HIV-1 Infection Alters the Retinol-Binding Protein:Transthyretin Ratio Even in the Absence of the Acute Phase Response[1]

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ABSTRACT   The ratio of retinol-binding protein (RBP) to transthyretin (TTR) has been proposed as an indirect method with which to assess vitamin A status in the context of inflammation. Few studies have been conducted among adults, and none examined the effect of HIV-1 infection. Our goal was to assess the RBP:TTR ratio among adults, including the effects of HIV-1 and the acute phase response. We used data from a cross-sectional study of 600 Kenyan women, of whom 400 had HIV-1. The effect of vitamin A supplementation among the HIV-1–infected participants was subsequently assessed in a randomized trial. Among HIV-1–uninfected women without an acute phase response, a RBP:TTR cut-off value of 0.25 had ~80% sensitivity and specificity to detect vitamin A deficiency (retinol <0.70 μmol/L). No RBP:TTR cut-off value demonstrated both high sensitivity and specificity among HIV-1 infected women without evidence of inflammation. HIV-1 infection and advanced HIV-1 disease were associated with higher RBP:TTR ratios. The effect of HIV-1 was independent of the acute phase response, which also increased the RBP:TTR ratio. Serum retinol increased with vitamin A supplementation among those with a low RBP:TTR ratio, although the effect was small and was not present among those with concurrent inflammation. Thus, the RBP:TTR ratio has modest ability to predict vitamin A deficiency among healthy adults, but HIV-1 infection alters the ratio, even in the absence of the acute phase response. Our results raise questions about the utility of this measurement given the high prevalence of HIV-1 infection in areas where vitamin A deficiency is common.   J. Nutr. 136: 1624–1629, 2006.

KEY WORDS: • vitamin A status • retinol-binding protein • transthyretin • HIV • inflammation

Vitamin A deficiency remains an important public health issue worldwide as a cause of morbidity and mortality among women and children (1). Reliable and accurate biochemical measures are necessary for the assessment of vitamin A deficiency in populations and for the monitoring of supplementation initiatives. Two biochemical indicators have traditionally been used for determining the extent of vitamin A deficiency within communities: serum retinol and serum retinol-binding protein (RBP),[3] which serves as a 1-to-1 carrier of retinol in the blood (2). Retinol is used more commonly and standard cut-off values have been established for defining vitamin A deficiency, whereas RBP is more stable under some field conditions and can potentially be measured at a lower cost. However, both retinol and RBP concentrations decline not only as a result of vitamin A deficiency but also in the context of the acute phase response, which accompanies inflammatory states such as infection or trauma (3). Thus, inflammation may confound the interpretation of retinol or RBP data, leading to inaccurate assessment of vitamin A deficiency (4).

The molar ratio of RBP to transthyretin (TTR) has been proposed as a method with which to assess vitamin A status indirectly in the context of inflammation or infection (5). TTR, also known as prealbumin, plays an important role in the transport of vitamin A and other molecules in the blood. Both RBP and TTR are synthesized by the liver, but unlike RBP, TTR secretion does not depend on vitamin A stores, and its serum concentration is not reduced as a result of vitamin A deficiency (6). However, both RBP and TTR are negative acute phase reactants, which means that their concentrations fall during inflammatory states. Thus, the RBP:TTR ratio should decrease during vitamin A deficiency, because RBP declines in parallel with retinol but TTR remains unchanged; this should not be the case during the acute phase response, when retinol, RBP, and TTR all decline. This difference in response of the

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RBP:TTR ratio should indicate whether a low retinol concentration is a result of true deficiency or a consequence of an inflammatory state. Only a small number of studies, primarily among children, investigated the RBP:TTR ratio among populations at risk for vitamin A deficiency (5,7–10).

We reported previously the results of 2 analyses from a large cross-sectional study of Kenyan women that examined the interaction between HIV-1 infection, the acute phase response, and measures of vitamin A status (3,11). In the first study, HIV-1 infection and the acute phase response were shown to depress serum retinol concentrations, consistent with other studies of the effect of infection and inflammation on circulating vitamin A levels (11). In the second study, serum RBP was shown to correlate strongly with serum retinol, even in the context of HIV-1 infection or the acute phase response, confirming that RBP and retinol levels react in parallel to inflammatory states (3). In the present analysis, we used data from this same cohort of women to examine the effect of HIV-1 infection and the acute phase response on the RBP:TTR ratio.

SUBJECTS AND METHODS

Study participants and procedures. Between September 1998 and June 2000, 400 HIV-1 infected women attending outpatient clinics at Coast Provincial General Hospital in Mombasa, Kenya were enrolled in a vitamin A supplementation trial (12). At the enrollment visit, data were collected for a cross-sectional study of the relation between vitamin A deficiency, HIV-1 infection, and the acute phase response (11). Randomly selected women ($n = 200$) who were also attending the outpatient clinics and who were screened for trial participation but were found to be HIV-1 uninfected also participated in the cross-sectional study. Serum and EDTA-anticoagulated blood were obtained and were light-protected after collection. All women provided written informed consent, and the study protocol was approved by the institutional review boards of the University of Washington and the University of Nairobi.

Study exclusion criteria included age <18 or >45 y or pregnancy, use of vitamin supplements, or use of oral contraceptive pills within the previous 3 mo. No women had ocular signs of vitamin A deficiency (xerophthalmia or Bitot's spots), and none of the HIV-1 seropositive women used antiretroviral therapy.

After completing the cross-sectional study, the HIV-1 seropositive women were randomly assigned in a 1-to-1 fashion to receive 6 wk of daily oral supplementation with either 10,000 IU vitamin A as retinyl palmitate or placebo, supplied as soft-gel capsules containing soybean oil (Tishcon) (12). This dosage is recommended by the WHO for correction of symptomatic vitamin A deficiency in women of childbearing age (13). The regimens were indistinguishable, and field researchers were unaware of treatment assignments. Women returned after 6 wk for follow-up, at which time blood samples were collected and a pill count was done to assess compliance.

Both HIV-1 seropositive and seronegative participants received 4 wk of daily 10,000 IU vitamin A after they completed the study to ensure treatment of subclinical deficiency (13).

Laboratory methods. HIV-1 serology was by ELISA (Detect HIV-1/2, BioChem ImmunoSystems, confirmed if positive by Recombigen, Cambridge Biotech). Absolute CD4 counts (Zymmune CD4/CD8 Cell Monitoring Kit, Bartels) were determined for HIV-1 seropositive women (14).

Serum was separated within 4 h of collection, stored in cryovials at −70°C, and shipped on dry ice to the University of Washington. HPLC was used to measure the concentration of serum retinol (15). Testing was performed with single samples on a minimum of 100 μL of sample. Retinol, after lipid extraction, was detected at 313 nm after separation using a C18 reversed-phase column. The column was eluted isocratically with a methanol:water mobile phase using retinyl acetate as an internal standard. Between-run precision was <4%. In addition to monitoring assay stability with in-laboratory control material, the testing laboratory participates in the National Institute of Standards

and Technology Triannual Survey. A standard cut-off value of <0.70 μmol/L was used to define vitamin A deficiency (2).

Serum RBP and TTR were measured by nephelometry (Dade Behring). The lower limits of quantification were 0.52 μmol/L for RBP and 0.36 μmol/L for TTR. Nephelometry was also used to measure serum concentrations of C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) (Dade Behring). An acute phase response was defined as concentrations of CRP >5 mg/L and/or AGP >1.0 g/L, as suggested by a recent metaanalysis (16). For all nephelometry measures, tests used 300–400 μL of serum, were done with single samples, and had CV <10%.

Statistical methods. Statistical analyses were conducted using SPSS 10.0. RBP and TTR concentrations below the limit of quantification were set at half that limit. Comparisons of categorical variables were conducted using χ^2 tests, and comparisons of continuous variables were conducted using Mann-Whitney U-tests, Spearman's correlation coefficient, and linear regression. A two-sided α of 0.05 was considered statistically significant.

Receiver operating characteristic (ROC) plots were constructed to explore the sensitivity and specificity of various RBP:TTR cut-off values to predict serum retinol concentrations <0.70 μmol/L (17). The area under the curve (AUC), a summary measure of test performance, was calculated. A perfect test has an AUC of 1.0, and a test with no predictive value has an AUC of 0.5. The utility of the RBP:TTR ratio for prediction of low retinol concentrations was first assessed among women with neither HIV-1 infection nor the acute phase response because serum retinol would be expected to accurately reflect vitamin A status among this group. An RBP:TTR cut-off value that had good sensitivity and specificity was selected and used to predict the prevalence of true vitamin A deficiency among women who had serum retinol concentrations <0.70 μmol/L and either an acute phase response, HIV-1 infection, or both. Additional analyses compared the contributions of HIV-1 infection and the acute phase response to the magnitude of the RBP:TTR ratio.

RESULTS

Study population. Four hundred HIV-1 seropositive women and 200 HIV-1 seronegative women were enrolled. One HIV-1 seropositive woman was excluded from the analysis because RBP concentration could not be determined. Among the remaining participants, the median age was 27 y (interquartile range [IQR] 23–32 y). HIV-1 seropositive women were slightly older, had fewer years of education, and were less likely to be currently married than HIV-1 seronegative women (**Table 1**). They also had had more pregnancies and were less likely to have characteristics associated with higher socioeconomic status, such as having running water in the home. Among the HIV-1 seropositive women, the median CD4 count was 226 cells/μL (IQR 109–364).

Serum retinol, RBP, and TTR concentrations were each lower among HIV-1 seropositive compared with seronegative women, as was the prevalence of retinol concentrations suggesting vitamin A deficiency (26 vs. 6%, respectively, $P <$ 0.001). In contrast, the median RBP:TTR ratio was higher (0.32 vs. 0.28, respectively, $P < 0.001$). RBP concentrations were below the limit of quantification for 47 (8%) women; only 1 woman had a TTR concentration (<1%) below its limit of quantification. The acute phase response was more common among HIV-1 seropositive than seronegative women (58 vs. 24%, respectively, $P < 0.001$). There was a weak, but significant, correlation between serum retinol concentrations and the RBP:TTR ratio overall (Spearman's $r = 0.16$, $P < 0.001$), which was considerably stronger among the HIV-1 seronegative women (Spearman's $r = 0.57$, $P < 0.001$) than among the HIV-1 seropositive women (Spearman's $r = 0.16$, $P = 0.002$).

RBP:TTR ratio among HIV-1 seronegative women. The utility of the RBP:TTR ratio to predict vitamin A status was

TABLE 1

Population characteristics[1]

Characteristic	HIV-1 seropositive	HIV-1 seronegative	*P*-value[2]
	n = 399	*n = 200*	
Demographic characteristics			
Age, *y*	28 (23–33)	26 (23–30)	0.006
Education, *y*	7 (4–8)	8 (6–12)	<0.001
Currently married	206 (52%)	133 (67%)	0.001
Gravidity	2 (1–4)	1 (0–3)	<0.001
Running water in home	19 (5%)	27 (14%)	<0.001
Measures of vitamin A status			
Retinol, *μmol/L*	0.96 (0.68–1.22)	1.28 (1.00–1.51)	<0.001
<0.70 *μ*mol/L (vitamin A deficiency)	103 (26%)	11 (6%)	<0.001
RBP, *μmol/L*	0.90 (0.67–1.19)	1.10 (0.86–1.33)	<0.001
TTR, *μmol/L*	2.91 (2.00–3.69)	3.93 (3.24–4.47)	<0.001
RBP:TTR ratio	0.32 (0.28–0.39)	0.28 (0.25–0.31)	<0.001
Acute phase proteins			
CRP, *mg/L*	3.10 (1.20–10.60)	1.00 (0.40–2.78)	<0.001
AGP, *g/L*	1.03 (0.76–1.56)	0.69 (0.52–0.87)	<0.001
Acute phase response[3]	233 (58%)	47 (24%)	<0.001

[1] Values are medians (interquartile range) or *n* (%).
[2] Comparisons of categorical variables were conducted using χ^2 tests and comparisons of continuous variables using Mann-Whitney U-tests.
[3] Defined as CRP >5 mg/L and/or AGP >1.0 g/L.

first assessed among the 153 HIV-1 seronegative women who did not have an acute phase response, 10 (6.5%) of whom had retinol concentrations <0.70 *μ*mol/L. The median RBP:TTR ratio was 0.27 (IQR 0.25–0.31), and was lower for women with retinol <0.70 *μ*mol/L compared with those with retinol ≥0.70 *μ*mol/L (0.21 vs. 0.28, *P* < 0.001, respectively). An ROC plot was constructed to assess the ability of RBP:TTR to detect retinol <0.70 *μ*mol/L. Its AUC was 0.91 (95% CI 0.83–0.98) (**Fig. 1A**), demonstrating excellent prediction. A cut-off value of 0.25 had 80% sensitivity (8/10) and 79% specificity (113/143). In this population, the positive predictive value of this cut-off value was modest (21%, 8/38) but its negative predictive value was high (98%, 113/115). Higher cut-off values improved sensitivity, but at significant expense to specificity, whereas lower cut-off values provided higher specificity, with significant loss of sensitivity. For example, a RBP:TTR cut-off value of 0.30 (5,7) had 100% sensitivity but only 32% specificity.

There was evidence of an acute phase response in 47 HIV-1 seronegative women. The acute phase response was associated with lower retinol, RBP, and TTR concentrations, and a significantly higher median RBP:TTR ratio among HIV-1 seronegative women (Table 2). Only 1 HIV-1 seronegative woman with an acute phase response had a serum retinol concentration <0.70 *μ*mol/L, and her RBP:TTR ratio was 0.12, suggesting true vitamin A deficiency.

RBP:TTR ratio among HIV-1 seropositive women. To assess the effect of HIV-1 infection on the RBP:TTR ratio, its relation to serum retinol was assessed among the 166 HIV-1 seropositive women who did not have an acute phase response; of these, 20 women (12%) had serum retinol concentrations <0.70 *μ*mol/L. The median RBP:TTR ratio was 0.30 (IQR 0.26–0.33), and was lower for women with retinol <0.70 *μ*mol/L compared with those with retinol ≥0.70 *μ*mol/L (0.25 vs. 0.30, *P* = 0.001, respectively). An ROC plot evaluating the RBP:TTR ratio to detect retinol <0.70 *μ*mol/L had only marginal predictive value, with an AUC of 0.73 (95% CI 0.59–0.87) (Fig. 1B). No RBP:TTR cut-off value demonstrated both high

(e.g., >70%) sensitivity and specificity to detect retinol <0.70 *μ*mol/L. Using a RBP:TTR cut-off value of 0.25, 10 of 20 women (50%) with retinol <0.70 *μ*mol/L were predicted to have true vitamin A deficiency.

The effect of HIV-1 disease stage was also examined among women without an acute phase response. Women with CD4 counts <200 cells/*μ*L had higher RBP:TTR ratios (0.31 vs. 0.29, respectively, *P* = 0.009) and tended to have lower retinol concentrations (1.02 vs. 1.13 *μ*mol/L, respectively, *P* = 0.1) than women with CD4 counts ≥200 cells/*μ*L. Even among those without advanced immunosuppression (i.e., CD4 counts ≥200 cells/*μ*L), the RBP:TTR ratio was able to predict retinol <0.70 *μ*mol/L with only marginal accuracy (area under ROC curve 0.76, 95% CI 0.59–0.92).

There was evidence for an acute phase response in 233 HIV-1 seropositive women, and this was associated with significantly lower retinol, RBP, and TTR concentrations, and significantly higher RBP:TTR ratios (**Table 2**). Among the subgroup of 83 HIV-1 seropositive women with retinol concentrations <0.70 *μ*mol/L, 19 (23%) were predicted to have vitamin A deficiency using a RBP:TTR cut-off value of 0.25.

Combined effects of HIV-1 infection and the acute phase response on the RBP:TTR ratio. HIV-1 infection was associated with significantly higher median RBP:TTR ratios, both for women with (0.35 vs. 0.30, *P* = 0.002) and without (0.30 vs. 0.27, *P* < 0.001) an acute phase response (Table 2). Both HIV-1 infection and the acute phase response increased the RBP:TTR ratio and reduced the correlation between the RBP:TTR ratio and retinol (**Fig. 2**). In multivariate linear regression analysis, HIV-1 infection and the acute phase response independently increased the RBP:TTR ratio (regression coefficients +0.04, 95% CI 0.02–0.06, *P* < 0.001 and +0.06, 95% CI 0.04–0.08, *P* < 0.001, respectively). There was no interaction between HIV-1 infection and the acute phase response on the RBP:TTR ratio (*P* = 0.08).

Effect of vitamin A supplementation among HIV-1 seropositive women. As described elsewhere (12), after completion of the cross-sectional study, the 400 HIV-1 seropositive women
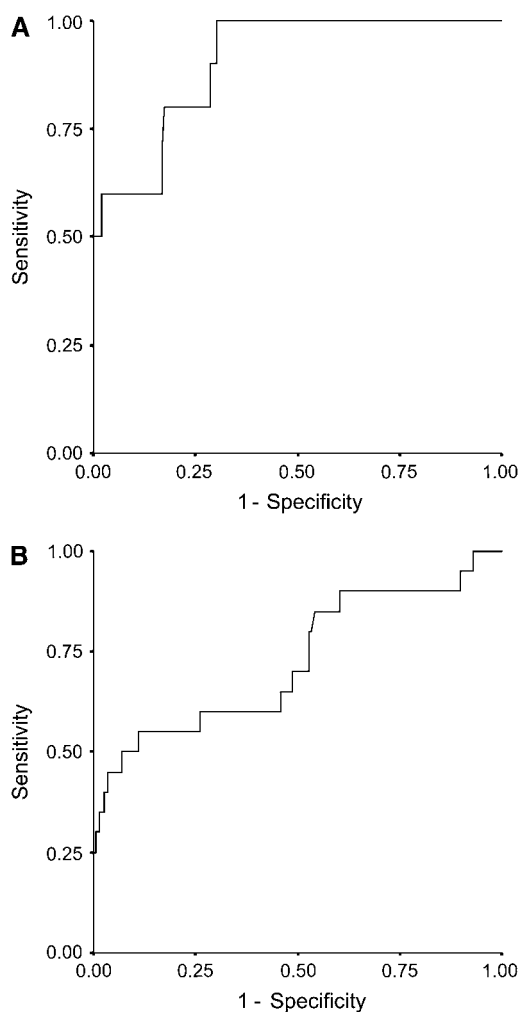
FIGURE 1 ROC plot for the molar RBP:TTR ratio for prediction of vitamin A deficiency (defined as retinol <0.70 μmol/L) among HIV-1 seronegative (*A*) and seropositive (*B*) women who did not have an acute phase response. The AUC is 0.91 (95% CI 0.83–0.98) for (*A*) and 0.73 (95% CI 0.59–0.87) for (*B*).

with the intervention was excellent, with ~95% of women taking ≥95% of their assigned pills.

Overall, vitamin A supplementation resulted in a modest, but significant, increase in serum retinol concentrations (**Table 3**). In subgroup analysis, this effect was restricted to those women who had a RBP:TTR ratio <0.25 at baseline, and there was a trend for an effect of supplementation only for those in this subgroup who did not have an acute phase response. Whether they received vitamin A or placebo, women who had had an acute phase response at baseline had higher retinol levels at the 6-wk follow-up visit, perhaps reflecting a resolving inflammatory state (5).

DISCUSSION

In this study of 600 Kenyan women, the RBP:TTR ratio demonstrated reasonable accuracy for prediction of vitamin A deficiency among HIV-1 seronegative women without an acute phase response. However, among HIV-1 seropositive women, even in the absence of elevated inflammatory markers, the RBP:TTR ratio had significantly less ability to predict vitamin A status. HIV-1 infection and the acute phase response independently increased the RBP:TTR ratio and decreased the correlation between the RBP:TTR ratio and serum retinol concentrations. A low RBP:TTR ratio was associated with response to vitamin A supplementation among the HIV-1–infected women, although the effect was modest.

Few studies have examined the performance of the RBP:TTR ratio to assess vitamin A deficiency among adults, and no standard RBP:TTR cut-off value has been established. One study of 100 healthy volunteers and 31 individuals undergoing elective surgery found that the RBP:TTR ratio was adequate for predicting vitamin A deficiency (area under the ROC curve of 0.82) (8). Serum retinol <0.70 μmol/L was present in 8% of the study population, and a RBP:TTR cut-off value of 0.37 achieved 82% sensitivity, 79% specificity, and positive and negative predictive values of 27 and 98%, respectively, for predicting deficiency. In comparison, among healthy women in our study, the RBP:TTR ratio provided a slightly higher predictive value (from ROC testing) and similar predictive percentiles when a cut-off value of 0.25 was used. The only study that used liver biopsy (the gold standard for assessment of vitamin A status) to evaluate the relation between the RBP:TTR ratio and liver vitamin A stores found that a cut-off value of 0.36 had 71% sensitivity and 50% specificity for detecting deficiency among 15 Bangladeshi

were randomly assigned to 6 wk of daily vitamin A or placebo. Demographic and medical characteristics and follow-up were comparable between the 2 randomization groups; 354 women (89%) returned for follow-up, at a median of 42 d. Compliance

TABLE 2

Serum retinol, RBP, and TTR concentrations and RBP:TTR ratios, stratified by HIV-1 and acute phase response status[1]

| | Median (IQR) | | | | P-value for comparison of medians[2] | | | |
| | | | | | Effect of APR | | Effect of HIV-1 | |
	HIV−/APR−, n = 153	HIV−/APR+, n = 47	HIV+/APR−, n = 166	HIV+/APR+, n = 233	HIV−/APR+ vs. HIV−/APR−	HIV+/APR+ vs. HIV+/APR−	HIV+/APR− vs. HIV−/APR−	HIV+/APR+ vs. HIV−/APR+
Retinol, μmol/L	1.29 (1.04–1.50)	1.19 (0.97–1.53)	1.10 (0.89–1.34)	0.84 (0.58–1.12)	0.6	<0.001	<0.001	<0.001
RBP, μmol/L	1.10 (0.88–1.31)	1.05 (0.86–1.33)	1.02 (0.81–1.25)	0.86 (0.57–1.19)	0.6	<0.001	0.03	0.001
TTR, μmol/L	4.02 (3.42–4.56)	3.67 (2.82–4.25)	3.43 (2.82–4.06)	2.40 (1.60–3.20)	0.004	<0.001	<0.001	<0.001
RBP:TTR	0.27 (0.25–0.31)	0.30 (0.28–0.35)	0.30 (0.26–0.33)	0.35 (0.29–0.42)	<0.001	<0.001	<0.001	0.002

[1] APR is defined as positive when C-reactive protein >5 mg/L and/or AGP >1.0 g/L.
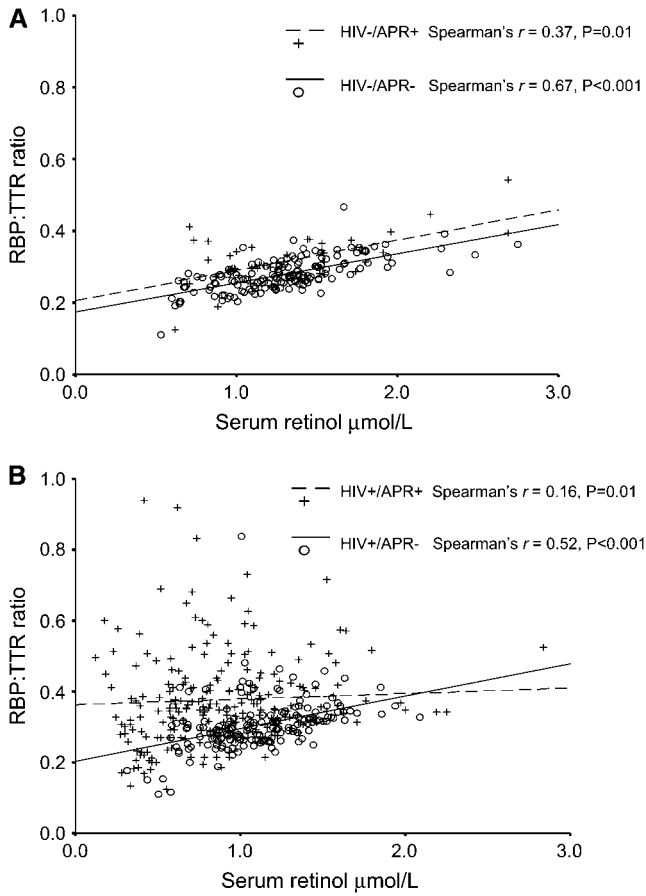[2] Comparisons by Mann-Whitney U-tests.

FIGURE 2 Relation between the RBP:TTR ratio and serum retinol among 599 Kenyan women, stratified by HIV-1 and acute phase response (APR) status. HIV-1 seronegative women are presented in (*A*), and HIV-1 seropositive women are in (*B*). Lines depict the best-fit linear regression of the data.

adults (9). Studies of children have used RBP:TTR cut-off values of 0.30 (5,7), 0.36 (9), and 0.47 (10) with variable success. In one study, among children whose vitamin A status was assessed using both the modified relative dose response test and serum retinol levels, a RBP:TTR cut-off value of 0.30 did not achieve the sensitivity and specificity levels that would make the test useful as an assessment tool (7).

Among the HIV-1 uninfected women in this study, the acute phase response was associated with lower retinol, RBP, and TTR concentrations and higher RBP:TTR ratios. These effects were more pronounced among the HIV-1 infected women. In previous studies, in both animal model systems and children, inflammation depressed retinol, RBP, and TTR levels, but RBP:TTR ratios were unchanged or often decreased (5,7). This unexpected decrease in the RBP:TTR ratio was postulated to occur because TTR changes as a result of inflammation may be more modest and delayed compared with RBP changes (7). However, some degree of TTR suppression as a result of the acute phase response is essential for the theoretical ability of the RBP:TTR ratio to differentiate vitamin A deficiency (which depresses RBP but not TTR) from inflammation (which depresses both RBP and TTR, and thus should not significantly depress their ratio). Our results suggest that differential suppression of RBP compared with TTR as a result of inflammation may not be as substantial in adults as in children. Alternatively, the results may indicate measurement at a later period in the course of an inflammatory event, when sufficient time has elapsed for TTR depression to occur. This scenario seems plausible because our study participants were outpatients, whereas those in some earlier studies were hospitalized with acute disease (7).

The most novel findings of our study concern the effect of HIV-1 on the RBP:TTR ratio. Among HIV-1 infected women without evidence of an acute phase response, the RBP:TTR ratio had only marginal ability to detect serum retinol <0.70 μmol/L, and a cut-off value of 0.25 predicted that the majority of women with serum retinol <0.70 μmol/L in this group would not have true vitamin A deficiency. HIV-1 infection, both among women with and without an acute phase response, was associated with lower concentrations of retinol, RBP, and TTR, and with higher RBP:TTR ratios. Furthermore, the effect on the RBP:TTR ratio was independent of evidence for a concurrent inflammatory state and was related to the degree of immunosuppression. We demonstrated previously that HIV-1 infection and, among infected individuals, more active or advanced disease were associated with the acute phase response (11). The present study implies that HIV-1 infection may have additional effects on measures of vitamin A status beyond those resulting from induction of an inflammatory reaction. Whether the RBP:TTR ratio can differentiate true deficiency among HIV-1 infected individuals is unknown, especially in the context of a concurrent acute phase response, when both conditions may simultaneously alter the ratio.

TABLE 3

Effect of vitamin A supplementation on serum retinol levels among HIV-1 seropositive women, stratified by RBP:TTR ratio and acute phase response status at enrollment[1]

| | Median serum retinol concentration, μmol/L | | | | | |
| | Enrollment | | | Follow-up | | |
Status at enrollment visit	Vitamin A	Placebo	*P*-value[2]	Vitamin A	Placebo	*P*-value[2]
All women, *n = 400*	0.95	0.97	0.4	1.03	0.94	0.03
RBP:TTR <0.25 *n = 54*	0.74	0.62	0.3	0.92	0.74	0.05
RBP:TTR ≥0.25 *n = 345*	1.01	1.03	0.8	1.04	0.98	0.2
RBP:TTR <0.25 and APR+ *n = 28*	0.52	0.44	0.3	0.78	0.75	0.4
RBP:TTR <0.25 and APR− *n = 26*	0.85	0.78	0.7	1.01	0.71	0.08

[1] APR is defined as positive when C-reactive protein >5 mg/L and/or AGP >1.0 g/L.
[2] Comparisons by Mann-Whitney U-tests.

Among HIV-1 infected women in this study, vitamin A supplementation increased serum retinol levels, especially among those who had RBP:TTR ratios <0.25 at baseline. Previous studies showed that individuals with low RBP:TTR ratios respond to vitamin A supplementation but those with higher ratios do not, suggesting that a low RBP:TTR ratio does to some extent indicate vitamin A deficiency (5,9). However, not all studies demonstrated that the RBP:TTR ratio correlates with response to supplementation (10). Furthermore, our results did not demonstrate a significant effect of supplementation among those who had evidence for an acute phase response in addition to a RBP:TTR ratio <0.25.

Previous studies of the RBP:TTR ratio showed that on a population level, it can predict the response to vitamin A supplementation, suggesting that a low ratio is broadly indicative of vitamin A deficiency (5,9). However, these studies generally did not assess how the RBP:TTR ratio could be used to define the vitamin A status among individual study participants. Several investigations examined the sensitivity and specificity of the RBP:TTR ratio to ascertain vitamin A status among healthy individuals (7–9), but only one assessed its prediction among individuals who were ill and those with subclinical inflammation (7). Our results suggest that the RBP:TTR ratio may be increased by HIV-1 infection, which could weaken its ability to predict vitamin A status in populations in which HIV-1 is prevalent.

The present study is the largest to date to examine the relation between the RBP:TTR ratio and other biomarkers of vitamin A status, and it had several strengths in addition to its size. First, it was conducted among African women, a population traditionally at risk for nutritional deficiency (1). Second, it included both HIV-1 infected and uninfected individuals, allowing for demonstration of novel effects of HIV-1 infection on the RBP:TTR ratio. Third, its population was drawn from an outpatient clinic setting, which permitted recruitment of individuals with and without inflammatory states, but none so sick as to require hospitalization. As a result, this cohort may more closely reflect populations recruited for community surveys of vitamin A status than cohorts of hospitalized individuals.

RBP concentrations and consequently RBP:TTR ratios were lower in our study than those in other populations. Among Canadian adolescents, the 2.5th–97.5th percentile range for the RBP:TTR ratio was 0.38–0.53 (9), and among healthy adults from Argentina with serum retinol ≥0.70 μmol/L, the RBP:TTR 2.5th–97.5th percentile range was 0.24–0.81 (8). In our study, among HIV-1 uninfected women without an acute phase response and with serum retinol ≥0.70 μmol/L, the 2.5th–7.5th percentile range was 0.21–0.37. A striking feature of these comparisons is the choice of study population. Our "healthy" group was defined by being HIV-1 uninfected and without evidence of an acute phase response, but all were African women, who are often at considerable risk of subclinical malnutrition from childbearing, poverty, and intermittent infections (e.g., malaria). Thus, our reference population, chosen from within our larger study population, was likely the best comparison group for this study. Disagreement remains about the appropriate methods for measuring RBP, and variability across studies is the principal reason that RBP (which can be easier to measure than retinol) has not replaced retinol for survey measures of vitamin A status (2,3).

Biochemical markers with standard cut-off values that will accurately define an individual's vitamin A status, especially in the context of inflammation or infection, are required to prevent misclassification of vitamin A deficiency in population studies (18). Our results confirm that the RBP:TTR ratio has modest predictive power among healthy individuals, but they raise questions about the utility of this measurement among HIV-1 infected individuals. Future studies will have to assess the ability of the RBP:TTR ratio to predict response to vitamin A supplementation in a variety of populations, and additional studies correlating biochemical markers with physiologic measures of vitamin A status, including liver biopsy, should be considered. Given the high prevalence of HIV-1 infection in areas in which vitamin A deficiency is common (19), our findings are important in planning surveys of vitamin A status and in monitoring supplementation initiatives.

LITERATURE CITED

1. World Bank. World Development Report 1993: Investing in health. New York: Oxford University Press; 1993.

2. de Pee S, Dary O. Biochemical indicators of vitamin A deficiency: serum retinol and serum retinol binding protein. J Nutr. 2002;132: 9 Suppl:2895S–901.

3. Baeten JM, Richardson BA, Bankson DD, Wener MH, Kreiss JK, Lavreys L, Mandaliya K, Bwayo JJ, McClelland RS. Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response. Am J Clin Nutr. 2004;79:218–25.

4. Tanumihardjo SA. Assessing vitamin A status: past, present and future. J Nutr. 2004;134:290S–3.

5. Rosales FJ, Ross AC. A low molar ratio of retinol binding protein to transthyretin indicates vitamin A deficiency during inflammation: studies in rats and a posterior analysis of vitamin A-supplemented children with measles. J Nutr. 1998;128:1681–7.

6. Navab M, Smith JE, Goodman DS. Rat plasma prealbumin. Metabolic studies on effects of vitamin A status and on tissue distribution. J Biol Chem. 1977;252:5107–14.

7. Filteau SM, Willumsen JF, Sullivan K, Simmank K, Gamble M. Use of the retinol-binding protein: transthyretin ratio for assessment of vitamin A status during the acute-phase response. Br J Nutr. 2000;83:513–20.

8. Zago LB, Dupraz H, Sarchi MI, Rio ME. The molar ratio of retinol-binding protein to transthyretin in the assessment of vitamin A status in adults. Proposal of a cut-off point. Clin Chem Lab Med. 2002;40:1301–7.

9. Rosales FJ, Chau KK, Haskell MH, Shankar AH. Determination of a cut-off value for the molar ratio of retinol-binding protein to transthyretin (RBP:TTR) in Bangladeshi patients with low hepatic vitamin A stores. J Nutr. 2002;132:3687–92.

10. Donnen P, Dramaix M, Brasseur D, Bitwe R, Bisimwa G, Hennart P. The molar ratio of serum retinol-binding protein (RBP) to transthyretin (TTR) is not useful to assess vitamin A status during infection in hospitalised children. Eur J Clin Nutr. 2001;55:1043–7.

11. Baeten JM, McClelland RS, Richardson BA, Bankson DD, Lavreys L, Wener MH, Overbaugh J, Mandaliya K, Ndinya-Achola JO, et al. Vitamin A deficiency and the acute phase response among HIV-1 infected and uninfected women in Kenya. J Acquir Immune Defic Syndr. 2002;31:243–9.

12. Baeten JM, McClelland RS, Overbaugh J, Richardson BA, Emery S, Lavreys L, Mandaliya K, Bankson DD, Ndinya-Achola JO, et al. Vitamin A supplementation and HIV-1 shedding in women: results of a randomized clinical trial. J Infect Dis. 2002;185:1187–91.

13. WHO/UNICEF/IVACG. Vitamin A supplements: a guide to their use in the treatment and prevention of vitamin A deficiency and xerophthalmia. 2nd edition. Geneva: World Health Organization; 1997.

14. Johnson D, Hirschkorn D, Busch MP. Evaluation of four alternative methodologies for determination of absolute CD4+ lymphocyte counts. The National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study. J Acquir Immune Defic Syndr Hum Retrovirol. 1995;10:522–30.

15. Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr. 1979;32:2143–9.

16. Thurnham DI, McCabe GP, Northrop-Clewes CA, Nestel P. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: meta-analysis. Lancet. 2003;362:2052–8.

17. McNeil BJ, Keller E, Adelstein SJ. Primer on certain elements of medical decision making. N Engl J Med. 1975;293:211–5.

18. Stephensen CB, Gildengorin G. Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. Am J Clin Nutr. 2000;72:1170–8.

19. UNAIDS/WHO. AIDS Epidemic Update: December 2004. [Cited 2004 December 30]. Available at: http://www.unaids.org/wad2004/EPI_1204_pdf_en/ EpiUpdate04_en.pdf.