Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response

Jared M Baeten, Barbra A Richardson, Daniel D Bankson, Mark H Wener, Joan K Kreiss, Ludo Lavreys, Kishorchandra Mandalaya, Job J Bwayo, and R Scott McClelland

ABSTRACT

Background: Serum retinol is the most commonly used indicator of vitamin A status. Retinol is transported in a 1-to-1 complex with retinol-binding protein (RBP). RBP is easy and inexpensive to measure, and studies have shown a high correlation between concentrations of RBP and concentrations of retinol. The performance of RBP in the context of infection or protein malnutrition, however, has not been evaluated.

Objective: Our aim was to determine whether RBP is a good surrogate measure for retinol in the context of HIV-1 infection, protein malnutrition, and the acute phase response.

Design: The relation between RBP and retinol was examined in a cross-sectional study of 600 Kenyan women.

Results: There was a high correlation between concentrations of RBP and those of retinol (r = 0.88). When equimolar cutoffs were used, RBP predicted marginal vitamin A status (retinol < 1.05 μmol/L) with 93% sensitivity and 75% specificity and vitamin A deficiency (retinol < 0.70 μmol/L) with 91% sensitivity and 94% specificity. Similarly high sensitivities and specificities were found among subgroups with HIV-1 infection, a positive acute phase response, and protein malnutrition. Protein malnutrition and a positive acute phase response were common, especially among HIV-1–infected women, and were independently and synergistically associated with lower RBP concentrations.

Conclusions: Equimolar RBP cutoffs predict vitamin A deficiency with high sensitivity and specificity, even in the context of infection and protein malnutrition. Like retinol, RBP may not accurately identify true vitamin A status under all conditions, because the acute phase response and protein malnutrition depress RBP concentrations. However, RBP may be a simple, inexpensive tool for assessment of vitamin A deficiency in population studies.

KEY WORDS Vitamin A, retinol, micronutrient deficiency, retinol-binding protein, nutritional assessment, nutritional status

INTRODUCTION

Vitamin A deficiency is a cause of significant morbidity and mortality worldwide, especially among women and children (1). Simple, reliable techniques for evaluating vitamin A status in populations are necessary to assess the need for vitamin A supplementation initiatives and to monitor their effectiveness. The most commonly used indicator of vitamin A status is the serum retinol concentration (2). However, retinol is unstable when exposed to heat or light, and the techniques used to quantify its concentration in serum, such as HPLC, require expensive and complicated laboratory equipment (3). Alternative biochemical indexes would allow field studies of vitamin A status to be done more easily.

The serum concentration of retinol-binding protein (RBP), the carrier for retinol in the blood, has been proposed as a surrogate measure for serum retinol (3). RBP is a 21-kDa protein synthesized by the liver and released into the circulation in a 1-to-1 complex with retinol (2). The techniques used to quantify serum RBP are easier and less expensive than those used for retinol, and RBP is considerably more stable with respect to light and temperature (4–6). Early studies showed good correlation between concentrations of RBP and those of retinol (7), and RBP has shown reasonable sensitivity and specificity in predicting vitamin A deficiency (4, 5, 8).

It is not clear whether RBP is a good marker for retinol in all contexts, however (5, 9). RBP concentrations fall in the setting of protein malnutrition or the acute phase response (which accompanies inflammatory states) (10, 11). Thus, the RBP-retinol relation could be confounded by inflammation, infection, or protein malnutrition (5). Moreover, the acute phase response depresses retinol concentrations, and low serum retinol may not indicate true deficiency in this context (12). Because of the complex interrelations between RBP, retinol, hypoalbuminemia, and the acute phase response, it is important to test whether RBP can be used as a surrogate marker for...
retinol in settings in which infection and malnutrition are common.

We previously reported the results of a large cross-sectional study of serum retinol, HIV-1 infection, and the acute phase response among Kenyan women (13). Two important findings emerged. First, HIV-1 infection and the acute phase response were associated with each other and with lower retinol concentrations. Second, among HIV-1-infected women, the presence of an acute phase response appeared to block any increase in serum retinol after vitamin A supplementation. These data suggest that HIV-1 and the acute phase response depress retinol concentrations, implying that serum retinol may not reflect true vitamin A status under these clinical conditions.

The objective of the present study was to examine the relation between serum retinol and RBP concentrations in this same cohort of Kenyan women. We also evaluated the influence of protein malnutrition, HIV-1 infection, and the acute phase response on the RBP-retinol relation and on RBP concentrations.

SUBJECTS AND METHODS

Study participants and procedures

Between September 1998 and June 2000, we enrolled 400 HIV-1–infected women attending outpatient clinics at Coast Provincial General Hospital in Mombasa, Kenya, in a randomized clinical trial of vitamin A supplementation (14). At the women’s enrollment visits, we collected data for a cross-sectional study of the relation between vitamin A deficiency, HIV-1 infection, and the acute phase response (13). Two hundred randomly selected HIV-1–seronegative women also participated in the cross-sectional study. The women were interviewed with a standard questionnaire that covered demographic characteristics and medical history. Serum and EDTA-anticoagulated blood were collected and were protected from light after collection. The institutional review boards of the University of Washington and the University of Nairobi approved the study protocol, and all women provided written informed consent.

Women who were younger than 18 y or older than 45 y were excluded from the study, as were women who had been pregnant, taken vitamin supplements, or used oral contraceptive pills within the prior 3 mo. None of the HIV-1–seropositive women used antiretroviral therapy. No women had ocular signs of vitamin A deficiency (xerophthalmia or Bitot spots), but all were provided with 4 wk of 10 000 IU vitamin A/d after completion of the study to ensure adequate treatment of subclinical vitamin A deficiency (15).

Laboratory methods

HIV-1 serology was performed by using serial enzyme-linked immunosorbent assays [Detect HIV-1/2 (BioChem ImmunoSystems, Montreal), with positive results confirmed by Recombigen (Cambridge Biotech, Worcester, MA)]. Absolute CD4 counts were determined for HIV-1–seropositive women (Zymmune CD4/CD8 Cell Monitoring Kit; Bartels Inc, Issaquah, WA).

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 (16). Marginal vitamin A status was defined as concentrations <1.05 μmol/L, as was done in other studies of vitamin A conducted among adults (17, 18). A standard cutoff of <0.70 μmol/L was used to define vitamin A deficiency (6). Serum RBP was measured by nephelometry (Dade Behring, Marburg, Germany). The lower limit of quantification was 0.52 μmol/L.

We also used nephelometry to measure serum concentrations of albumin, C-reactive protein, and α1-acid glycoprotein (Dade Behring, Marburg, Germany; 19). We used a standard definition of <3.5 g albumin/dL to define protein malnutrition (20). We defined a positive acute phase response as concentrations of C-reactive protein ≥10 mg/L, concentrations of α1-acid glycoprotein ≥1.2 g/L, or both, which is consistent with definitions used in other studies of the relation between vitamin A and the acute phase response (13, 18, 21).

Data analysis

Statistical analyses were conducted by using SPSS 10.0 (SPSS Inc, Chicago). RBP concentrations below the limit of quantification were set at half that limit. Comparisons of categorical variables were conducted by using chi-square tests, and comparisons of continuous variables were conducted by using Mann-Whitney U tests, Spearman’s correlation coefficients, and linear regression. Receiver operating characteristic plots were constructed to explore the sensitivity and specificity of various RBP cutoffs to predict marginal vitamin A status and vitamin A deficiency. The area under the curve, a summary measure of test performance, was calculated for each receiver operating characteristic plot. A perfect test has an area under the curve of 1.

RESULTS

Study population

The median age of the women enrolled in the study was 27 y (range: 18–45 y). HIV-1–seropositive women were slightly older, less educated, and less likely to be married than were 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 (range: 25–1117 cells/μL).

Seroologic evidence of vitamin A deficiency was common in this population, especially among the HIV-1–seropositive women, who were significantly more likely than the 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 (range: 25–1117 cells/μL).

Correlation between concentrations of serum retinol and serum RBP

Overall, there was a high correlation between serum concentrations of retinol and those of RBP (Spearman’s r = 0.88, P < 0.001; Figure 1). This correlation was also high, although less strong, among the subgroups of women with serologic
evidence of marginal vitamin A status ($r = 0.80$, $P < 0.001$; $n = 290$) or vitamin A deficiency ($r = 0.73$, $P < 0.001$; $n = 114$). Forty-seven (8%) women had RBP concentrations below the limit of quantification of 0.52 μmol/L, and the RBP concentration could not be determined for one HIV-1-seropositive woman.

### Use of serum RBP to predict marginal vitamin A status and vitamin A deficiency

Previous studies calculated the sensitivity and specificity of various RBP cutoffs for identifying vitamin A deficiency, as

![FIGURE 1. Relation between serum concentrations of retinol and those of retinol-binding protein (RBP) in 599 Kenyan women. Spearman’s correlation coefficient = 0.88, $P < 0.001$. The solid line represents the best-fit linear regression of the data.](image-url)
defined by serum retinol (4, 5, 8). Because RBP and retinol exist in the circulation in equimolar concentrations, we used RBP cutoffs of 1.05 and 0.70 μmol/L to identify marginal vitamin A status (retinol < 1.05 μmol/L) and vitamin A deficiency (retinol < 0.70 μmol/L), respectively (5, 22). With the use of these cutoffs, serum RBP had 93% sensitivity for predicting marginal vitamin A status (Table 2) and 91% sensitivity for predicting vitamin A deficiency (Table 3). The corresponding specificities were 75% and 94%, respectively. These sensitivity and specificity measures were comparable or superior to those generated if linear regression was used instead to calculate a modeled relation between RBP and retinol concentrations in this dataset, which is another method that has been used to develop RBP cutoffs for defining vitamin A deficiency (4, 8). Because there is often a slight excess of RBP in the circulation that does not contain retinol, we also considered RBP cutoffs that were raised by 15%, i.e., <1.21 and <0.81 μmol/L for prediction of marginal vitamin A status and vitamin A deficiency, respectively (5, 6). These raised cutoffs showed slightly higher sensitivities, 97% (280/290) for marginal vitamin A status and 96% (110/114) for vitamin A deficiency, but considerably lower specificities, 52% (160/309) and 83% (402/485), respectively.

We constructed receiver operating characteristic plots to further characterize RBP as a surrogate for retinol. Examination of these plots showed that RBP had strong agreement with retinol for predicting marginal vitamin A status (plot not shown) and vitamin A deficiency (Figure 2). The area under the curve for both plots was >0.9.

**Effect of HIV-1 status, the acute phase response, and protein malnutrition on the retinol-RBP correlation**

The effects of HIV-1 infection, the acute phase response, and protein malnutrition on the correlation between serum retinol and serum RBP were assessed by calculating Spearman’s correlation coefficients for 8 strata defined by these factors (Table 4). Overall, the correlations were high (r>0.8) but were weaker for the strata containing women with any one of these factors than for the stratum containing women who had none (r = 0.95). To assess whether each of these differences had a significant influence on the relation between retinol and RBP, multivariate linear regression models were constructed according to the following formula:

\[
\text{Retinol} = \beta_0 + \beta_1 \text{(RBP)} + \beta_2 \text{(factor)} + \beta_3 \text{(RBP X factor)} (I)
\]

where factor is either HIV-1 infection, the acute phase response, or protein malnutrition. In each model, the interaction term (\(\beta_3\)) was significant (\(P < 0.001\) for all 3 models), suggesting that the retinol-RBP relation differed in relation to the presence or absence of HIV-1 infection, acute phase response, and protein malnutrition. In a multivariate model containing all 3 factors plus their interaction terms with RBP, the coefficients for the interaction terms for the acute phase response and protein malnutrition remained highly significant (\(P < 0.01\) for both) and that for HIV-1 status was of marginal significance (\(P = 0.06\), suggesting that each had an independent influence on the relation between retinol and RBP. Further adjustment of these multivariate models for the demographic characteristics presented in Table 1 had no substantial effect on these results (data not shown).

**HIV-1 status, the acute phase response, protein malnutrition, and RBP concentrations**

HIV-1–infected women had lower RBP concentrations than uninfected women (median: 0.90 compared with 1.10 μmol/L, respectively; \(P < 0.001\)). Similarly, RBP concentrations were significantly different among women with and without an acute phase response (median: 0.81 compared with 1.05 μmol/L, respectively; \(P < 0.001\)), as well as among women with and without protein malnutrition (median: 0.86 compared with 1.10 μmol/L, respectively; \(P < 0.001\)). HIV-1 status, the acute phase response, and protein malnutrition were also significantly associated with each other (data not shown; all compar-

---

**TABLE 2**

Use of retinol binding protein (RBP) to predict marginal vitamin A status, as determined by serum retinol (retinol <1.05 μmol/L)\(^1\)

<table>
<thead>
<tr>
<th>Retinol</th>
<th>(&lt;1.05 \mu\text{mol/L})</th>
<th>(\geq 1.05 \mu\text{mol/L})</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>(&lt;1.05 \mu\text{mol/L})</td>
<td>270</td>
<td>78</td>
<td>348</td>
</tr>
<tr>
<td>(\geq 1.05 \mu\text{mol/L})</td>
<td>20</td>
<td>231</td>
<td>251</td>
</tr>
<tr>
<td>Total</td>
<td>290</td>
<td>309</td>
<td>599</td>
</tr>
</tbody>
</table>

\(^1\) Sensitivity of RBP for detecting marginal vitamin A status = 93% (270/290); specificity = 75% (231/309). Chi-square = 283, \(P < 0.001\).

---

**FIGURE 2.** Receiver operating characteristic plot for the use of retinol-binding protein to predict vitamin A deficiency (retinol < 0.70 μmol/L). The area under the curve is 0.96. The receiver operating characteristic curve for predicting marginal vitamin A status (retinol < 1.05 μmol/L) was similar (area under the curve: 0.93) and thus is not shown.
table 4
correlation between retinol and retinol-binding protein (rbp) by hiv-1, acute phase response, and protein malnutrition status

<table>
<thead>
<tr>
<th>hiv-1</th>
<th>acute phase response</th>
<th>protein malnutrition</th>
<th>n</th>
<th>retinol-rbp correlation coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>158</td>
<td>0.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>negative</td>
<td>negative</td>
<td>positive</td>
<td>20</td>
<td>0.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>negative</td>
<td>positive</td>
<td>negative</td>
<td>14</td>
<td>0.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>positive</td>
<td>negative</td>
<td>positive</td>
<td>8</td>
<td>0.82</td>
<td>0.01</td>
</tr>
<tr>
<td>positive</td>
<td>negative</td>
<td>negative</td>
<td>96</td>
<td>0.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>positive</td>
<td>negative</td>
<td>positive</td>
<td>27</td>
<td>0.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>130</td>
<td>0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>positive</td>
<td>positive</td>
<td>positive</td>
<td>146</td>
<td>0.87</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 defined as c-reactive protein \( \geq 10 \text{ mg/L} \), \( \alpha_1 \)-acid glycoprotein \( \geq 1.2 \text{ g/L} \), or both.
2 defined as albumin \( < 3.5 \text{ g/L} \).
3 spearman’s correlation.

Isotopes \( P < 0.001 \). In multivariate linear regression analysis including all 3 factors, the acute phase response and protein malnutrition were independently associated with lower rbp concentrations (regression coefficients: \(-0.09 \text{ µmol/L}, P = 0.04; \) and \(-0.18 \text{ µmol/L}, P < 0.001, \) respectively), although hiv-1 status was not \((P = 0.4)\). Inclusion of all two-way interactions among these 3 factors showed that women who had both an acute phase response and protein malnutrition had significantly lower rbp concentrations than did women with either of these factors alone \((P = 0.004)\). The three-way interaction among these factors was not significant \((P = 0.6)\). Further adjustment for demographic characteristics had essentially no effect on these results.

We performed multivariate linear regression analyses using serum retinol as the dependent variable to compare the effects on rbp and retinol concentrations of hiv-1 infection, the acute phase response, and protein malnutrition. All 3 factors were associated with lower retinol concentrations (regression coefficients: \(-0.15 \text{ µmol/L}, P < 0.001; -0.19 \text{ µmol/L}, P < 0.001; \) and \(-0.20 \text{ µmol/L}, P < 0.001, \) respectively). Thus, hiv-1 infection influenced retinol but not rbp concentrations, the acute phase response influenced both but to a greater extent for retinol, and protein malnutrition had similar effects on the 2 measures. Inclusion of interaction terms in multivariate analysis gave generally similar results for retinol as were found for rbp (data not shown).

We also assessed the effect of hiv-1 infection, the acute phase response, and protein malnutrition on the relative concentrations of rbp and retinol. For the entire study population, the median ratio of rbp to retinol concentrations was 0.91 (range: 0.22–4.02), and two-thirds of the women (383/599) had ratios between 0.85 and 1.15. Ratios slightly <1.0 have been commonly seen in other populations \((5)\). The median ratio of rbp to retinol was slightly higher for hiv-1–infected women than for uninfected women \((0.95 \text{ compared with } 0.87; P < 0.001)\), for women with than without an acute phase response \((0.98 \text{ compared with } 0.90; P < 0.001)\), and for women with than without protein malnutrition \((0.95 \text{ compared with } 0.89; P < 0.001)\). Women with low vitamin a concentrations \((<1.05 \text{ µmol/L})\) also had higher ratios \((0.93 \text{ compared with } 0.90; P < 0.001)\) for women with retinol \(>1.05 \text{ µmol/L}; P = 0.03)\), suggesting decreased rbp saturation for women with low circulating retinol concentrations. This may in part explain the higher ratios of rbp to retinol for women with hiv-1 infection, a positive acute phase response, or protein malnutrition than for women without these factors. In multivariate linear regression analysis, hiv-1 infection and the acute phase response were associated with higher ratios \((P = 0.006 \) and \( P < 0.001, \) respectively), whereas protein malnutrition was not \((P = 0.4)\).

Effect of hiv-1, the acute phase response, and protein malnutrition on rbp for identification of marginal vitamin a status and vitamin a deficiency

Because hiv-1 infection, the acute phase response, and protein malnutrition had somewhat different effects on the absolute concentrations of serum rbp and retinol, we examined the influence of these conditions on the ability of rbp to predict marginal vitamin a status and vitamin a deficiency. Within strata defined by hiv-1, acute phase response, or protein malnutrition status, the sensitivity and specificity of equimolar rbp cutoffs for identifying marginal vitamin a status \((<1.05 \text{ µmol/L})\) and vitamin a deficiency \((<0.70 \text{ µmol/L})\) were similar to those for the entire study population \((table 5)\).

Discussion

In this population of 600 kenyan women, we found that rbp was a sensitive and specific marker for serum retinol concentrations indicative of marginal vitamin a status and vitamin a deficiency, even in the context of hiv-1 infection, the acute phase response, and protein malnutrition. The correlation between concentrations of retinol and those of rbp was lower among women with a positive acute phase response or protein malnutrition. Moreover, the acute phase response and protein malnutrition were independently and synergistically associated with lower rbp concentrations, suggesting that these conditions suppress rbp.

The measurement of rbp has several advantages over retinol for assessing the vitamin a status of populations. First, hplc methods for serum retinol quantification require complicated and expensive laboratory equipment and reagents. In contrast, relatively simple, inexpensive immunologic methods are available for measurement of rbp, even with the use of finger-prick blood samples. We used nephelometry to quantify rbp in this study, and other techniques, such as radial immunodiffusion and enzyme-linked immunosorbent assays, are also widely available. These methods have excellent reliability for...
TABLE 5
Effect of HIV-1, acute phase response, and protein malnutrition status on the use of equimolar retinol-binding protein (RBP) cutoffs to predict marginal vitamin A status and vitamin A deficiency

<table>
<thead>
<tr>
<th>HIV-1 status</th>
<th>RBP cutoff &lt;1.05 μmol/L to predict marginal vitamin A status (retinol &lt;1.05 μmol/L)</th>
<th>RBP cutoff &lt;0.70 μmol/L to predict vitamin A deficiency (retinol &lt;0.70 μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity Specificity % (n/total n)</td>
<td>Sensitivity Specificity % (n/total n)</td>
</tr>
<tr>
<td>Positive</td>
<td>91 (213/233) 75 (125/166)</td>
<td>91 (94/103) 93 (276/296)</td>
</tr>
<tr>
<td>Negative</td>
<td>100 (57/57) 74 (106/143)</td>
<td>91 (10/11) 94 (178/189)</td>
</tr>
<tr>
<td>Acute phase response</td>
<td>91 (126/138) 81 (51/63)</td>
<td>89 (69/78) 92 (113/123)</td>
</tr>
<tr>
<td>Positive</td>
<td>95 (144/152) 73 (180/246)</td>
<td>97 (35/36) 94 (341/362)</td>
</tr>
<tr>
<td>Negative</td>
<td>92 (184/201) 74 (72/97)</td>
<td>91 (88/97) 92 (185/201)</td>
</tr>
<tr>
<td>Protein malnutrition</td>
<td>97 (86/89) 75 (159/212)</td>
<td>94 (16/17) 95 (269/284)</td>
</tr>
</tbody>
</table>

1 Defined as C-reactive protein ≥10 mg/L, α1-acid glycoprotein ≥1.2 g/L, or both.
2 Defined as albumin <3.5 g/L.

quantifying RBP (23–26). Second, whereas blood samples collected for measurement of serum retinol must be continuously shaded from light and kept cool, RBP is considerably less photo- and heat-sensitive than is retinol (5). Because many studies of vitamin A status are conducted in tropical areas, often with limited laboratory and refrigeration capacities, more accurate assessment of vitamin A status might be obtained by measurement of RBP instead of retinol. Finally, the ease and reliability of RBP quantification techniques could allow for RBP measurement onsite where vitamin A surveys are conducted.

A reliable and widely applicable cutoff is necessary for RBP to be used in population surveys of vitamin A deficiency (6). Previous studies showed a strong correlation between concentrations of RBP and those of retinol in serum (4, 5, 7, 8). The 2 exist in approximately equimolar concentrations, because RBP has a single binding site for retinol and acts as its carrier in the circulation (27). However, some have questioned whether RBP can be a reliable surrogate marker for retinol, because a small but variable proportion of RBP can circulate without retinol, and factors such as infection, inflammation, and malnutrition could alter the binding of retinol to RBP (6). Previous studies used different RBP cutoffs to define vitamin A deficiency (4, 5, 8). In this population of Kenyan adults, we found that equimolar RBP cutoffs were robust in their ability to detect retinol concentrations indicating marginal vitamin A status or vitamin A deficiency, even among subgroups with HIV-1 infection, a positive acute phase response, or protein malnutrition. Equimolar cutoffs have the considerable advantage of being easily applied, compared with more population-specific cutoffs that have thus far limited the use of RBP as a biochemical marker for vitamin A deficiency (6).

Protein malnutrition and the acute phase response reduced the RBP-retinol correlation in our study, and HIV-1 infection and the acute phase response had different effects on the absolute concentrations of RBP and retinol, as well as their ratio. In contrast, another study found no effect of C-reactive protein, α1-acid glycoprotein, or albumin concentrations on the retinol-RBP relation (8), although the moderate correlation described (r = 0.55) may have limited the ability to detect an effect of these factors. Despite high prevalences of HIV-1 infection, a positive acute phase response, and protein malnutrition in our study population, however, we found that RBP was a sensitive and specific predictor of low retinol concentrations. We were unable to explore the influence of other factors that influence the circulation of RBP, including liver function and other nutrients (such as zinc), although their effects would be of interest for future study (2).

The physiologic significance of low retinol concentrations in the context of a positive acute phase response or malnutrition is unclear (19). In one study of Thai adults with malaria, low retinol concentrations were common despite high intakes of vitamin A and no clinical signs of deficiency (12). Others found that elevated concentrations of acute phase response proteins are associated with lower serum retinol concentrations (18), even during subclinical infection (13, 28). These results suggest that the acute phase response suppresses retinol concentrations, independent of total vitamin A stores, and thus that low retinol concentrations may not signify true vitamin A deficiency in this context. In our study, protein malnutrition and the acute phase response were also associated with lower RBP concentrations. These results suggest that among populations in whom malnutrition and concurrent infections are common, RBP measurements, like retinol measurements, may overestimate the prevalence of true vitamin A deficiency.

The presence of an acute phase response has been used to adjust the results of large survey studies that used serum retinol to define vitamin A deficiency (18), and similar techniques may be helpful in interpreting RBP findings from populations. Another approach has used measurement of the ratio of RBP to prealbumin as a way to assess vitamin A deficiency in the setting of inflammation (29).

The role of vitamin A in HIV-1 infection has been of great interest during the past 10 y (30). Early observational studies found strong associations between low serum retinol concentrations and HIV-1 disease progression and infectivity (17, 31, 32). However, randomized trials subsequently failed to find any effect of vitamin A supplementation on HIV-1 plasma viral load, CD4 counts, genital HIV-1 shedding, or HIV-1 transmission (14, 33, 34). One proposed explanation for the disparity between the observational data and the supplementation trials is that low vitamin A concentrations reflect more active HIV-1 infection rather than true vitamin A deficiency (34). In support of this hypothesis, we reported that HIV-1–infected women had lower retinol concentrations than did uninfected women and a higher prevalence of a positive acute phase response,
especially among those with more advanced HIV-1 disease (lower CD4 count, higher HIV-1 plasma viral load, and symptomatic HIV-1 infection; 13). In the current analysis, HIV-1 was associated with both protein malnutrition and the acute phase response, and these were in turn associated with significantly lower serum RBP concentrations. Because HIV-1 infection is increasingly common in settings where nutritional deficiencies are prevalent, a better understanding of the interactions between HIV-1, the acute phase response, and vitamin A status (especially if defined by serum retinol or RBP) is necessary to conduct accurate assessments of the need for and potential benefit of vitamin A supplementation.

The present study had several strengths. First, it was conducted among a population traditionally at risk of vitamin A deficiency, namely, poor women in sub-Saharan Africa with evidence of nutritional compromise. Second, inclusion of both HIV-1–infected and uninfected women as well as the high prevalences of a positive acute phase response and protein malnutrition allowed for a detailed examination of their effects on the RBP-retinol relation. Such an examination has been called for by others who previously studied this relation (5). Finally, the large sample size permitted multiple adjusted analyses with good statistical power.

Overall, the results of the present study suggest that serum RBP is a sensitive and specific surrogate measure for serum retinol. These 2 markers of vitamin A status are highly correlated, although protein malnutrition and the acute phase response diminish the strength of this relation. Moreover, the acute phase response and protein malnutrition, both of which were significantly more common among HIV-1–infected women, were associated with lower RBP concentrations. This suggests that low RBP concentrations in the context of these clinical conditions may not always indicate true vitamin A deficiency. However, for large population studies, even in areas where HIV-1, other concurrent infections, and protein malnutrition are common, serum RBP appears to be a reasonably simple and inexpensive tool for the assessment of vitamin A deficiency.

We thank the clinical and laboratory staff in Seattle and Mombasa for their dedication and Coast Pro vincial General Hospital for the use of its facilities. We are especially grateful to the women who made this study possible.

JMB was involved in the conception, design, and coordination of the study; completed the statistical analyses; and compiled the manuscript. BAR supervised the statistical analyses and was involved in the design and coordination of the study. DDB was involved in the design of the study and supervised the retinol testing. MHW oversaw the laboratory testing of the nutritional markers and advised on data interpretation. IKK secured funding for the study and oversaw its design and execution. LL was the site director in Kenya and participated in study design and coordination. KM was involved in the coordination of the study and oversaw laboratory work in Mombasa. JJB participated in the initiation of the study and contributed to the interpretation of the analysis. RSM participated in the design and coordination of the study and advised the analyses and data interpretation. All authors contributed to the writing of the manuscript. The authors had no conflicts of interest.

REFERENCES