Impact of vitamin A supplementation on anaemia and plasma erythropoietin concentrations in pregnant women: a controlled clinical trial


Abstract: Introduction: Although studies suggest that vitamin A or its metabolites influence the synthesis of erythropoietin in vitro and in animal models, it is unclear whether vitamin A supplementation increases plasma erythropoietin concentrations in humans. Objective: To determine whether daily vitamin A supplementation increases plasma erythropoietin concentrations in pregnant women with a high prevalence of anaemia. Methods: A randomized, double-blind, controlled clinical trial was conducted to examine the effect of daily vitamin A (3000 μg retinol equivalent), iron (30 mg), and folate (400 μg) versus iron (30 mg) and folate (400 μg) (control) on haemoglobin and plasma erythropoietin concentrations in 203 pregnant women in Malawi, Africa. Results: Mean gestational age at enrollment was 23 wk, at which time 50% of the women were anaemic (haemoglobin <110 g/L). Mean (±SEM) change in haemoglobin from enrollment to 38 wk was 4.7±1.6 g/L (p=0.003) and 7.3±2.3 g/L (p=0.003) in the vitamin A and control groups, respectively. Mean change in plasma erythropoietin concentrations from enrollment to 38 wk was 2.39±5.00 (p=0.63) and −2.87±3.92 IU/L (p=0.46) in the vitamin A and control groups, respectively. There were no significant differences between vitamin A and control groups in the slope of the regression line between log₁₀ erythropoietin and haemoglobin at enrollment or 38 wk, and between enrollment and follow-up within either group. Conclusions: Vitamin A supplementation does not appear to increase haemoglobin and plasma erythropoietin concentrations among pregnant women with a high prevalence of anaemia in Malawi.

Vitamin A is known to play a role in hematopoiesis, but the biological mechanisms by which vitamin A influences hematopoiesis are not well understood (1, 2). Controlled trials in Guatemala (3), Indonesia (4), and Belize (5) demonstrated that vitamin A supplementation increases haemoglobin in preschool children. A large controlled community trial in Indonesia showed that improvement of vitamin A status using vitamin A-fortified monosodium glutamate was associated with an increase in haemoglobin of about 10 g/L among preschool children (6). In pregnant women, daily vitamin A (3 mg retinol equivalent, RE) and iron (60 mg) increased haemoglobin more than daily iron (60 mg) supplementation alone in a controlled trial (7). Pregnant women in India who received daily iron (60 mg elemental iron), folate (500 μg), and a single dose of vitamin A (60 mg retinol equivalent), had a higher increase in mean haemoglobin than women who received iron and folate (8). In Bangladesh, pregnant women supplemented with daily iron (60 mg elemental iron) and zinc (15 mg)
plus a single dose of vitamin A (60 mg retinol equivalent), had significantly better improvement in anaemia in 2 months compared with women receiving iron plus zinc, or daily iron alone (9).

Recently, it has been proposed that vitamin A may increase the synthesis of erythropoietin (10, 11). Retinoic acid, the active metabolite of vitamin A, has been shown to increase production of erythropoietin in a human hepatoma cell line as well as increase serum erythropoietin in vitamin A-depleted rats (12). Vitamin A was also associated with a dose-dependent increase in erythropoietin in a human hepatoma cell line (10). Regulation of erythropoietin gene expression appears to involve both hypoxia and reactive oxygen species (13), and in perfused rat kidneys, vitamin A was shown to increase erythropoietin production (11). Studies in embryonal carcinoma cells suggest that retinoic acid stimulates erythropoietin gene transcription in an oxygen-dependent manner (14). We conducted a randomized, double-masked, controlled clinical trial in Malawi, Africa, to test the hypotheses that vitamin A supplementation (i) increases haemoglobin concentrations, (ii) increases plasma erythropoietin concentrations, and (iii) changes the relationship between plasma erythropoietin and haemoglobin concentrations among pregnant women with a high prevalence of anaemia.

Subjects and methods
Pregnant women from 18 to 28 wk gestation were seen at the antenatal clinic of the Queen Elizabeth Central Hospital in Blantyre, Malawi, from November 1995 through December 1996. The Queen Elizabeth Central Hospital is the main hospital for Blantyre, a city of approximately 300,000 inhabitants. Pregnant women received instructions in prenatal care, AIDS education, HIV testing, pre- and post-test HIV counselling, physical examination, treatment for sexually transmitted diseases, and malaria prophylaxis. All women received two doses of fansidar during pregnancy as per the guidelines of Malawi Ministry of Health. Gestational age was estimated using the last reported menstrual period. Weight and height were measured at enrollment.

Venous blood samples were drawn at screening for HIV testing, and women returned one week later for the results of the HIV testing. After determination of HIV serostatus, HIV-negative women were randomized to receive either a daily supplement containing iron (30 mg elemental iron), folate (400 µg), and vitamin A (3000 µg retinol equivalent), or iron (30 mg) and folate (400 µg) (Tishcon Corporation, Salisbury, Maryland) from enrollment until delivery. Treatment assignment was determined using a computer random-number generator, and treatment assignment was concealed by pre-packing study supplements in sequentially numbered opaque bottles with supplements. Supplements containing vitamin A, iron, and folate were identical in appearance to the supplements containing iron and folate. Tablet counts were conducted every 4 wk to assess compliance with supplementation.

The planned outcome measures of the trial were haemoglobin concentrations and plasma erythropoietin concentrations at 38 wk gestation. Because plasma erythropoietin concentrations are regulated in a feedback loop with haemoglobin concentrations, the slope of the regression line between log₁₀ plasma erythropoietin concentrations, as widely used in clinical studies of erythropoietin, was examined as a secondary outcome measure. The sample size of the study was originally estimated to give 80% power to detect a 6 g/L difference in mean haemoglobin concentrations at 38 wk gestation, given 20% loss to follow-up, a two-sided test, and \( \alpha = 0.05 \).

Written, informed consent was obtained from all study participants. The study protocol was in accordance with the Helsinki Declaration of 1975 and was approved by ethical review committees: the Johns Hopkins School of Medicine; the Malawi Health Sciences Research Committee, Ministry of Health and Population, Government of Malawi; and the National Cancer Institute, National Institutes of Health. Final approval was given by the Office for Protection from Research Risk, National Institutes of Health, Bethesda, Maryland.

Laboratory methods
The study included only women who consented to HIV testing and counselling and were HIV-negative. Serum was separated for the presence of HIV-1 antibody using enzyme-linked immunosorbent assay (EIA) (Wellcozyme, Wellcome Diagnostics, Dartford, Kent, UK, and Genetic Systems EIA, Seattle, WA, USA). Both EIAs were required to be positive for a woman to be considered HIV-1-positive. Immunoblotting (Bio-Rad Laboratories, Hercules, CA, USA) was used to confirm HIV-1 status in women with equivocal HIV-1 testing by EIA. One week later, blood samples were drawn by venipuncture, and plasma was separated immediately and frozen at \(-70^\circ\text{C}\). A complete blood count including haemoglobin was
performed using an automated hematology analyzer (Coulter, Hialeah, FL, USA). Anaemia was defined as a haemoglobin concentration <110 g/L according to standard guidelines (15).

Plasma was kept frozen at —70 °C until time of the analyses. Pooled human plasma was analyzed in each run of the laboratory analyses in order to assess between assay coefficient of variation (CV). It was not possible to measure every single analyte for all plasma samples because of the limited volume of plasma available for some of the subjects. Plasma erythropoietin concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (American Laboratory Products Company, Windham, NH, USA) with a between-assay CV of 2.9%. Plasma ferritin was measured by enzyme-linked immunosorbent assay (Human Ferritin Enzyme Immunoassay Test Kit, American Laboratory Products Company, Windham, NH, USA) with a between-assay CV of 5.7%. Plasma ferritin <12 µg/L was considered consistent with iron deficiency (16). Because some recent studies suggest that ferritin <30 µg/L may be a better indicator for iron storage (17, 18), this cut-off point was also included in the analyses. Plasma α1-acid glycoprotein (AGP) was measured using radial immunodiffusion (Bindarid, The Binding Site, Birmingham, UK) with a between-assay CV of 3.5%. C-Reactive protein (CRP) was measured by enzyme-linked immunosorbent assay (Virgo CRP 150, Hemagen Diagnostics, Waltham, MA, USA) with a between-assay CV of 4.1%. Plasma AGP was considered to be elevated if >1 g/L, and plasma CRP was considered to be elevated if >5 mg/L (19). Plasma vitamin A concentrations were measured using high performance liquid chromatography (20) with a between-assay CV of 4.7%.

**Statistical analysis**

Comparisons between groups were made using Student’s t-test for continuous variables. Appropriate variable transformations were made to normalize distributions. Chi-square tests or Fisher’s exact tests were used to compare categorical variables between groups. Spearman correlation was used to examine the relationship between selected variables. Linear regression models were used to adjust for potential confounders at enrollment where the dependent variable was haemoglobin concentration at 38 wk. A mixed model using a heterogeneous compound symmetric covariance matrix controlling for repeated measures was used to compare the relationship between plasma erythropoietin concentration and haemoglobin concentration at enrollment and follow-up visits, using the model

\[ \log_{10} \text{erythropoietin} = \beta_0 + \beta_1 \text{haemoglobin} + \beta_2 \text{treatment} + \beta_3 \text{visit}_i + \beta_4 \text{haemoglobin} \times \text{treatment} + \beta_5 \text{haemoglobin} \times \text{visit}_i + \beta_6 \text{treatment} \times \text{visit}_i + \beta_7 \text{haemoglobin} \times \text{treatment} \times \text{visit}_i + \epsilon, \]

where treatment = 0 or 1, visit = 0 (enrollment) or 1 (38 week), and \(i,...,N\). The mixed model allows comparison of the slope between \(\log_{10}\) plasma erythropoietin and haemoglobin within each treatment group, and follow-up, and comparison of the slope between each treatment group between enrollment and follow-up. Analyses were conducted with SAS software (SAS Institute, Inc., Cary, NC, USA).

**Results**

Two hundred and three pregnant women were enrolled in the study, the overall follow-up was 67.5% at 38 wk gestation (137 of 203 women). In the vitamin A and control groups, the follow-up was 69.7% (76 of 109 women) and 64.9% (61 of 94 women), respectively. Of the 33 women in the vitamin A group who did complete the study follow-up at 38 wk, 21 missed the study visit, 6 did not have their haemoglobin analyzed, and 6 moved out of the study area. Of the 33 women in the control group who did not complete the follow-up at 38 wk, 21 missed the study visit, 3 did not have their haemoglobin analyzed, and 9 moved out of the study area. Of the 33 women in the two treatment groups at enrollment are shown in Table 1. There were no significant differences in age, parity, gestational age, haemoglobin, ferritin, plasma erythropoietin concentration, α1-acid glycoprotein, C-reactive protein, or CD4+ lymphocyte count between the two treatment groups at enrollment. Plasma vitamin A concentrations were significantly lower in the vitamin A group compared to the control group at enrollment. About half of the women in the study were anaemic (haemoglobin <110 g/L) at enrollment. The prevalence of iron deficiency anaemia, as defined by haemoglobin and ferritin concentrations, was not significantly different between the two treatment groups. There were no significant differences in age, parity, gestational age, haemoglobin, ferritin, erythropoietin, α1-acid glycoprotein, C-reactive protein, CD4+ lymphocyte count, or vitamin A concentrations between women who were not seen at 38 wk compared with women who completed follow-up through 38 wk (data not shown).

Mean haemoglobin concentrations at enrollment and 38 wk gestation are shown in Fig. 1A. Mean (SEM) change in haemoglobin from enrollment to 38 wk was 4.7±1.6 g/L (p=0.005) in vitamin A.
Table 1. Characteristics of pregnant women in the two treatment groups at enrollment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vitamin A</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23.3 ± 0.5 [109]</td>
<td>24.2 ± 0.6 [94]</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>23.5 ± 0.3 [109]</td>
<td>23.0 ± 0.3 [94]</td>
</tr>
<tr>
<td>Parity</td>
<td>34.8 [109]</td>
<td>26.6 [94]</td>
</tr>
<tr>
<td>Parity 1-2 (%)</td>
<td>31.1 [109]</td>
<td>37.2 [94]</td>
</tr>
<tr>
<td>Parity &gt;3 (%)</td>
<td>33.9 [109]</td>
<td>36.1 [94]</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>107.9 ± 1.5 [100]</td>
<td>109.7 ± 1.4 [91]</td>
</tr>
<tr>
<td>Haemoglobin &lt;110 g/L (%)</td>
<td>51.0 [100]</td>
<td>48.4 [91]</td>
</tr>
<tr>
<td>Haemoglobin &lt;100 g/L (%)</td>
<td>28.0 [100]</td>
<td>20.9 [91]</td>
</tr>
<tr>
<td>Haemoglobin &lt;90 g/L (%)</td>
<td>11.0 [100]</td>
<td>5.5 [91]</td>
</tr>
<tr>
<td>Log_{10} ferritin (ng/L)</td>
<td>18.4 (5.5, 61.2) [108]</td>
<td>23.9 (7.7, 74.9) [90]</td>
</tr>
<tr>
<td>Haemoglobin &lt;110 g/L and ferritin &lt;12 μg/L (%)</td>
<td>18.2 [99]</td>
<td>27.3 [88]</td>
</tr>
<tr>
<td>Haemoglobin &lt;110 g/L and ferritin &lt;30 μg/L (%)</td>
<td>36.3 [99]</td>
<td>27.3 [88]</td>
</tr>
<tr>
<td>Log_{10} erythropoietin (IU/L)</td>
<td>1.57 ± 0.33 [102]</td>
<td>1.95 ± 0.29 [88]</td>
</tr>
<tr>
<td>Log_{10} α2-acid glycoprotein (g/L)</td>
<td>2.71 ± 0.01 [105]</td>
<td>2.73 ± 0.01 [89]</td>
</tr>
<tr>
<td>α2-Acid glycoprotein &gt;1 g/L (%)</td>
<td>1.9 [105]</td>
<td>2.2 [89]</td>
</tr>
<tr>
<td>Log_{10} C-reactive protein (mg/L)</td>
<td>0.4 ± 0.05 [105]</td>
<td>0.47 ± 0.06 [88]</td>
</tr>
<tr>
<td>C-reactive protein &gt;5 mg/L (%)</td>
<td>26.6 [105]</td>
<td>34.1 [88]</td>
</tr>
<tr>
<td>CD4^+ lymphocyte count (cells/μL)</td>
<td>831 ± 36 [105]</td>
<td>803 ± 40 [84]</td>
</tr>
<tr>
<td>Plasma vitamin A (μmol/L)</td>
<td>0.81 ± 0.02 [109]</td>
<td>0.91 ± 0.03 [94]</td>
</tr>
<tr>
<td>Plasma vitamin A &lt;1.05 μmol/L (%)</td>
<td>85.3 [109]</td>
<td>70.9 [94]</td>
</tr>
<tr>
<td>Plasma vitamin A &lt;0.70 μmol/L (%)</td>
<td>34.8 [109]</td>
<td>24.7 [94]</td>
</tr>
</tbody>
</table>

1 For continuous variables, mean ± SEM.
2 [μg/L]
3 In comparison between vitamin A and placebo groups, for all variables p>0.05 except mean plasma vitamin A (p = 0.013) and plasma vitamin A <1.05 μmol/L (p = 0.013).

Group (n=63) and 7.3 ± 2.3 g/L (p = 0.003) in control group (n=52), by paired t-test. At 38 wk gestation, the prevalence of anaemia (haemoglobin <110 g/L) was 28.9% in the vitamin A group and 35.2% in the control group (p = 0.46 by chi-square). Mean haemoglobin concentrations were not significantly different between the vitamin A and control group at 38 wk. Multiple linear regression models were used to adjust for baseline vitamin A deficiency (plasma vitamin A <1.05 μmol/L), iron deficiency anaemia (hemoglobin <110 g/L and ferritin <30 μg/L), and elevated acute phase proteins (α2-acid glycoprotein >1 g/L and/or C-reactive protein >5 mg/L), where the dependent variable was haemoglobin. These models showed no significant effect of vitamin A supplementation on haemoglobin concentrations at 38 wk after adjusting for these baseline variables (data not shown).

Geometric mean plasma erythropoietin concentration at enrollment and 38 wk gestation are shown in Fig. 1B. Mean (SEM) change in erythropoietin from enrollment to 38 wk was 2.39 ± 5.00 IU/L in the vitamin A group (n = 71) (p = 0.63) and -2.87 ± 3.92 IU/L in the control group (n = 62) (p = 0.46), by paired t-test. At 38 wk follow-up, there were no significant differences in mean plasma erythropoietin concentrations between the vitamin A and control groups. Multiple linear regression

---

**Fig. 1.** (A) Haemoglobin concentrations at enrollment and 38 wk. Bars indicate 95% confidence intervals. Differences between treatment groups at enrollment (p = 0.40) and 38 wk (p = 0.25) are shown. (B) Plasma erythropoietin concentrations at enrollment and 38 wk. Bars indicate SEM. Differences between treatment groups at enrollment (p = 0.09) and 38 wk (p = 0.045) are shown.
Vitamin A and erythropoietin

mean to 38 wk ($p = 0.37$). In the control group, there was a change of $-0.0022$ in the slope of the regression line between \( \log_{10} \) erythropoietin and haemoglobin from enrollment to 38 wk ($p = 0.53$). Additional models were run to determine whether vitamin A deficiency at enrollment had an effect on the slope between plasma erythropoietin concentrations and haemoglobin, and these results were not significant (data not shown).

At enrollment, the Spearman correlation between \( \log_{10} \) erythropoietin and haemoglobin was $r = -0.49$, $p < 0.0001$ and $r = -0.313$, $p = 0.003$ in the vitamin A and control group, respectively. At 38 wk gestation, the Spearman correlation between \( \log_{10} \) erythropoietin and haemoglobin was $r = -0.545$, $p < 0.0001$ and $r = -0.502$, $p < 0.0001$ in the vitamin A and control group, respectively. At enrollment, the Spearman correlations between \( \log_{10} \) erythropoietin and AGP and CRP were $r = 0.147$, $p = 0.045$ and $r = 0.091$, $p = 0.21$, respectively.

Discussion

To our knowledge, this was the first study to examine the relationship between vitamin A supplementation and plasma erythropoietin concentrations in humans. Vitamin A supplementation did not appear to modulate the relationship between haemoglobin and plasma erythropoietin concentrations in this group of pregnant women with a high prevalence of anaemia. Because erythropoietin production by the kidney is influenced in a feedback loop by haemoglobin concentrations, it is more appropriate to characterize changes in the slope of
the regression line between log_{10} plasma erythropoietin concentration and haemoglobin rather than absolute plasma concentrations of erythropoietin (21). In the study, the slope of this regression line was not significantly different between treatment groups at follow-up, nor was there a significant change between enrollment and follow-up in the slope of the regression line within each treatment group.

Both treatment groups received daily iron and folate supplementation, and there was a significant increase in haemoglobin in both groups by 38 wk gestation, which is consistent with iron supplementation. In contrast to a previous study in Indonesia (7), vitamin A and iron supplementation did not result in a larger increase in haemoglobin compared with iron supplementation alone, but these studies differed in that the present study utilized 30 mg rather than 60 mg of daily oral iron. It may be possible that vitamin A supplementation has a potentiating effect on increasing haemoglobin concentrations only when larger daily doses of iron are given. Another major difference between the two studies was that malaria, another major cause of anaemia, is endemic in Malawi but not in West Java. A limitation of the present study is that malaria parasitaemia was not measured; however, acute phase proteins were measured and generally correlate well with malaria parasitaemia.

During normal pregnancy, the red cell mass increases greatly to meet the needs of the growing fetus (22). The increase in red cell mass is largely regulated by the production of erythropoietin from renal cortical cells. Among healthy pregnant women in Finland who received iron supplementation, 60–100 mg/d, plasma erythropoietin concentration increased before 24 wk gestation and then declined towards term (23). In a longitudinal study that followed healthy, pregnant women from conception to delivery, plasma erythropoietin concentration increased steadily from about 8 wk gestation until delivery (24, 25). Another study conducted in Belgium among 317 pregnant women with a high prevalence of anaemia suggested that plasma erythropoietin concentration increases steadily during pregnancy and then remain stable during the third trimester (26). In the present study, conducted in a population where about half the women were anaemic and there was a high prevalence of iron deficiency, the mean plasma erythropoietin concentration did not change significantly between the second and third trimester.

The 3'-enhancer region for the erythropoietin gene contains a sequence homologous to DR-2, a steroid-responsive element that appears to be regulated by retinoic acid (12). In vitamin A-depleted rats, intragastric administration of all-

trans retinoic acid was associated with a large increase in serum erythropoietin concentration within 4 h of dosing, but within 24 h, the serum erythropoietin concentration returned to original level (12). Vitamin A, but not vitamin E or vitamin C, was shown to have a dose-related effect on the production of erythropoietin in human hepatoma cell lines HepG2 and Hep3B (10). Observations of the modulation of erythropoietin by vitamin A have currently been limited in that a renal cell culture model has not been established, and current in-vitro models utilize human hepatoma cell lines. Alternatively, modulation of erythropoietin production by vitamin A has been studied in isolated, perfused rat kidneys, and perfusion with vitamin A or the antioxidant desferrioxamine was shown to increase renal erythropoietin synthesis (11). The present study in humans does not corroborate these in-vitro and animal model studies that suggest that vitamin A increases erythropoietin production. Further trials are currently in progress in young children and adults to determine whether vitamin A or other micronutrients are involved in the modulation of erythropoietin production.

**Acknowledgements**

We thank the mothers who participated in this study, the staff of the Johns Hopkins Project and Queen Elizabeth Central Hospital, the Malawi Health Sciences Research Committee, Anne Willoughby and Robert Nugent, National Institute for Child Health and Human Development, Kenneth Bridbord, Fogarty International Center, and the National Institutes of Health, for their continuing support and encouragement. Financial support is acknowledged from the National Institutes of Health (HD32247, HD30042, HIVNET contract N01-AI-35173–117), the Fogarty International Center, and the United States Agency for International Development (Cooperative Agreement HRN-A-00–97–00015–00).

**References**


6. **MUHILAL,** PERMEISIII D, IDJRADINATA YR, MUIHERDIYANTINGIWI, KARYADI D. Vitamin A-fortified mono-


Vitamin A and erythropoietin