervention (groups)</th>
<th>Time (months)</th>
<th>Results</th>
<th>Conclusions</th>
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<tbody>
<tr>
<td>Graham et al. (2007)</td>
<td>Nepal</td>
<td>96</td>
<td>Fortified rice with 850 μg of RAE + capsule with 10 mg of Fe + 6 mg of Riboflavin 6 days/week</td>
<td>1.5</td>
<td>Differences between baseline and post-supplementation</td>
<td>Significant differences for ferritin, erythrocyte riboflavin, ferritin/albumin (p &lt; 0.001), and pupillary threshold (p &lt; 0.05).</td>
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<td></td>
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<td>Fortified rice with 850 μg of RAE + placebo 6 days/week</td>
<td></td>
<td>↑ Retinol = 0.04, ferritin/albumin = 1.87, hemoglobin/albumin = 0.16, erythrocyte riboflavin = 92.2, ferritin = 3.9, ↓ Hb = −2.7, albumin = −0.2, pupillary threshold = −57.1</td>
<td></td>
</tr>
<tr>
<td>Muñoz et al. (2000)</td>
<td>Mexico</td>
<td>219</td>
<td>Children (1.5–3 years)</td>
<td>6</td>
<td>Differences between baseline and post-supplementation</td>
<td>Fe supplementation alone was more effective in increasing serum retinol and RBP (p &lt; 0.05).</td>
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<td></td>
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<td>20 mg of Zn/day</td>
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<td>↑ Hb = 8.0, plasma Zn = 3.63, RBP = 1.9, TTR = 42.0, retinol = 0.08, ferritin = 2.3</td>
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<td>20 mg of Fe/day</td>
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<td>↑ Hb = 14.0, plasma Zn = 0.28, RBP = 8.4, TTR = 13.0, retinol = 0.27, ferritin = 16.0</td>
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<td>20 mg of Fe + 2 mg of Zn/day</td>
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<td>↑ Hb = 13.0, plasma Zn = 1.62, RBP = 2.0, TTR = 23.0, retinol = 0.08, ferritin = 18.6</td>
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<td>Placebo</td>
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<td>↑ Hb = 8.0, plasma Zn = 0.18, RBP = 0.9, TTR = 14.0, retinol = −0.05, ferritin = −4.6</td>
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<tr>
<td>Shatrugna et al. (1997)</td>
<td>India</td>
<td>82</td>
<td>Pregnant women † (17–24 weeks’ gestation)</td>
<td>3–4</td>
<td>Differences between baseline and post-supplementation</td>
<td>Significant reduction in VAD in the 120 mg Fe and VA + Fe groups.</td>
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<td>60 mg of Fe + 500 μg of folic acid/day</td>
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<td>↑ Hb = 8.5, retinol = 0.01, ↓ VAD prevalence = 5.0%</td>
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<td>120 mg of Fe + 500 μg of folic acid/day</td>
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<td>↑ Hb = 9.2, retinol = 0.12, ↓ VAD prevalence = 20.7%</td>
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<td>60 mg of Fe + 500 μg of folic acid + 1800 μg of VA/day</td>
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<td>↑ Hb = 8.9, retinol = 0.20, ↓ VAD prevalence = 31.6%</td>
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</table>

Abbreviations and units of measure: albumin (g/dL), erythrocyte riboflavin (μmol/L), Fe, elementary iron; Hb, hemoglobin (g/L); plasma ferritin (μg/L); plasma Zn, plasma zinc (μg/dL); pupillary threshold, impaired pupillary threshold (≤ 1.11 log cd/m²); RAE, retinol activity equivalent; retinol, serum or plasma retinol (μmol/L), RBP, retinol binding protein (mg/L); TTR, transthyretin (mg/L); VAD, vitamin A deficiency; Zn, elementary zinc.

*8–10 weeks’ gestation. †17–24 weeks’ gestation.
was found to be more effective in increasing serum retinol and RBP compared to supplementation with zinc and iron.\(^4\)

Wieringa et al.\(^5\) conducted a randomized, double-blind, placebo-controlled supplementation trial on 256 Indonesian infants aged four months with supposed marginal vitamin A deficiency. The children were randomly assigned to receive iron (10 mg), zinc (10 mg), beta-carotene (2.4 mg), or placebo, five days a week for six months. After the intervention, plasma retinol concentration was significantly lower and liver vitamin A store was greater in the iron and iron + zinc groups. The authors suggest that a possible redistribution of serum retinol and impairment in VAD may explain the differences observed after supplementation. However, baseline serum retinol was not assessed, which lead to a limited interpretation of results. Systematic differences between groups at baseline can mislead the results of supplementation in these groups of children. Therefore, it was not possible to evaluate the impact of the intervention, so this article was not included in Table 2.

In a study conducted by Shatrugna et al.\(^19\) in India, 82 pregnant women (gestational stage 17–24 weeks) were supplemented with iron (60 mg/day) + folic acid (500 μg/day), iron (120 mg/day) + folic acid (500 μg/day), or iron (60 mg/day) + folic acid (500 μg/day) + vitamin A (1800 μg/day) for three to four months (Table 2). An increase of serum retinol concentration of 0.21 and 0.12 μmol/L was observed in the groups receiving vitamin A and 120 mg of iron, respectively. Thus, a significant decrease in the prevalence of vitamin A deficiency was observed in both the vitamin A group (31.6%) and the group receiving 120 mg iron (20.7%) at the end of treatment. A limitation of the study is that the authors did not describe the nutritional status of the women at baseline, they just noted that 50% of the subjects had vitamin A levels lower than 30 μg/dL.

Graham et al.\(^20\) conducted a randomized, double-blind, placebo-controlled supplementation trial on 96 night-blind Nepalese pregnant women. The women were randomly assigned to receive vitamin A-fortified rice (850 μg of retinol activity equivalent [RAE]) with 30 mg of iron + 6 mg of riboflavin (capsule) or vitamin A-fortified rice (850 μg of RAE) with a placebo capsule (control), six days a week for one and a half months (Table 2). The prevalence of VAD (plasma retinol < 0.7 μmol/L) was 18% in the control group and 27% in the experimental group at baseline. There was no significant reduction in the prevalence of VAD after supplementation \((p = 0.52)\). At the end of the treatment period, all of the women reported recovery from night blindness. However, 30.2% of the women supplemented with vitamin A + iron + riboflavin and 39.5% of the women from the control group still had an abnormal dark adaptation threshold at the end of the study. The final prevalence of impaired pupillary threshold was not significantly different among the groups, but the reduction was greater in the vitamin A + iron + riboflavin group \((\geq 1.11 \log \text{cd/m}^2)\) than in the control group (Table 2). The change in pupillary threshold was evaluated for interactions between treatment group and baseline status of vitamin A, iron, riboflavin, and iron deficiency anemia. All of them except riboflavin \((p = 0.13)\) interact significantly with the treatment group. Interestingly, women with poor iron status at baseline who were supplemented with vitamin A + iron + riboflavin had a significant improvement in the pupillary threshold compared to the women from the control group \((-1.41 \pm 0.20\) vs \(-0.65 \pm 0.25 \log \text{cd/m}^2, p = 0.05)\).

**CONCLUSIONS**

Although the results from three studies on rats indicate that iron deficiency is associated with reduced serum retinol concentration and increased hepatic vitamin A, there is no consensus among them and the results from studies on humans. According to Muñoz et al.\(^4\) and Shatrugna et al.\(^19\) iron supplementation had a significant impact on the vitamin A indicators investigated. The studies by Muñoz et al.\(^4\) and Wieringa et al.\(^5\) were both randomized, double-blind, placebo-controlled trials involving children from developing countries. Nevertheless, there were some methodological differences that could account for their conflicting results. In the study by Wieringa et al.\(^5\) the biochemical parameters were not evaluated at baseline; thus, it is not possible to assure that the changes described really occurred.

The results described by Muñoz et al.\(^4\) were somewhat surprising. In their trial, zinc supplementations (alone or combined with iron) were less effective than iron supplementation alone. The interactions between zinc and vitamin A are well described.\(^21\) Thus, a greater impact of the intervention with this mineral was expected. Moreover, the interaction between iron and zinc could interfere with the results. One recent trial found that supplementation with zinc and iron together reduced the prevalence of anemia by 21% and zinc deficiency by 10%, but it was less effective than supplementation with either iron alone (28% reduction in anemia) or zinc alone (18% reduction in zinc deficiency).\(^22\) Therefore, it is possible that in the trial by Muñoz et al.\(^4\) supplementation with iron and zinc together reduced the efficacy of each micronutrient on serum retinol and RBP improvement. Furthermore, it was interesting to note that iron alone had a greater impact on the serum retinol and RBP levels than zinc alone. The prevalence of iron deficiency anemia was almost three times higher than the
prevalence of zinc deficiency at baseline (73% vs 25%). It is possible that the impact of iron supplementation was more expressive due to this difference.

Only one study assessed the impact of iron supplementation on night blindness and dark adaptation threshold. Night blindness is recognized as a functional sign of VAD, but zinc, riboflavin, and omega-3 fatty acid are also involved in the visual process. In the trial by Graham et al., the prevalence of impaired pupillary threshold was significantly lower in the vitamin A + iron + riboflavin group compared to the controls (vitamin A only). Adjusting the analysis according to the baseline nutritional status, the authors found a higher impact in women who were iron and/or vitamin A deficient. The result suggests a possible role of iron in the dark adaptation process and deserves further investigation.

Several lines of evidence indicate that vitamin A metabolism is altered in situations of iron deficiency, characterized by low serum retinol concentrations and increased hepatic retinyl ester or retinol stores. These changes probably result from a reduction in the activity of retinyl ester hydrolases, with a consequent decrease in vitamin A mobilization, or from an increase in retinol sequestration to the liver. Thus, micronutrient supplementation programs in regions where vitamin A and iron deficiencies coexist should consider this interaction.

The antagonistic or synergistic effects between two or more micronutrients need to be investigated in detail considering that micronutrient deficiencies occur concomitantly, especially zinc, iron, and vitamin A deficiencies. The precise relationship between vitamin A and iron depends on many factors, such as the degree of micronutrient deficiencies, presence of inflammation, acute or chronic diseases, etc. Further studies involving different populations are necessary to elucidate the interaction between these micronutrients. We recommend conducting randomized controlled clinical trials comparing the effects of vitamin A supplementation alone or in combination with iron and/or zinc in areas where both deficiencies coexist. The impact of supplementation regimens should be monitored with more biochemical markers of vitamin A and iron status than serum retinol and hemoglobin concentrations, i.e., serum RBP, molar ratio of serum RBP to TTR, retinyl esters hepatic stores, serum ferritin, serum transferrin receptor, zinc protoporphyrin/heme ratio, divalent metal transporter-1, ferroportin-1. The evaluation of visual parameters such as night blindness and pupillary threshold might also be useful to clarify the mechanisms whereby these micronutrients interact.

REFERENCES


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