HIV and other predictors of serum β-carotene and retinol in pregnancy: a cross-sectional study in Zimbabwe[1–3]

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ABSTRACT

Background: Vitamin A status during pregnancy is important to maternal and infant health.

Objective: Our goal was to identify predictors of serum β-carotene and retinol.

Design: This was a cross-sectional study of 1669 women (22–35 wk of gestation) in Harare, Zimbabwe, who were receiving prenatal care. The statistical effects of age, season, gestational age, gravidity, HIV-1 infection, malaria parasitemia, and serum α_1-antichymotrypsin (ACT) on serum β-carotene ($\log_{10}$ transformed) and retinol were estimated by using multiple linear regression analyses.

Results: HIV infection was found in 31.5% of the women; 0.4% had malaria. Serum β-carotene concentrations (geometric $\bar{x}$: 0.19 μmol/L) were lower in HIV-infected women than in uninfected women ($10^{\beta} = 0.78$; 95% CI: 0.72, 0.84) and increased with age ($10^{\beta} = 1.05$; 1.02, 1.07) in gravida 1 but not in gravida ≥2 (*P* for interaction = 0.00002). Serum retinol ($\bar{x}$: 0.92 μmol/L) increased with age (β = 0.004; 0.0001, 0.008) in uninfected women but not in HIV-infected women (*P* for interaction = 0.02) and was 0.05-μmol/L (0.02, 0.09) lower in HIV-infected women than in uninfected women at 24 y of age. Furthermore, gestational age, season, use of prenatal supplements, and malaria were predictors of serum β-carotene. Serum retinol was lower in women carrying male (β = –0.04; –0.08, –0.00005) and multiple (β = –0.21; –0.35, –0.08) fetuses. Serum ACT concentrations of 0.3–0.4, 0.4–0.5, and >0.5 g/L were associated with 3%, 11%, and 44% lower serum β-carotene and 0.04-, 0.15-, and 0.41-μmol/L lower serum retinol. Serum ACT (g/L) was higher in women with malaria than in those without (β = 0.10; 0.03, 0.16) and in gravida 1 than in gravida ≥2 (β = 0.012; 0.003, 0.021), but was not higher in HIV-infected women than in uninfected women (β = 0.001; −0.008, 0.011).

Conclusions: HIV infection, malaria, gravidity, and gestational age were predictors of serum β-carotene and retinol. Serum ACT was an important predictor of both and was associated with gravidity and gestational age. *Am J Clin Nutr* 2001;73:1058–65.

KEY WORDS Vitamin A, retinol, β-carotene, reproduction, pregnancy, gestational age, gravidity, HIV, malaria, acute phase response, α_1-antichymotrypsin, women, Zimbabwe

INTRODUCTION

Vitamin A deficiency is common in women of reproductive age and young children in developing countries and is an important determinant of morbidity and mortality (1, 2). The main dietary sources of vitamin A are provitamin A carotenoids in green leafy vegetables and yellow-orange fruit (3); foods rich in preformed vitamin A are rarely affordable in developing countries. Women need adequate stores of vitamin A to meet the increased requirements of pregnancy and lactation (4). Vitamin A plays an important role in reproduction in that it is essential to embryogenesis, yet is teratogenic if the fetus is excessively exposed early in pregnancy (4, 5). Accordingly, only little vitamin A passes through the placenta to the fetus and newborns rely on breast milk to fill up their stores. Infants of well-nourished mothers consume breast milk containing >2 μmol vitamin A/L, allowing vitamin A stores to accumulate from 5 μmol at delivery to 100 μmol at the end of the first year (6). In contrast, because the breast milk of vitamin A–deficient mothers contains only ≈1 μmol/L, the children of these mothers, although rarely xerophthalmic, may have grossly inadequate stores at the end of infancy. These children may suffer more severe morbidity from infections, which can then precipitate clinical vitamin A deficiency and establish a vicious circle (1).

In addition to effects on the vitamin A status of breast-fed infants, maternal vitamin A deficiency may cause intrauterine growth retardation and other adverse pregnancy outcomes (6) and maternal morbidity and mortality (2). Although severe vitamin A deficiency is known to result in resorption of the fetus or abortion in animals, the reproductive strategy of humans is strikingly different (7). Because humans carry only one off-

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spring at a time and have a long reproductive cycle, sacrificing the offspring is unlikely to be of evolutionary benefit; a priori, effects on the mother rather than the offspring are to be expected. Accordingly, a large cluster-randomized vitamin A study in Nepal found no effects of vitamin A supplements on birth weight (ML Dreyfuss, KP West Jr, J Katz, et al, unpublished observations, 1997) and fetal loss (8), but showed significantly reduced maternal mortality among women of reproductive age (2).

Consequently, identification of predictors of vitamin A status in pregnant women is of importance in terms of rationally designing and targeting appropriate interventions. In addition to inadequate dietary intake, infections and obstetric history may contribute to the low vitamin A status of women of reproductive age. Infections, and maybe pregnancy, may also disturb the extent to which serum retinol reflects the hepatic stores of vitamin A.

As part of a micronutrient supplementation trial, baseline data on serum concentrations of β-carotene and retinol were available for 1580 (94.7%) of 1669 pregnant women. In the present study, we report on multiple linear regression analyses performed to identify and estimate the statistical effects of obstetric history, HIV infection, and other predictors on serum β-carotene and retinol, while controlling for the confounding effects of the acute phase response.

SUBJECTS AND METHODS

Study area and population

The study was conducted in the Mbare residential area of Harare, the capital of Zimbabwe. Harare is 1500 m above sea level and has a tropical climate with a mean daily maximum temperature of 25 °C and a minimum of 12 °C. There are 4 main seasons: early (June to August) and late (September to November) dry and early (December to February) and late (March to May) rainy. The common diet is maize meal porridge taken with vegetables and occasionally meat. Unemployment is very high in Mbare, especially among women, and most of those employed are general laborers. Malaria is not endemic in the study area because of the high altitude, but most women often return to their rural homes outside Harare, where malaria transmission may occur.

Information about the study was given to women registering for routine prenatal care at Edith Oppermann Maternity Hospital, and those between 22 and 36 wk of gestation were invited to participate. Women were informed about the HIV testing and were counseled before they gave their written consent to participate. Counseling was provided by a nongovernmental organization, the AIDS Counseling Trust. HIV results were made available within 2 wk of blood sampling and those willing to know their results were counseled again. A questionnaire was administered and clinical examinations and blood sampling were performed. The structured questionnaire, administered by a research nurse, was used to gather demographic data and medical and obstetric history. Gestational age was calculated from the first day of the last menstruation. When this was not possible, gestational age was estimated by fundus height.

Permission to conduct the study was obtained from the ethical and scientific committees of the Medical Research Council of Zimbabwe, the Harare City Health Department, and the Ministry of Health. The study was also approved by the Danish Central Medical Ethics Committee. The national Ministry of Health recommendations were adhered to when giving women information on breast-feeding. Women found to be sick during the clinical examination were referred for treatment to Edith Oppermann Maternity Hospital or Harare Central Hospital.

Clinical examinations and HIV testing

Clinical examinations were carried out as part of the routine prenatal care. Height and weight were measured with the women barefoot and wearing light clothing. Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg. Venous blood was collected between 0900 and 1200. Five milliliters of blood was collected in tubes containing EDTA and 5 mL in plain tubes. The samples were placed in ice-cooled insulated boxes immediately after collection and were delivered to the laboratory within 4 h. Thick and thin blood slides were made for malaria parasitemia testing. Serum was separated and stored in liquid nitrogen at −196 °C until tested for antibodies against HIV-1/2 or was shipped to the Research Department of Human Nutrition, Denmark, for micronutrient analyses. Samples for which negative results were obtained with the Genelavia Mixt (Sanofi, France) HIV-1/2 enzyme immunoassay kit were reported as being negative. Positive and indeterminate samples were tested further with use of the Recombigen HIV-1/2 (Cambridge Biotech, Worcester, MA) enzyme immunoassay with different antigens from the Genelavia kit. Samples for which positive results were obtained with the Recombigen kit were reported as being positive.

Positive results were confirmed at the Department of Clinical Chemistry, Aalborg University Hospital, Denmark, by using a modification of a reverse transcriptase–polymerase chain reaction method developed in-house (9). HIV-1 RNA was isolated from 125 μL plasma. The following primers from the *gag* region of the virus were used: 5′-primer, AGTTGGAGGACATCAAG CAGCCATGCAAAT, and 3′-primer, TGCTATGTCAGTTCCC CTTGGTTCTCT. All samples were analyzed in 2 independent extractions and runs. Samples with discordant results were retested in duplicate. The limit of detection was 40 000 genome equivalents/L.

Measurement of retinol, β-carotene, and α_1-antichymotrypsin

HPLC was used to measure serum concentrations of retinol and β-carotene. First, 400 μL serum was added to 400 μL ethanol (product no. 1.11727.2500; Merck, Darmstadt, Germany) and 800 μL ethyl acetate (product no. C 2513; Lab-Scan Sciences, Dublin). The extracts were dehydrated with 40 mg Na_2SO_4 (product no. 1.06649.0500; Merck) at −20 °C for 20–30 min. After centrifugation at 13000 × *g* for 5 min at room temperature, 1200 μL supernatant fluid was evaporated at 37 °C under N_2 and the residue was reconstituted in 300 μL ethyl acetate:ethanol (1:1, by vol) by ultrasonication for 5 min. The reconstituted extract was filtered through a 0.45-μm filter (polytetrafluoroethylene membrane no. 17820; Sartorius, Goettingen, Germany) before 20 μL was injected into the HPLC system.

The chromatography system consisted of a refrigerated (4 °C) autosampler (model 9300; Varian, Palo Alto, CA), a pump (model 9012; Varian), a column oven (29 °C; Croco-Cil, Riemerling, Germany), a guard column (model 69080; Varian), a 250 × 4.6 mm octadecylsilane (C_{18}) analytic column packed with 5-μm particles (Varian Res Elut, 90 Å), and an ultraviolet-visual light detector

TABLE 1
Background characteristics of the 1669 women by HIV status[1]

	HIV negative (n = 1113)	HIV positive (n = 526)	P
Age (y)	24.0 (14–45)[2]	25.0 (15–45)	0.0004
Gravidity (%)			
1	46	26	<0.00001
2	24	37	
3	15	22	
≥4	15	15	
Gestational age (wk)	29.2 (29.0, 29.3)[3]	28.9 (28.6, 29.2)	0.18
Season (%)[4]			
Early rainy	19	18	0.97
Late rainy	13	12	
Early dry	40	40	
Late dry	28	30	
Height (cm)	161.3 (160.9, 161.6)	161.5 (161.0, 162.0)	0.56
Weight (kg)	65.1 (64.5, 65.7)	65.3 (64.2, 65.9)	0.85
Use of prenatal supplements (%)	3.7	3.7	0.98

[1] HIV status was indeterminate in 30 women.
[2] $\bar{x}$; range in parentheses.
[3] $\bar{x}$; 95% CI in parentheses.
[4] Season was defined as early (June to August) and late (September to November) dry and early (December to February) and late (March to May) rainy.

(model 9050; Varian). The mobile phase was acetonitrile (product no. C 2502; Lab-Scan Sciences):ethanol (65:35, by vol) added to 0.05% triethylamine (product no. 23, 962-3; Aldrich Chemical Co, Milwaukee); the flow rate was 1.5 mL/min (10). Both compounds were eluted within 13 min. Retinol was measured at 325 nm and β-carotene at 450 nm. Linear calibration curves of retinol (product no. RO 01-4955; Roche, Basel, Switzerland) and β-carotene (product no. RO 01-8300; Roche) were prepared from mixed solutions of the analytes in different concentrations. Detection limits were 0.11 and 0.04 μmol/L for retinol and β-carotene, respectively. The interrun CVs were 4.3% for retinol and 7.3% for β-carotene. Concentrations of both retinol and β-carotene were within certified values of a reference serum (Standard Reference Material 968b; National Institute of Standards and Technology, Gaithersburg, MD) used to measure the accuracy of the analysis.

Serum α_1-antichymotrypsin (ACT) was measured by automated turbidimetry (Cobas Mira Plus; Roche). Rabbit antihuman ACT (DAKO, Glostrup, Denmark) was used to precipitate ACT and turbidity was measured at 345 nm after incubation for 8.3 min at 37°C. The results are given as g/L serum on the basis of a standard curve from commercial calibrators (DAKO). The interassay CV was 3% and the accuracy was verified by frequent runs of low (0.21–0.29 g/L) and high (0.55–0.75 g/L) commercial serum controls (DAKO). The test range for the assay was 0.05–1.20 g/L. ACT was arbitrarily categorized as ≤0.3, 0.3–0.4, 0.4–0.5, and >0.5 g/L.

Statistical analysis

The distribution of serum retinol conformed to normality as assessed by rankit plots. Before $\log_{10}$ transformation of the serum β-carotene data, values below the detection limit of 0.04 μmol/L were randomly assigned values between 0.01 and 0.04 μmol/L. Two-sample t tests and one-way analyses of variance with Scheffe's multiple comparison post hoc tests were used to test for differences in means. The chi-square test was used to test for differences in proportions between groups. Multiple linear regression analysis was used to identify and estimate effects of predictors of serum retinol and β-carotene. The variables assessed were age, gravidity, gestational age, season, use of prenatal supplements, HIV infection, elevated serum ACT, and malaria parasitemia. All possible two-way interactions were tested for. When interactions were identified, interaction terms were computed to allow estimation of the effect of one of the variables separately for each level of the effect-modifying variable. Dummy variables were used to assess the effect of categorical variables or continuous variables when the relation was not linear. Residual analysis was performed by assessing normal plots and by plotting standardized residuals against predicted values and continuous independent variables. The level of significance used was 0.05. SPSS for WINDOWS (version 10; SPSS Inc, Chicago) was used to analyze the results.

RESULTS

Of 1669 pregnant women between 22 and 35 wk of gestation included in the study, measurements of serum retinol and β-carotene concentrations were available for 1580 women (94.7%). Most of the women were enrolled in the early (39.9%) and late (28.8%) dry seasons, compared with the early (18.5%) and late (12.7%) rainy seasons. The mean age of the women was 24.4 y (range: 14–45 y) and the median number of previous pregnancies was 1 ($\bar{x}$: 1.2; range: 0–8), with 39.6% being gravida 1, 27.9% gravida 2, 17.1% gravida 3, and 14.9% gravida ≥4. The mean (range) age was 20.6 (14–40) y in gravida 1, 23.5 (16–36) y in gravida 2, 27.1 (18–43) y in gravida 3, and 32.1 (20–45) y in gravida ≥4. The mean gestational age was 29.1 wk (range: 22–35 wk). Intake of prenatal vitamin or mineral supplements before enrollment was reported by 59 women (3.7%): 35 (2.1%) had taken iron supplements, either as iron alone (n = 7) or together with folic acid (n = 21) or with folic acid and multivitamins (n = 7), and 24 (1.5%) had taken supplements of other or unknown composition. Thus, only 7 women (0.4%) were known to have taken multivitamin supplements likely to have contained vitamin A.

Infection

Of the 1669 women, 526 (31.5%) were HIV-1 positive, 1113 (66.7%) were HIV-1 negative, and HIV infection status was indeterminate in 30 (1.8%). HIV-infected women were older and had higher gravidity than did uninfected women, whereas there were no significant differences between these 2 groups in mean gestational age, seasonal distribution of enrollment, weight, height, and reported intake of prenatal supplements (**Table 1**). Only 7 women (0.4%) had malaria parasitemia.

Serum ACT

The women's mean serum ACT concentration was 0.33 g/L (95% CI: 0.33, 0.34). The range was 0.15–1.19 g/L and the 90th and 95th percentiles were at 0.41 and 0.47 g/L, respectively. The mean serum ACT was slightly, but significantly, higher in the dry seasons than in the rainy seasons (**Table 2**), as was the prevalence of elevated serum ACT. The mean serum ACT in the HIV-infected and uninfected women was not significantly different, although the distribution was marginally different, with slightly more HIV-infected women in the lowest and highest serum ACT categories (**Table 3**). The effects of season, infection, and obstetric variables on serum ACT

TABLE 2
Serum α_1-antichymotrypsin (ACT), β-carotene, and retinol concentrations in HIV-uninfected, pregnant women by season[1]

	Early rainy (Dec to Feb) (n = 203)	Late rainy (Mar to May) (n = 159)	Early dry (Jun to Aug) (n = 440)	Late dry (Sept to Nov) (n = 311)	P
ACT (g/L)[2]	0.32 (0.31, 0.33)	0.32 (0.31, 0.33)	0.34 (0.33, 0.35)	0.33 (0.32, 0.34)	0.001
<0.3 (%)	43 (36, 50)	46 (38, 53)	36 (31, 40)	36 (30, 41)	0.021
0.3–0.4 (%)	50 (43, 57)	47 (39, 55)	51 (46, 56)	53 (47, 59)	
0.4–0.5 (%)	6 (3, 9)	6 (2, 10)	8 (6, 11)	10 (7, 13)	
>0.5 (%)	1 (0, 3)	1 (0, 3)	5 (3, 7)	2 (1, 3)	
β-Carotene (μmol/L)[3]	0.34 (0.31, 0.38)	0.23 (0.21, 0.26)	0.18 (0.17, 0.19)	0.16 (0.15, 0.17)	<0.00001
Retinol (μmol/L)[4]	0.99 (0.94, 1.04)	0.86 (0.80, 0.93)	0.94 (0.91, 0.97)	0.93 (0.89, 0.96)	0.01
<0.70 (%)	21 (16, 27)	32 (24, 40)	22 (18, 26)	26 (21, 31)	0.09

[1] 95% CI in parentheses.
[2] $\bar{x}$; n = 1103.
[3] Geometric $\bar{x}$; n = 1052.
[4] $\bar{x}$; n = 1052.

were assessed in multiple linear regression analyses (**Table 4**). Serum ACT was 0.02-g/L higher in the dry seasons than in the wet seasons and 0.10-g/L higher in women with malaria parasitemia than in women without. Furthermore, gravida 1 had slightly but significantly higher serum ACT than did gravida ≥2, whereas women between 22 and 25 wk of gestation had marginally significantly higher serum ACT than did women ≥25 wk of gestation. HIV status had no significant effect on serum ACT (β = 0.001; 95% CI:− 0.008, 0.011; P = 0.80).

Serum β-carotene

Among HIV-uninfected women, the geometric mean serum β-carotene concentration was 0.20 μmol/L (95% CI: 0.19, 0.21). The geometric mean serum β-carotene differed by age (P = 0.001), with lower concentrations in women aged <20 y (0.17 μmol/L) and >32 y (0.20 μmol/L) than in women between the ages of 20 and 32 y (0.22 μmol/L). In contrast, there were no significant differences over categories of gestational age or parity. Women with serum ACT concentrations <0.3 g/L had a mean serum β-carotene concentration of 0.20 μmol/L, compared with 0.19, 0.16, and 0.10 μmol/L in women with serum ACT concentrations of 0.3–0.4, 0.4–0.5, and >0.5 g/L, respectively (test for linearity: P < 0.00001).

There were significant differences in serum β-carotene by season (P < 0.00001), with values doubling from the lowest concentrations (0.16 μmol/L) in the dry seasons to the highest (0.34 μmol/L) in the early rainy season (Table 2). Serum β-carotene was lower in HIV-infected women than in uninfected women (Table 3). In multiple linear regression analyses, season, gravidity, age, and HIV infection were independent predictors of β-carotene, even when elevated serum ACT was included in the model (**Table 5**). In contrast, gestational age was neither a predictor as a continuous variable nor as a dummy variable. When β-carotene was $\log_{10}$ transformed, the regression coefficient β corresponded to a 10^{β} times change in mean serum β-carotene per unit increase in the explanatory variable. For example, the regression coefficient β of the early dry season was −0.26, which corresponded to a $10^{-0.26}$ or 0.55 times lower β-carotene in that season than in the reference season. In other words, serum β-carotene concentrations in the dry and the late rainy seasons were approximately one-half and two-thirds, respectively, of the concentration in the early rainy season. The estimated effects of season did not change considerably if ACT was taken out of the model.

A strong interaction was found between age and gravidity (P = 0.00002): age was a predictor of serum β-carotene in gravida 1, but not in gravida 2, 3, or ≥4 (P > 0.47), which were combined as gravida ≥2. The regression coefficient of 0.02 for age in gravida 1 corresponds to a $10^{0.02}$ or 1.05 times higher β-carotene concentration for each 1-y increase in age. Accordingly, the association between gravidity and serum β-carotene depended on the age of comparison. The mean serum β-carotene in gravida ≥2 was 0.84 times that of gravida 1 at 24 y of age, and 0.55 times that of gravida 1 at 32 y of age.

Serum β-carotene was 0.78 times lower in HIV-infected women than in uninfected women. Malaria parasitemia was associated with a 0.60 times lower concentration; however, because only 7 women were infected, this estimate had a wide CI and was not significant. The acute phase response had little influence on the estimated effect of HIV on serum β-carotene, but a large influence on the estimated effect of malaria. In fact, when ACT was taken out of the model, the 10^{β} of HIV infection changed only from 0.78 to 0.77, whereas the 10^{β} of malaria changed from 0.60 to 0.52. However, ACT itself was a strong predictor of serum β-carotene, in that women with a serum ACT concentration >0.5 g/L had a mean serum β-carotene concentration that was only one-half of that of women with an ACT concentration <0.3 g/L. There were no other two-way interactions between any of the variable assessed.

TABLE 3
Serum α_1-antichymotrypsin (ACT), β-carotene, and retinol concentrations in pregnant women by HIV status[1]

	HIV negative (n = 1113)	HIV positive (n = 526)	P
ACT (g/L)[2]	0.33 (0.33, 0.34)	0.33 (0.32, 0.34)	0.91
<0.3 (%)	38 (35, 41)	42 (38, 46)	0.08
0.3–0.4 (%)	51 (48, 54)	45 (41, 49)	
0.4–0.5 (%)	8 (6, 10)	8 (6, 11)	
>0.5 (%)	3 (2, 4)	5 (3, 7)	
β-Carotene (μmol/L)[3]	0.20 (0.19, 0.21)	0.16 (0.15, 0.17)	<0.00001
Retinol (μmol/L)[4]	0.93 (0.91, 0.95)	0.87 (0.84, 0.90)	0.0004
<0.70 (%)	24 (22, 27)	34 (30, 38)	0.0001

[1] 95% CI in parentheses. HIV status was indeterminate in 30 women.
[2] $\bar{x}$; n = 1615.
[3] Geometric $\bar{x}$; n = 1556.
[4] $\bar{x}$; n = 1556.

TABLE 4
Regression coefficients (β) and 95% CIs for predictors of serum α_1-antichymotrypsin (g/L) in pregnant women[1]

Variable	β (95% CI)	*P*
Season[2]		
Dry	0.02 (0.01, 0.03)	0.00001
Malaria parasitemia[3]	0.10 (0.03, 0.16)	0.003
Gravidity[4]		
1	0.012 (0.003, 0.021)	0.009
Gestational age (wk)[5]		
22–25	0.011 (−0.002, 0.024)	0.07

[1] n = 1637. Adjusted R^2 = 0.023.
[2] Coded as 1 for dry (June to November) and 0 for rainy (December to May).
[3] Coded as 0 for absent and 1 for present.
[4] Coded as 1 for gravida 1 and 0 for gravida ≥2.
[5] Coded as 1 for 22–25 wk and 0 for 25–35 wk.

Serum retinol

Among HIV-uninfected women, the mean serum retinol concentration was 0.93 μmol/L (95% CI: 0.91, 0.95); 65% of the women had values <1.05 μmol/L and 24% had values <0.70 μmol/L. Serum retinol decreased with increasing gestational age (test for linearity: $P < 0.00001$): mean serum retinol was 1.03 μmol/L in women <25 wk of gestation, and 0.94, 0.91, and 0.82 μmol/L in women at 25–28, 28–32, and >32 wk of gestation, respectively. Serum retinol did not differ significantly by categories of gravidity or age. In HIV-uninfected women, serum retinol did differ by season ($P = 0.001$), with the lowest concentrations in the late rainy season and the highest in the early rainy season (Table 2).

The mean serum retinol concentration was 0.06-μmol/L (95% CI: 0.02, 0.09) lower in HIV-infected women than in uninfected women, and a considerably higher proportion of HIV-infected women had values below the cutoff of 0.70 μmol/L (Table 3). Mean serum retinol was 0.96 μmol/L in women with serum ACT <0.3 g/L, but 0.93, 0.80, and 0.53 μmol/L in women with serum ACT concentrations of 0.3–0.4, 0.4–0.5, and >0.5 g/L, respectively (test for linearity: $P < 0.00001$).

In the multiple linear regression analysis, season, age, gestational age, HIV status, elevated serum ACT, malaria parasitemia, and use of supplements were independent predictors of serum retinol (**Table 6**), whereas parity was not. Serum retinol was 0.05-μmol/L lower in women enrolled in the early and late dry seasons and 0.11-μmol/L lower in women enrolled in the late rainy season than in women enrolled in the early rainy season. Interestingly, when serum β-carotene was included in the model, the early (0.01; 95% CI: −0.04, 0.60; $P = 0.61$) and late (0.02; 95% CI: −0.03, 0.07; $P = 0.43$) dry seasons dropped out of the model, whereas the regression coefficient of late rainy season was reduced (−0.08; 95% CI: −0.14, −0.016; $P = 0.01$). Gestational age was a strong predictor of serum retinol, with a 0.02-μmol/L decline in serum retinol per week increase in gestational age. If assessed using dummy variables with gestation between weeks 22 and 25 as the reference category, serum retinol was found to be 0.10-μmol/L (95% CI: 0.03, 0.16), 0.12-μmol/L (95% CI: 0.06, 0.18), and 0.20-μmol/L (95% CI: 0.13, 0.27) lower in weeks 25–28, 29–32, and 33–35, respectively. In contrast, there were no significant effects of gravidity.

Age and HIV status were found to interact ($P = 0.02$) and age was therefore assessed separately for HIV-infected and uninfected women. The interaction was due to an increase in serum retinol with age in uninfected but not in HIV-infected women. Accordingly, the difference in serum retinol between uninfected and HIV-infected women increased with age. For example, there was no significant difference between HIV-infected and uninfected women at 18 y of age (−0.01 μmol/L; 95% CI: −0.07, 0.04), whereas at 32 and 42 y of age the difference was −0.12 μmol/L (95% CI: −0.18, −0.06; $P = 0.0001$) and −0.20 μmol/L (95% CI: −0.32, −0.08; $P = 0.001$), respectively. Intake of prenatal supplements was associated with a 0.10-μmol/L higher serum retinol concentration. Women with malaria parasitemia had a 0.21-μmol/L lower serum retinol concentration than did women without, but because only 7 women had malaria, the estimate had little precision and was not significant ($P = 0.08$).

Because there are no established cutoffs for serum ACT, the effect of different serum ACT concentrations was estimated through use of dummy variables. When serum ACT <0.3 g/L was used as the reference category, concentrations between 0.3 and 0.4 g/L were associated with a 0.04-μmol/L lower serum retinol concentration, whereas serum ACT concentrations between 0.4 and 0.5 and >0.5 g/L were associated with 0.15- and 0.41-μmol/L lower serum retinol. The 95% CI around the mean serum retinol concentration increased from 0.90–0.93 to 0.94–0.99 μmol/L with adjustment for the acute phase response.

To evaluate the magnitude of the confounding the acute phase response would have caused if not controlled for in the analyses, we assessed the changes in the regression coefficients when serum ACT was taken out of the model. This analysis showed that the acute phase response was a positive confounder of the effect of malaria on serum retinol because the effect of malaria was −0.31 without and −0.21 with ACT in the model. In contrast, the regression coefficients of season, HIV, and other predictors were little affected, but the R^2 dropped from 0.11 to 0.06 when serum ACT

TABLE 5
Regression coefficients (β) and 95% CIs for predictors of serum β-carotene (μmol/L; $\log_{10}$ transformed) in pregnant women[1]

Variable	10^{β} (95% CI)	*P*
Season[2]		
Late rainy	0.68 (0.60, 0.78)	<0.00001
Early dry	0.55 (0.49, 0.60)	<0.00001
Late dry	0.48 (0.43, 0.52)	<0.00001
Age (y)[3]		
In gravida 1	1.05 (1.02, 1.07)	<0.00001
In gravida ≥2	1.00 (0.99, 1.01)	0.45
Gravidity[4]		
≥2	0.84 (0.76, 0.92)	0.0004
HIV infection[5]	0.78 (0.72, 0.84)	<0.00001
Malaria parasitemia[5]	0.60 (0.35, 1.02)	0.065
Serum ACT (g/L)[6]		
0.3–0.4	0.97 (0.92, 1.07)	0.77
0.4–0.5	0.89 (0.78, 1.02)	0.10
>0.5	0.56 (0.46, 0.68)	<0.00001

[1] n = 1419. Adjusted R^2 = 0.16.
[2] Season was defined as early (June to August) and late (September to November) dry and late rainy (March to May), with use of early rainy (December to February) as the reference category.
[3] The effect of age was significantly different in gravida 1 and gravida ≥2 ($P = 0.00002$).
[4] Effect of gravida ≥2 assessed at a mean age of 24 y.
[5] Coded as 0 for absent and 1 for present.
[6] Serum α_1-antichymotrypsin (ACT) ≤0.3 g/L was used as the reference category.

TABLE 6
Regression coefficients (β) and 95% CIs for predictors of serum retinol (μmol/L) in pregnant women[1]

Variable	β (95% CI)	*P*
Season[2]		
Late rainy	−0.11 (−0.18, −0.05)	0.0004
Early dry	−0.05 (−0.09, −0.001)	0.044
Late dry	−0.05 (−0.10, −0.002)	0.042
Age (y)[3]		
In HIV infected	−0.004 (−0.010, 0.002)	0.15
In HIV uninfected	0.004 (0.0001, 0.008)	0.03
Gestational age (wk)	−0.02 (−0.027, −0.018)	<0.00001
Use of prenatal supplements[4]	0.10 (0.01, 0.18)	0.03
HIV infection[5]	−0.05 (−0.09, −0.02)	0.003
Malaria parasitemia[6]	−0.21 (−0.45, 0.026)	0.08
Serum ACT (g/L)[7]		
0.3–0.4	−0.04 (−0.08, −0.008)	0.016
0.4–0.5	−0.15 (−0.21, −0.08)	<0.00001
>0.5	−0.41 (−0.51, −0.31)	<0.00001

[1] n = 1419. Adjusted R^2 = 0.11.
[2] Season was defined as early (June to August) and late (September to November) dry and late rainy (March to May), with use of early rainy (December to February) as the reference category.
[3] The effect of age was significantly different in HIV-infected and uninfected women (P = 0.02).
[4] Previous intake of any micronutrient supplement in current pregnancy coded as 1 versus 0.
[5] Coded as 0 for absent and 1 for present. The effect was estimated at a mean age of 24 y.
[6] Coded as 0 for absent and 1 for present.
[7] Serum α_1-antichymotrypsin (ACT) ≤0.3 g/L was used as the reference category.

was excluded from the model. No interactions between age and parity or between any of the other variables assessed were found.

Plurality and sex of the fetus were later determined in 1197 (71.7%) of the 1580 women for whom measurements of serum β-carotene and retinol were available. When the relation was assessed in the final models, women carrying a male fetus had a mean serum retinol concentration that was 0.04-μmol/L (95% CI: 0.00005, 0.08; P = 0.048) lower than that of women expecting a girl. The effect of fetal sex was not explained by the sex differences in size of the fetus. In fact, birth weight was a marginally significant independent predictor (β = −0.03; 95% CI: −0.08, 0.003; P = 0.07), but inclusion in the model did not change the estimated effect of fetal sex. With plurality coded as 0 for singletons, 1 for twins, and 2 for triplets, the effect was a 0.21-μmol/L (95% CI: 0.08, 0.35; P = 0.002) lower maternal serum retinol concentration per additional fetus. When assessed with use of dummy variables, similar effects were seen: the 16 women carrying twins had a 0.21-μmol/L (95% CI: 0.04, 0.39; P = 0.017) lower mean serum retinol concentration, whereas the 2 women carrying triplets had a 0.44-μmol/L (95% CI: 0.02, 0.86; P = 0.04) lower mean serum retinol concentration, than did the women with a singleton pregnancy when other factors were controlled for in the model. In contrast, neither sex nor plurality was a predictor of serum β-carotene.

DISCUSSION

Serum retinol remains the most widely used measure of vitamin A status. The main limitation of this measure is that the serum retinol concentration is homoeostatically controlled over a wide range of liver retinol concentrations, wherefore serum retinol is a better measure of status in deficiency than in sufficiency (3). Furthermore, serum retinol declines during the acute phase response because of reduced synthesis of retinol binding protein (RBP) (11), urinary excretion of retinol bound to RBP (12, 13), and probably fluxing of retinol and RBP to the extravascular space, making retinol available to tissues (14). Accordingly, the acute phase response leads to an underestimation of vitamin A status and confounded estimates of the effects of low serum retinol. It is therefore recommended to simultaneously measure an acute phase protein to control for potential confounding. C-reactive protein (15), amyloid A (16), α_1-acid glycoprotein (AGP) (17), and ACT (PI Paracha, A Jamil, K Belbase, et al, unpublished observations, 1999) have all been used for this purpose, but it is not clear which most closely follows the changes in serum retinol during the acute phase response.

Although higher concentrations of acute phase proteins are seen in AIDS patients (18), HIV infection was not associated with ACT in our study of pregnant women. This is not necessarily surprising because the study participants were generally asymptomatic, fertile women. Interestingly, serum ACT was higher in gravida 1 than in gravida ≥2 and was marginally significantly higher in midgestation than in late gestation. Elevated serum ACT was an important independent predictor of serum β-carotene and retinol and explained a considerable proportion of the variation in these micronutrients. There are no established cutoffs for serum ACT, so we assessed the effects of serum ACT by using dummy variables. With use of <0.3 g/L as the reference category, only values >0.5 g/L were associated with reductions in serum β-carotene. For serum retinol bound to the negative acute phase proteins RBP and transthyretin, serum ACT concentrations of 0.3–0.4, 0.4–0.5, and >0.5 g/L were associated with 0.05-, 0.14-, and 0.38-μmol/L lower serum retinol concentrations, respectively.

Ghanian children with elevated serum AGP (>1 g/L) had a 24% lower serum retinol concentration than did children with normal serum AGP concentrations (16). In Bangladeshi children with shigella dysentery, neither serum C-reactive protein nor AGP was a predictor of serum retinol at the time of admission to the hospital, but the former was a predictor at discharge (19). Among children in Papua New Guinea, a 1-SD increase in serum AGP corresponded to a 0.12-μmol/L reduction in serum retinol (20). In pregnant Nepali women, elevated serum AGP (≥1 g/L) and C-reactive protein (≥5 mg/L) were associated with a 0.2–0.3-μmol/L lower mean serum retinol concentration (21).

Our finding that serum ACT concentrations as low as 0.3–0.4 g/L were associated with low serum retinol was surprising in view of a population mean between 0.33 and 0.34 g/L in women without malaria and HIV. This observation, and the fact that gravidity and gestational age were predictors of serum ACT, suggests that the serum ACT concentration changes during pregnancy and may serve to regulate various metabolic and immunologic processes.

HIV and malaria infection

Lower serum β-carotene and retinol in individuals with HIV (22, 23) and malaria (24–26) than in healthy persons is well described, although HIV infection had no effect on carotenoid concentrations in pregnant Malawian women (27). The magnitude of the estimated effects of malaria and HIV varies considerably, probably because of a lack of control for the acute phase response and other confounders and a lack of assessment of effect modifiers. Interestingly, we found that the negative statistical

effect of HIV infection on serum retinol, but not β-carotene, increased with age, probably because age was a proxy of length of time with HIV infection.

Season

Serum β-carotene varied considerably by season, with concentrations in the dry seasons only one-half of those in the early rainy season (December to February), when mangoes and papaya are available in Zimbabwe. Less pronounced seasonal differences were observed for serum retinol, most likely because of homoeostatic control of retinol. Large seasonal variations in vitamin A intake and concentrations of plasma carotenoids, but not retinol, were reported in The Gambia (28).

Age and obstetric history

The effects of age on serum β-carotene depended on gravidity, in that serum β-carotene increased 1.05 times per 1-y increase in age, but only in gravida 1. That is, serum β-carotene was higher in gravida 1 than in gravida ≥2, except in the very young women, and the difference increased with age. It is hard to explain this finding, but given the strength of the statistical association, it cannot be neglected. It is conceivable but cannot be substantiated that this finding has something to due with taboos in relation to intake of provitamin A carotenoid-rich foods during pregnancy that differ between gravida 1 and ≥2. However, a corresponding age-gravidity interaction on retinol was not found. The increase in serum retinol with age in uninfected women is similar to that found in female, but not male, schoolchildren in Kenya (26). In the present study, if this finding were due to higher intake, then an increase in serum β-carotene with age in not only gravida 1 but also in gravida ≥2 would be expected. Serum retinol, but not serum β-carotene, decreased with increasing gestational age. This probably was due to the effects of hemodilution, the transfer of vitamin A from the mother to the fetus, and increased utilization of vitamin A by the mother. The net increase in plasma volume was estimated to increase from 50 mL at 10 wk of gestation to 800, 1200, and 1500 mL at 20, 30, and 40 wk, respectively (29). In contrast, the weight of the fetus increased from 5 g at 10 wk of gestation to 300, 1500, and 3400 g at 20, 30, and 40 wk, respectively. In other words, hemodilution is likely to explain more of the effect of the 0.10-μmol/L lower serum retinol concentration in weeks 25–28 than in weeks 22–25, whereas transfer of vitamin A to the fetus may explain most of the 0.20-μmol/L lower concentration in women between 32 and 35 wk of gestation.

That the decline in serum retinol was explained mainly by transfer of vitamin A to the fetal stores and not merely by physiologic changes in pregnancy is supported by the estimated effects of carrying twins and triplets. Although lower serum retinol in cord blood from male than from female neonates has been shown (30), an effect of fetal sex on maternal serum retinol has to our knowledge not been previously reported. The effect was not explained by the slightly greater weight of the male fetuses and, hence, liver stores, and may rather have been due to hormonal factors and organogenesis.

Women reporting intake of supplements before enrollment had higher concentrations of serum retinol, but not of serum β-carotene, than did women who did not take supplements. The effect on serum retinol was surprising because few women reported consuming supplements likely to have contained vitamin A. Alternatively, reported use of prenatal supplements could be a marker of higher socioeconomic status and hence higher intake of preformed vitamin A.

Conclusion

Season and obstetric variables are important predictors of not only serum β-carotene and retinol but also serum ACT. Furthermore, serum ACT is a strong predictor of serum β-carotene and retinol and may have metabolic and immunologic regulatory functions. HIV infection was associated with lower serum β-carotene and retinol, but these effects did not seem to be mediated by the acute phase response as assessed by serum ACT.

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REFERENCES

1. Fawzi WW, Chalmers TC, Herrera MG, Mosteller F. Vitamin A supplementation and child mortality. A meta-analysis. JAMA 1993; 269:898–903.
2. West KP Jr, Katz J, Khatry SK, et al. Double blind, cluster randomised trial of low dose supplementation with vitamin A or beta carotene on mortality related to pregnancy in Nepal. The NNIPS-2 Study Group. BMJ 1999;318:570–5.
3. Olson JA, Loveridge N, Duthie GG, Shearer MJ. Fat-soluble vitamins. In: Garrow JS, James WPT, Ralph A, eds. Human nutrition and dietetics. Edinburgh: Churchill Livingstone, 2000:211–47.
4. Ross AC, Gardner EM. The function of vitamin A in cellular growth and differentiation, and its roles during pregnancy and lactation. Adv Exp Med Biol 1994;352:187–200.
5. Azais-Braesco V, Pascal G. Vitamin A in pregnancy: requirements and safety limits. Am J Clin Nutr 2000;71(suppl):1325S–33S.
6. Stoltzfus RJ. Vitamin A deficiency in the mother-infant dyad. SCN News 1994;11:25–7.
7. Prentice AM, Goldberg GR, Poppitt SD. Reproductive stresses in undernourished and well-nourished women. Bibl Nutr Dieta 1996; 53:1–10.
8. Katz J, West KP Jr, Khatry SK, et al. Maternal low-dose vitamin A or β-carotene supplementation has no effect on fetal loss and early infant mortality: a randomized cluster trial in Nepal. Am J Clin Nutr 2000;71:1570–6.
9. Krarup HB, Drewes AM, Madsen PH. A quantitative HCV-PCR test for routine diagnostics. Scand J Clin Lab Invest 1998;58:415–22.
10. Sowell AL. Simple, rapid method for assessment of serum vitamin A concentration. Clin Lab Sci 1994;7:160–1.
11. Rosales FJ, Ritter SJ, Zolfaghari R, Smith JE, Ross AC. Effects of acute inflammation on plasma retinol, retinol-binding protein, and its mRNA in the liver and kidneys of vitamin A-sufficient rats. J Lipid Res 1996;37:962–71.
12. Stephensen CB, Alvarez JO, Kohatsu J, Hardmeier R, Kennedy JI Jr, Gammon RB Jr. Vitamin A is excreted in the urine during acute infection. Am J Clin Nutr 1994;60:388–92.
13. Mitra AK, Alvarez JO, Guay-Woodford L, Fuchs GJ, Wahed MA, Stephensen CB. Urinary retinol excretion and kidney function in children with shigellosis. Am J Clin Nutr 1998;68:1095–103.
14. Thurnham DI, Singkamani R. The acute phase response and vitamin A status in malaria. Trans R Soc Trop Med Hyg 1991;85:194–9.
15. Friis H, Ndhlovu P, Kaondera K, et al. Serum concentration of micronutrients in relation to schistosomiasis and indicators of infection: a cross-sectional study among rural Zimbabwean schoolchildren. Eur J Clin Nutr 1996;50:386–91.
16. Filteau SM, Morris SS, Abbott RA, et al. Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. Am J Clin Nutr 1993;58:192–7.

17. Filteau SM, Morris SS, Raynes JG, et al. Vitamin A supplementation, morbidity, and serum acute-phase proteins in young Ghanaian children. Am J Clin Nutr 1995;62:434–8.
18. Jahoor F, Gazzard B, Phillips G, et al. The acute-phase protein response to human immunodeficiency virus infection in human subjects. Am J Physiol 1999;276:E1092–8.
19. Mitra AK, Alvarez JO, Wahed MA, Fuchs GJ, Stephensen CB. Predictors of serum retinol in children with shigellosis. Am J Clin Nutr 1998;68:1088–94.
20. Rosales FJ, Topping JD, Smith JE, Shankar AH, Ross AC. Relation of serum retinol to acute phase proteins and malarial morbidity in Papua New Guinea children. Am J Clin Nutr 2000;71:1582–8.
21. Christian P, Schulze K, Stoltzfus RJ, West KP Jr. Hyporetinolemia, illness symptoms, and acute phase protein response in pregnant women with and without night blindness. Am J Clin Nutr 1998; 67:1237–43.
22. Allard JP, Aghdassi E, Chau J, Salit I, Walmsley S. Oxidative stress and plasma antioxidant micronutrients in humans with HIV infection. Am J Clin Nutr 1998;67:143–7.
23. Baum M, Cassetti L, Bonvehi P, Shor-Posner G, Lu Y, Sauberlich H. Inadequate dietary intake and altered nutrition status in early HIV-1 infection. Nutrition 1994;10:16–20.
24. Das BS, Thurnham DI, Das DB. Plasma α-tocopherol, retinol, and carotenoids in children with falciparum malaria. Am J Clin Nutr 1996;64:94–100.
25. Davis TM, Binh TQ, Danh PT, et al. Serum vitamin A and E concentrations in acute falciparum malaria: modulators or markers of severity? Clin Sci (Colch) 1994;87:505–11.
26. Friis H, Mwaniki D, Omondi B, et al. Serum retinol concentrations and *Schistosoma mansoni*, intestinal helminths, and malarial parasitemia: a cross-sectional study in Kenyan preschool and primary school children. Am J Clin Nutr 1997;66:665–71.
27. Lan Y, Kumwenda N, Taha TE, et al. Carotenoid status of pregnant women with and without HIV infection in Malawi. East Afr Med J 1999;76:133–7.
28. Bates CJ, Prentice AM, Paul AA. Seasonal variations in vitamins A, C, riboflavin and folate intakes and status of pregnant and lactating women in a rural Gambian community: some possible implications. Eur J Clin Nutr 1994;48:660–8.
29. King JC. Physiology of pregnancy and nutrient metabolism. Am J Clin Nutr 2000;71(suppl):1218S–25S.
30. Yassai MB, Malek F. Newborns vitamin A in relation to sex and birth weight. J Trop Pediatr 1989;35:247–9.