Original Research

Retinol-binding protein, retinol, and modified-relative-dose response in Ugandan children aged 12–23 months and their non-pregnant caregivers

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Impact statement

Retinol-binding protein (RBP), measured from serum, plasma, or dried blood spots (DBS), is used as a surrogate marker of retinol to assess vitamin A status in many large-scale, population-based nutrition and health surveys because it is assumed to have an approximately equimolar relationship with retinol and is logistically easier and less expensive to measure. However, there is lack of literature comparing different vitamin A indicators collected in population survey settings. Our findings question (a) the use of DBS as a matrix to measure RBP and (b) whether RBP is a suitable proxy for retinol in Uganda. These findings are important because RBP is often used to determine national vitamin A policies and programs. More research is needed to better understand the relationships between vitamin A indicators across contexts.

Abstract

Retinol-binding protein (RBP), retinol, and modified-relative-dose response (MRDR) are used to assess vitamin A status. We describe vitamin A status in Ugandan children and women using dried blood spot (DBS) RBP, serum RBP, plasma retinol, and MRDR and compare DBS-RBP, serum RBP, and plasma retinol. Blood was collected from 39 children aged 12–23 months and 28 non-pregnant mothers aged 15–49 years as a subsample from a survey in Amuria district, Uganda, in 2016. DBS RBP was assessed using a commercial enzyme immunoassay kit, serum RBP using an in-house sandwich enzyme-linked immunosorbent assay, and plasma retinol/MRDR test using high-performance liquid chromatography. We examined (a) median concentration or value (Q1, Q3); (b) R^2 between DBS-RBP, serum RBP, and plasma retinol; and (c) Bland-Altman plots. Median (Q1, Q3) for children and mothers, respectively, were as follows: DBS-RBP 1.15 μmol/L (0.97, 1.42) and 1.73 (1.52, 1.96), serum RBP 0.95 μmol/L (0.78, 1.18) and 1.47 μmol/L (1.30, 1.79), plasma retinol 0.82 μmol/L (0.67, 0.99) and 1.33 μmol/L (1.22, 1.58), and MRDR 0.025 (0.014, 0.042) and 0.014 (0.009, 0.019). DBS RBP-serum RBP R^2 was 0.09 for both children and mothers. The mean biases were −0.19 μmol/L (95% limits of agreement [LOA] 0.62, −0.99) for children and −0.01 μmol/L (95% LOA −1.11, −1.31) for mothers. DBS RBP-plasma retinol R^2 was 0.11 for children and 0.13 for mothers. Mean biases were 0.33 μmol/L (95% LOA −0.37, 1.03) for children, and 0.29 μmol/L (95% LOA −0.69, 1.27) for mothers. Serum RBP-plasma retinol R^2 was 0.75 for children and 0.55 for mothers, with mean biases of 0.13 μmol/L (95% LOA −0.23, 0.49) for children and 0.18 μmol/L (95% LOA −0.61, 0.96) for mothers. Results varied by indicator and matrix. The serum RBP-retinol R^2 for children was moderate (0.75), but poor for other comparisons. Understanding the relationships among vitamin A indicators across contexts and population groups is needed.

Keywords: Retinol, retinol-binding protein, vitamin A, dried blood spots, modified-relative-dose response, serum

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Introduction

The World Health Organization (WHO) recommends using at least two biological indicators, one of which is usually serum or plasma retinol, to assess vitamin A status. Alternatively, one biological indicator of deficiency can be assessed if at least four demographic and ecological risk factors are assessed—two of which are related to diet or nutrition.[1] Including serum retinol in population-based surveys is limited by the technical capacity required to carry out high-performance liquid chromatography (HPLC), and analysis costs. Although liver reserves are the gold standard for measuring vitamin A status, the modified-relative-dose response (MRDR) is a qualitative test that identifies low or adequate vitamin A liver reserves.[2] MRDR is challenging to apply in large-scale population-based surveys due to the lack of commercially available 3,4-didehydroretinyl acetate (vitamin A_2), the need to collect venous blood, the time delay of 4–6 h between dosing and blood collection, and the cost of materials and analysis.[2] Currently, serum retinol is the preferred measure for vitamin A deficiency (VAD) because it is the predominant form of vitamin A that circulates in the blood and it is the form of vitamin A measured most often in population-based surveys.[1] Retinol-binding protein (RBP) is commonly used as a surrogate marker of retinol[3–7] to assess vitamin A status in many nutrition and health surveys because it is assumed to have an approximately equimolar relationship with retinol[8] and is cheaper to analyze than either retinol or MRDR.

To further reduce logistical and financial constraints to vitamin A status assessment in low- and middle-income countries, innovative methods of specimen collection and storage have been used to assess RBP. Dried blood spots (DBS) generally do not require maintaining a cold chain for any analytes. Basic supplies, like special filter paper, drying racks, sealable plastic bags, and desiccant packs, are needed to ensure that DBS cards are protected from excessive humidity.[9,10] Further, fewer than 500 µL whole blood is needed when collecting DBS compared to >2 mL that is needed with venous blood sampling. For these reasons, several large-scale, population-based surveys have used DBS.[10,11] Among these, the Uganda Demographic Health Surveys (UDHS) used DBS in combination with a commercial RBP assay kit[4,11] in their 2006, 2011, and 2016 surveys.[12–14]

The 2016 Uganda Micronutrient Powder (MNP)/Infant and Young Child Feeding (IYCF) endline survey[15] occurred the same year as the 2016 UDHS, but the surveys were planning to use different biomarkers to assess vitamin A status. The endline survey evaluation design included serum RBP for all study participants and MRDR/retinol among a subsample. Recognizing that different biomarkers and methods may yield different results, the endline evaluation also included an additional subsample using the same methods as the UDHS (i.e. DBS in combination with the Scimedx commercial RBP assay kit) so the country could review the various biomarker results measured in the same individuals. The objectives for this analysis were to describe the results and examine the relationships among DBS RBP, serum RBP, and plasma retinol among Ugandan children aged 12–23 months and their non-pregnant female caregivers aged 15–49 years.

Materials and methods

Study design and sampling

Ethical approval for this survey was obtained from the School of Biomedical Sciences Higher Degrees, Research and Ethics Committee, College of Health Sciences, Makerere University, and research clearance was obtained from the Uganda National Council for Science and Technology. Informed consent was obtained from the mothers for the households, themselves, and the reference child prior to participation. In 2016, a cross-sectional population-based household survey was conducted in Amuria district as an endline for the Uganda MNP/IYCF impact evaluation.[15] Using probability proportionate to size sampling, 38 clusters were selected for the survey in the district. Following a household census that listed all eligible children aged 12–23 months per cluster, 22 children aged 12–23 months were randomly selected in each cluster. Among the 22 children selected per cluster, one randomly selected child along with his or her non-pregnant female caregiver aged 15–49 years (>90% were the mothers and are henceforth referred to as mothers) were invited to participate in the MRDR test, for a sample size of 38 children and 38 mothers. Because we needed a sample size of 49 children to identify a change in the standard deviation of the mean MRDR ratio—a primary objective of the survey, 11 clusters were randomly selected for an additional child/mother pair (for a total of two child/mother pairs in these clusters) to participate in the MRDR test. The MRDR test analytical method includes determination of retinol concentration, which was included in this comparative analysis. With the intention of inviting $n = 200$ per group, we collected and analyzed DBS RBP in the MRDR child/mother pairs and an additional 5 to 6 child/mother pairs per cluster who were selected using simple random sampling. There was no replacement of selected participants for any reason in the survey.[15]

Of the 49 child/mother pairs invited to participate in the MRDR subsample, blood samples were collected from 44 children and 33 from mothers. Our primary analytic samples included 39 children and 28 mothers who had complete information on DBS RBP (Scimedx), serum RBP (enzyme-linked immunosorbent assay [ELISA]), and plasma retinol/MRDR. The reasons for exclusion included loss to follow-up at the second blood collection for the MRDR test ($n = 16$), loss of sample due to reanalysis ($n = 3$), removal of mother's samples who were later identified as pregnant ($n = 2$), and a mother missing a cluster identifier ($n = 1$). In sensitivity analyses, we also analyzed larger samples of 162 children aged 12–23 months and 157 mothers for whom DBS RBP and serum RBP data were collected, regardless of whether or not they had MRDR and retinol measurements. The reasons for not achieving the full sample of $n = 200$ per group were due to refusal to

participate in the survey ($n = 38$ children and mothers) and refusal to provide a blood sample ($n = 5$ for mothers).

Specimen collection and analysis

At a central location in each cluster, trained laboratory technicians collected approximately 500 µL finger stick capillary blood on all survey participants. The blood was collected in two collection tubes for each child and mother; one tube contained no additive for serum collection, and one tube contained the anticoagulant potassium ethylenediaminetetraacetic acid (K_2EDTA) in case the subject was selected for DBS RBP. For the subsample of children and mothers selected for the MRDR test, after the capillary blood collection, an oral dose of vitamin A_2 mixed with one-half teaspoon of vegetable oil was administered to the individual with the aid of disposable syringes. At 4 h after dosing, the laboratory technician collected a 3-mL venous blood specimen using a blood collection tube containing K_2EDTA. From 2 h before the interview and data collection until 4 h after vitamin A_2 dosing, the participants were instructed not to consume any vitamin A-rich food or beverage sources. This was facilitated by giving participants selected for MRDR testing, non-vitamin A-rich foods and beverages and having them stay at the data collection site through the second venous blood collection.

All specimens were processed at a central location in the cluster 3–4 h after collection. For the children and mothers selected for DBS RBP testing, lab technicians prepared DBS samples from the capillary blood collection tube containing the K_2EDTA anticoagulant. The DBS cards were labeled with preprinted labels containing the selected participant's unique identification number and were placed into a drying rack, and 50 µL of whole blood from the tube was pipetted directly onto a 1-cm DBS card circle. Four circles on each card were prepared for each participant and allowed to dry overnight. Dried cards were placed into individual sealable plastic bags with desiccant, and samples from each cluster were placed together into a one-gallon sealable plastic bag with desiccant and then transported for storage in a −20°C freezer at Makerere University until analysis.

All capillary and venous blood collection tubes meant for serum or plasma were centrifuged by the end of each day. After centrifugation, the plasma and serum were transferred into storage vials labeled with a unique specimen identification number. Samples were temporarily stored at a regional level laboratory in a −20°C freezer until transported to Makerere University where they were stored in a −86°C freezer until shipped to laboratories for analysis. Storage vials containing serum from the first blood collection tube were shipped frozen to VitMin Lab (Germany) and analyzed for RBP, C-reactive protein (CRP), and α1-acid glycoprotein (AGP) using an in-house sandwich ELISA.[16] Storage vials containing plasma from the MRDR subsample were shipped frozen to University of Wisconsin-Madison (UW, USA) and analyzed for 3,4-didehydroretinol and retinol using a Waters C18 Reversed-Phase HPLC column and system equipped with a photodiode array detector. DBS samples were extracted for plasma and analyzed at Makerere University using the Scimedx Scanlisa RBP enzyme immunoassay kit (Scimedx Corporation, Denville, NJ), according to the manufacturer's directions.[17] All laboratories that were involved in the analysis of the biological specimens routinely test quality control (QC) pools along with the specimen analysis. The inter-assay coefficients of variation (CV) from Makerere Lab was 18.0% for DBS RBP; VitMin Lab was 3.6% for serum RBP; and UW was 5.0% for MRDR (includes retinol) (see Supplemental Text for additional information on internal and external QC).

Statistical analysis

We examined the median concentrations (Q1, Q3) for all biomarkers. We also compared the coefficient of determination (R^2) of the different assays using simple linear regression to examine the relationship among children and mothers who had both (a) DBS RBP and serum RBP values, (b) DBS-RBP and plasma retinol values, and (c) serum RBP and plasma retinol values. We created Bland Altman plots to evaluate the agreement between the different matrix-assay combinations (comparisons a–c). The plots include absolute bias and 95% limits of agreement (LOA), which are defined as the mean difference (bias) ± 1.96 of the differences. Additionally, we compared DBS RBP and serum RBP values in the larger sample of children and mothers who had complete information on DBS RBP and serum RBP but who may or may not have had MRDR/retinol measured. The cut-offs to define VAD are ≤0.70 µmol/L for serum retinol and ≥0.060 for the MRDR ratio, which is the molar ratio of vitamin A_2 to retinol in the serum or plasma. We planned to calculate sensitivity and specificity of DBS-RBP, serum RBP, and serum retinol to identify VAD compared to MRDR, but this was not possible because regardless of indicator there were two or fewer cases of VAD among children or mothers.

We conducted two sets of sensitivity analyses for the primary analytic sample for whom all biomarkers were tested to evaluate the roles of (a) inflammation and (b) statistical outliers on our findings. To account for the role of inflammation on select biomarkers of vitamin A, we regression-adjusted DSB RBP, serum RBP, and plasma retinol in children only per the guidance outlined in the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia method using CRP and AGP.[18] We then examined the median (Q1, Q3) concentrations, R^2, and Bland Altman plots for the inflammation-adjusted biomarkers. When comparing two analytical runs from two different assays, biologically plausible outliers should not be removed.[19] However, we assessed the influence of potential statistical outliers identified by studentized residuals[20,21] and examined the R^2 and Bland Altman plots excluding those outliers, where applicable.

We used SAS Version 9.4, IBM SPSS Statistics 24, and Analyze-It Version 5.11 statistical packages for all analyses. Median (Q1, Q3) concentrations or values for all biomarkers account for multistage complex sampling using PROC SURVEY procedures and weighting.

Results

The median (Q1, Q3) values for each vitamin A and inflammation biomarker for children ($n = 39$) and mothers ($n = 29$) are presented in Table 1 and suggest adequate vitamin A status in a context of high inflammation. Comparing DBS RBP to serum RBP in the primary analytic subsample for whom all biomarkers were tested, the R^2 was 0.09 for both children (Figure 1a and Table 2) and mothers (Figure 1c and Table 2). Bland-Altman plots for these assay comparisons are presented in Figure 1b (children) and Figure 1d (mothers). The mean biases were −0.19 μmol/L (95% LOA 0.62, −0.99) for children and −0.01 μmol/L (95% LOA −1.11, −1.31) for mothers. When we compared DBS RBP to serum RBP in the larger sample of children ($n = 162$) and mothers ($n = 157$) among whom DBS RBP and serum RBP were measured but whom may or may not have had MRDR/retinol measured, the median (Q1, Q3)

Table 1. Median concentration or value of indicators of vitamin A status and inflammation among children aged 12–23 months and non-pregnant mothers aged 15–49 years in the Uganda endline MNP/IYCF survey in Amuria district, 2016.

	Children (12–23 months) ($n = 39$)	Non-pregnant mothers (15–49 years) ($n = 28$)
	Median (Q1, Q3)	Median (Q1, Q3)
DBS-RBP, μmol/L	1.15 (0.97, 1.42)	1.73 (1.52, 1.96)
Serum RBP, μmol/L	0.95 (0.78, 1.18)	1.47 (1.30, 1.79)
Plasma retinol, μmol/L	0.82 (0.67, 0.99)	1.33 (1.22, 1.58)
MRDR	0.025 (0.014, 0.042)	0.014 (0.009, 0.019)
CRP, mg/L	2.14 (0.65, 15.60)	2.14 (0.66, 2.89)
AGP, g/L	0.99 (0.68, 1.67)	0.64 (0.55, 0.90)

Estimates account for complex sampling design.
AGP: α1-acid glycoprotein; CRP: C-reactive protein; DBS: dried blood spots; IYCF, infant and young child feeding; MNP, micronutrient powder; MRDR: modified-relative-dose response; RBP: retinol-binding protein.

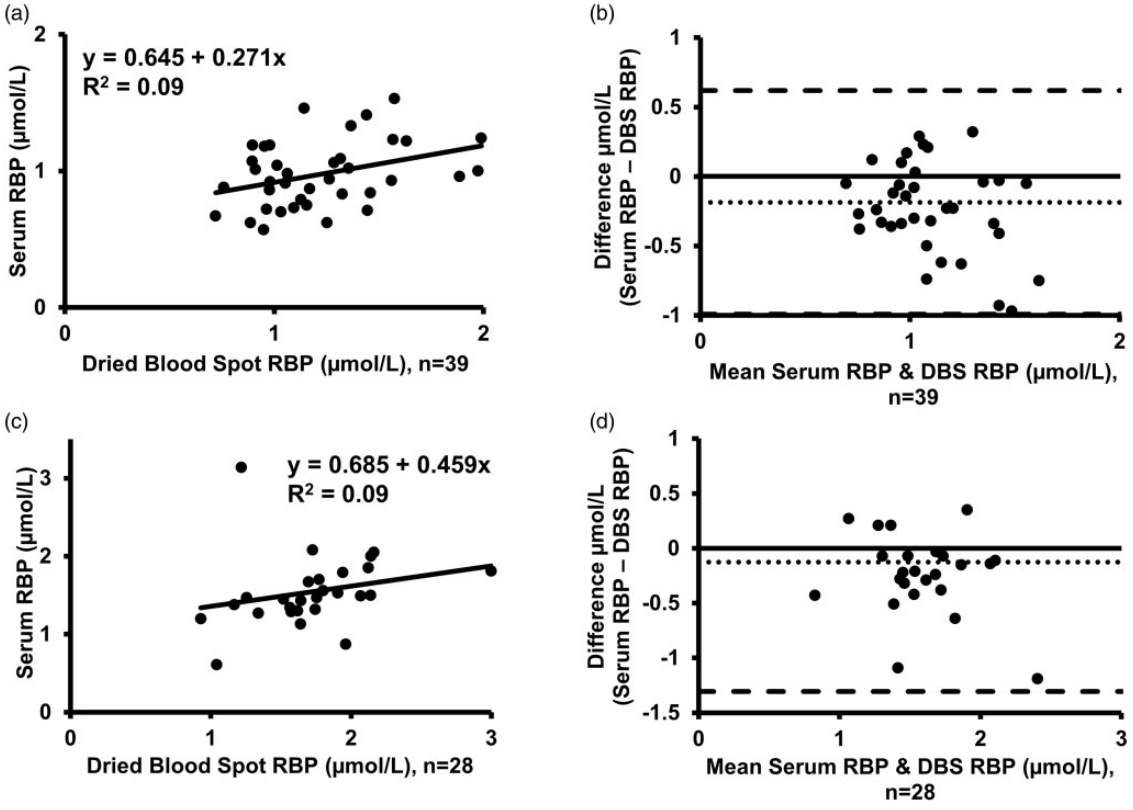

Figure 1. (a) Simple linear regression DBS-RBP Scimedx versus serum RBP ELISA, among children aged 12–23 months in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (b) Bland-Altman difference plot DBS-RBP Scimedx versus serum RBP ELISA, among children aged 12–23 months in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (c) simple linear regression DBS-RBP Scimedx versus serum RBP ELISA, among non-pregnant mothers aged 15–49 years in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (d) Bland-Altman difference plot DBS-RBP Scimedx versus serum RBP ELISA, among non-pregnant mothers aged 15–49 years in the Uganda endline MNP/IYCF survey in Amuria district, 2016.
DBS: dried blood spot; RBP: retinol-binding protein; Dashed line: limits of agreement; Dotted line: 95% mean bias.

Table 2. Correlations between DBS-RBP, serum RBP, and plasma retinol among children aged 12–23 months and non-pregnant mothers aged 15–49 years in the Uganda endline MNP/IYCF survey in Amuria district, 2016.

	Children (12–23 months) ($n = 39$)	Non-pregnant mothers (15–49 years) ($n = 28$)
	R^2	R^2
DBS-RBP—serum RBP	0.09	0.09
DBS-RBP—plasma retinol	0.11	0.13
Serum RBP—plasma retinol	0.75	0.55

DBS: dried blood spots; IYCF, infant and young child feeding; MNP, micronutrient powder; RBP: retinol-binding protein.

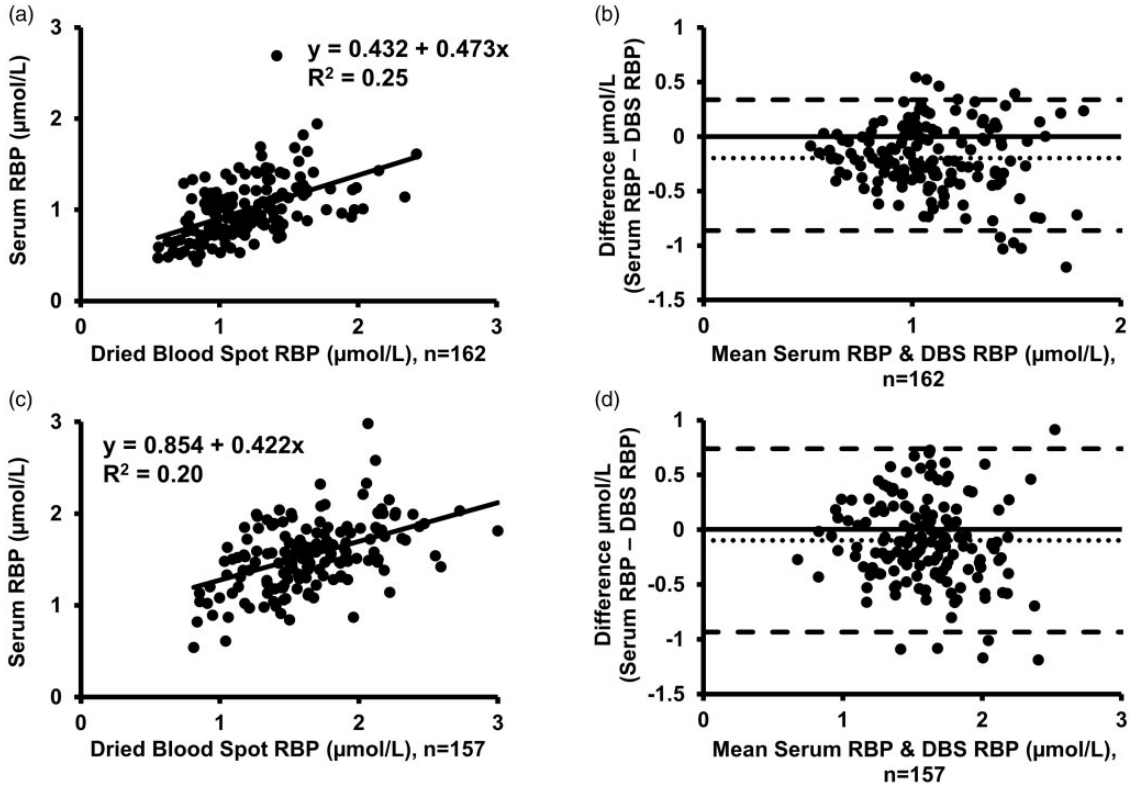

Figure 2. (a) Simple linear regression DBS-RBP Scimedx versus serum RBP ELISA, among all children aged 12–23 months (who may or may not have plasma retinol/modified-relative-dose response assessed) in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (b) Bland-Altman difference plot DBS-RBP Scimedx versus serum RBP ELISA, among all children aged 12–23 months (who may or may not have plasma retinol/modified-relative-dose response assessed) in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (c) simple linear regression DBS-RBP Scimedx versus serum RBP ELISA, among all non-pregnant mothers aged 15–49 years (who may or may not have plasma retinol/modified-relative-dose response assessed) in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (d) Bland-Altman difference plot DBS-RBP Scimedx versus serum RBP ELISA, among all mothers aged 15–49 years (who may or may not have plasma retinol/modified-relative-dose response results) in the Uganda endline MNP/IYCF survey in Amuria district, 2016.
DBS: dried blood spot; RBP: retinol-binding protein; Dashed line: limits of agreement; Dotted line: 95% mean bias.

concentrations were similar to those of the primary analytic subsample (Supplemental Table 1). R^2 values were higher in the larger samples compared to the primary analytic samples (0.25 vs. 0.09 [Figure 2a] in children and 0.20 vs. 0.09 [Figure 2c] in mothers). The Bland Altman mean biases were −0.20 µmol/L (95% LOA –0.94, 0.54) for children (Figure 2b) and –0.10 µmol/L (95% LOA –0.93, 0.74) for mothers (Figure 2d).

The simple linear regression comparisons for DBS RBP to plasma retinol are presented in Figure 3a and c. R^2 was 0.11 for children (Figure 3a and Table 2) and 0.13 for mothers (Figure 3c and Table 2). Bland-Altman plots from the assay comparisons of the DBS RBP compared to plasma retinol are presented in Figure 3b (children) and Figure 3d (mothers). The mean biases were 0.33 µmol/L (95% LOA –0.37, 1.03) for children and 0.29 µmol/L (95% LOA –0.69, 1.27) for mothers.

Simple linear regression plots comparing serum RBP and plasma retinol for children and mothers are presented in Figure 4a and c, respectively. R^2 was 0.75 for children (Figure 4a and Table 2) and 0.55 for mothers (Figure 4c and Table 2). Bland-Altman plots from the assay comparisons of serum RBP and plasma retinol are presented in Figure 4b (children) and Figure 4d (mothers). The mean biases were 0.13 µmol/L (95% LOA –0.23, 0.49) for children and 0.18 µmol/L (95% LOA –0.61, 0.96) for mothers.

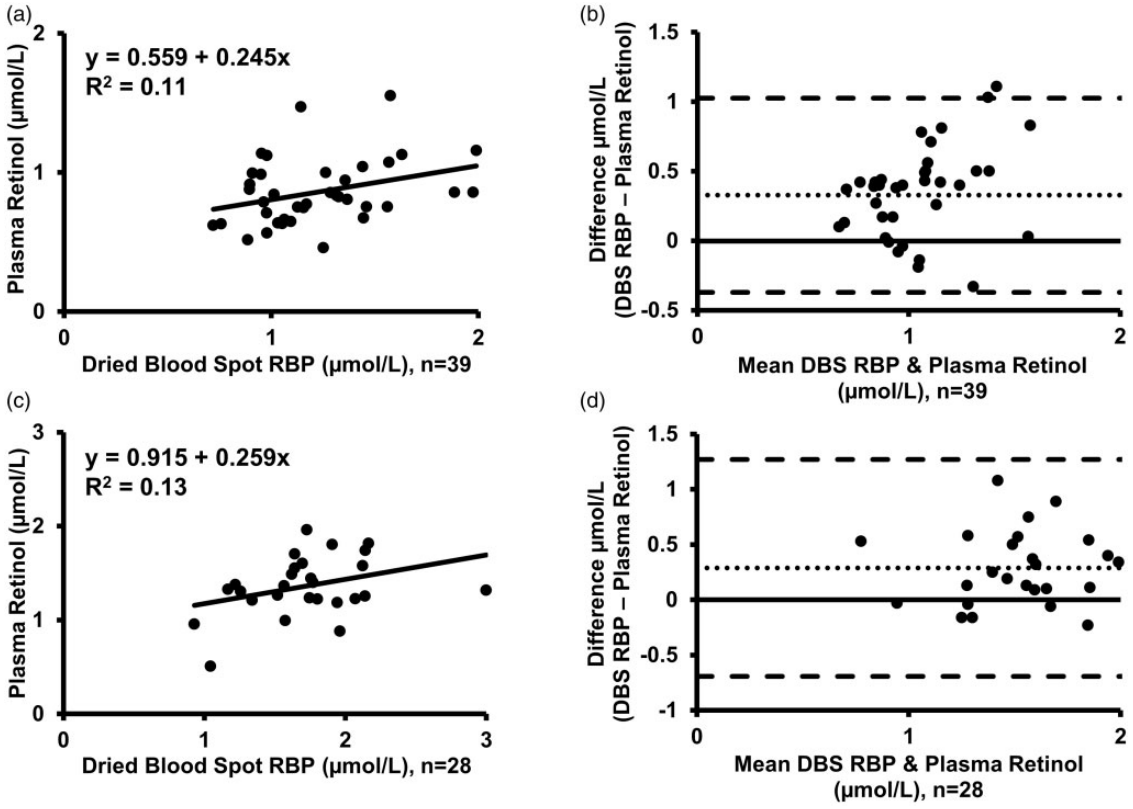

Figure 3. (a) Simple linear regression DBS-RBP Scimedx versus plasma retinol HPLC, among children aged 12–23 months in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (b) Bland-Altman difference plot DBS-RBP Scimedx versus plasma retinol HPLC, among children aged 12–23 months in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (c) simple linear regression DBS-RBP Scimedx versus plasma retinol HPLC, among non-pregnant mothers aged 15–49 years in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (d) Bland-Altman difference plot DBS-RBP Scimedx versus plasma-retinol HPLC, among non-pregnant mothers aged 15–49 years in the Uganda endline MNP/IYCF survey in Amuria district, 2016.
DBS: dried blood spot; RBP: retinol-binding protein; Dashed line: limits of agreement; Dotted line: 95% mean bias.

Inflammation-adjusted median (Q1, Q3) concentrations of each biomarker for children are presented in Supplemental Table 2. Median concentrations were higher after inflammation adjustment. The R^2 for serum RBP compared to plasma retinol was lower after adjusting both biomarkers for inflammation (0.57 adjusted vs. 0.75 unadjusted), while the R^2 for all other inflammation-adjusted comparisons were similar to the unadjusted results (Supplemental Table 3). The Bland Altman mean biases were −0.09 µmol/L (95% LOA −1.00, 0.82) for inflammation-adjusted DBS RBP and inflammation-adjusted serum RBP (Supplemental Figure 1a), 0.33 µmol/L (95% LOA −0.47, 1.14) for inflammation-adjusted DBS RBP and inflammation-adjusted plasma retinol (Supplemental Figure 1b), and 0.24 µmol/L (95% LOA –0.25, 0.73) for inflammation-adjusted serum RBP and inflammation-adjusted plasma retinol (Supplemental Figure 1c).

We identified several statistical outliers using studentized residuals: DBS RBP—serum RBP ($n = 1$ child and $n = 2$ mothers), DBS RBP—plasma retinol ($n = 1$ child and $n = 1$ mother), and serum RBP—plasma retinol ($n = 1$ child and $n = 2$ mothers). With the exception of the DBS RBP and serum RBP comparison among mothers where the $R^2 = 0.09$ with outliers and 0.33 without, the R^2 results excluding statistical outliers were similar to those in the main analyses (absolute difference in R^2 ranging 0.00–0.05) (Supplemental Table 4). Bland-Altman plots from the assay comparisons excluding statistical outliers for DBS RBP and serum RBP ($n = 1$ child and $n = 2$ mothers) are presented in Supplemental Figures 2a and 2b, respectively; for DBS RBP and plasma retinol ($n = 1$ child and $n = 1$ mother) are presented in Supplemental Figures 3a and 3b, respectively; and for serum RBP and plasma retinol ($n = 1$ child and $n = 2$ mothers) are presented in Supplemental Figures 4a and 4b, respectively. The mean biases for DBS RBP and serum RBP were –0.25 µmol/L (95% LOA –0.89, 0.40) for children and –0.26 µmol/L (95% LOA –0.96, 0.45) for mothers, for DBS RBP and plasma retinol were 0.33 µmol/L (95% LOA –0.37, 1.03) for children and 0.36 µmol/L (95% LOA –0.48, 1.20) for mothers, and for serum RBP and plasma retinol were 0.13 µmol/L (95% LOA –0.12, 0.38) for children and 0.12 µmol/L (95% LOA –0.35, 0.59) for mothers.

Discussion

Using data from children aged 12–23 months and their non-pregnant mothers participating in the 2016 Uganda MNP/IYCF endline survey, our comparisons of DBS-RBP, serum RBP, and plasma retinol found poor linear relationships for most comparisons, in the context of low VAD confirmed by the MRDR test and high inflammation. The median MRDR ratio of 0.025 in children is similar to that found in

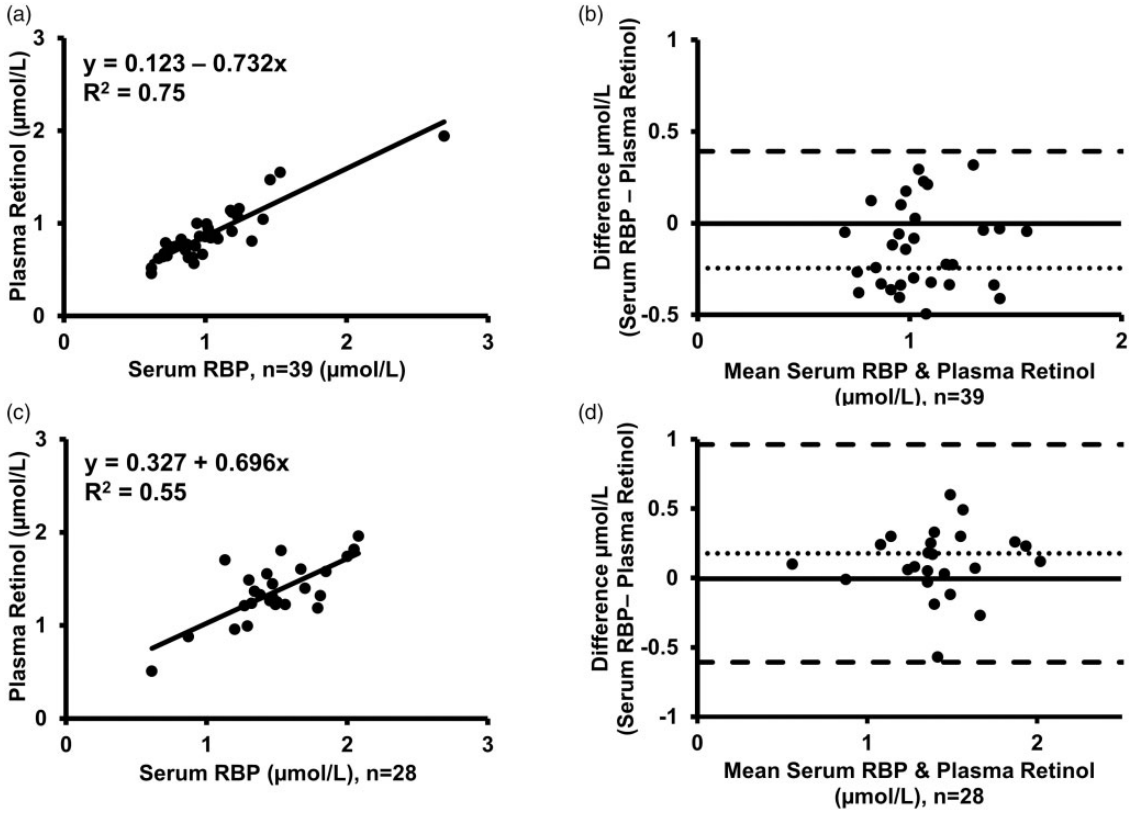

Figure 4. (a) Simple linear regression serum RBP ELISA versus plasma retinol HPLC, among children aged 12–23 months in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (b) Bland-Altman difference plot serum RBP ELISA versus plasma retinol HPLC, among children aged 12–23 months in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (c) simple linear regression serum RBP ELISA versus plasma retinol HPLC, among non-pregnant mothers aged 15–49 years in the Uganda endline MNP/IYCF survey in Amuria district, 2016; (d) Bland-Altman difference plot serum RBP ELISA versus plasma retinol HPLC, among non-pregnant mothers aged 15–49 years in the Uganda endline MNP/IYCF survey in Amuria district, 2016.
RBP: retinol-binding protein; Dashed line: limits of agreement; Dotted line: 95% mean bias.

well-nourished American children[22] and 0.019 for the women is lower than that found in low-income women residing in the United States, i.e., 0.026.[23] Serum RBP had a moderate positive relationship with retinol in children ($R^2 = 0.75$), suggesting that serum RBP was a potentially useful proxy for retinol; however, the relationship was weaker among mothers ($R^2 = 0.55$). R^2 values were ≤ 0.13 for all other biomarker comparisons. When DBS-RBP, serum RBP, and retinol were inflammation adjusted in children, correlations either stayed the same or declined; notably, the serum RBP−retinol relationship decreased from $R^2 = 0.75$ to $R^2 = 0.57$. The R^2 results excluding statistical outliers were similar to those in the main analyses, with the exception of the DBS RBP and serum RBP comparison among mothers, which saw an improvement in the correlations after excluding outliers ($R^2 = 0.09$ to 0.33).

We expected to find strong relationships between DBS RBP, serum RBP, and retinol. Because DBS RBP and serum RBP are both measures of the same protein, one would expect them to be approximately equivalent when measured in the same individual. Because RBP is the transport protein for retinol, they are assumed to have a 1:1 equimolar relationship. However, some health conditions can affect this relationship. Conditions such as abdominal obesity, insulin resistance, type 2 diabetes, and blood pressure have been associated with elevated RBP concentrations,[24–28] and adipose tissue releases apo-RBP (RBP without retinol bound to it) into circulation. Protein energy malnutrition and zinc deficiency interfere with hepatic synthesis of RBP.[29] Retinol concentration is homeostatically controlled and only reflects vitamin A liver stores once they are low or very high.[1]

It is unclear why the DBS RBP had such poor linear relationships with the other assays. Recent studies of DBS RBP and plasma retinol reported poor correlations, consistent with our analyses,[30] but we did not identify literature comparing DBS-RBP to serum RBP. The poor correlation in our study may be in part related to the extra level of difficulty of DBS extraction. Serum and DBS extract are the recommended samples for the Scimedx commercial kit.[17] The poor correlations may be in part due to the addition of fibrinogen clots in plasma that are not present in serum.[30] However, the inter-assay CV was 18% for the DBS-RBP laboratory analyses, whereas <10% is considered acceptable performance for this assay according to Scimedx. This highlights the importance of survey reports and other publications including descriptions of laboratory participation in external quality assurance programs, reporting internal quality assurance procedures and results for each assay, and the limits of detection of each assay. However, there currently are not any external quality assurance programs known to be available for RBP.

Our findings showed that serum RBP quantified using an in-house ELISA method and plasma retinol had a moderate positive linear relationship among children based on the R^2, suggesting that serum RBP was a potentially useful proxy for retinol in this population group. These R^2 findings agree with previous studies among children that have shown sufficient positive linear relationships between retinol and serum or plasma RBP quantified using different methods, such as ELISA and HPLC.[2,3,5,7,31]

Among mothers in our analyses, the R^2 for serum RBP and plasma retinol was 0.55, which contrasts with a study that showed a stronger positive relationship ($R^2 = 0.94$) between serum RBP and plasma retinol among women in Cameroon.[32] There is no WHO guidance on an acceptable R^2 threshold to conclude a sufficiently strong positive linear relationship to use RBP as a proxy for retinol. The R^2 values reported in this paper highlight the importance for survey reports and other publications to report the R^2 or r values when using RBP as a proxy for retinol. Considering the R^2 of 0.55 and moderate bias based on Bland Altman plots among Ugandan mothers in our analysis, the use of serum RBP as a proxy for plasma retinol in this population warrants further research. Recognizing the small sample sizes for retinol in our analyses, this issue may also warrant further exploration in Uganda and other settings.

The consensus emerging based on recent studies is that where there are acceptable correlations between RBP and retinol, the RBP cut-off to define VAD should be study-specific and based on a comparison with serum- or plasma retinol concentrations measured from the same samples in a subset using HPLC.[9,32,33] If the RBP-retinol correlations are not acceptable, then RBP is not a good proxy for retinol and should not be used for this purpose. For example, if we determined the 0.55 R^2 for serum RBP and plasma retinol among mothers was too low, then we would not have used the serum RBP data for mothers from Uganda as a proxy for retinol nor calculated a study-specific RBP cut-off for VAD. This has important consequences for country decision making and national vitamin A policies if RBP is the main indicator assessed among all participants. While not appropriate for this assay comparison,[19] in a survey setting, it might be appropriate to consider removing statistical outliers based on studentized residuals for the purpose of calculating retinol equivalent cut-off thresholds, in addition to biologically implausible values. In sensitivity analyses, removing statistical outliers for women in this study did not improve the R^2 of DBS-RBP or serum RBP compared to retinol, which were 0.12 and 0.55, respectively, excluding outliers.

The strength of this analysis is that we analyzed all samples using ELISA, HPLC, and enzyme immunoassay kits, which are internationally recognized standard laboratory methods. However, the inter-assay CV for DBS-RBP laboratory analysis was higher than considered acceptable for this assay according to Scimedx, whereas the inter-assay CV for the other methods was <10%, indicating acceptable laboratory performance. Using different matrices and blood sources (capillary vs. venous) in the assay comparison may have introduced variability. Serum and plasma are similar in the sense that they are both the liquid portion of blood once the cells are removed, and in many surveys, plasma is often used as a replacement matrix for serum. Retinol is transported by RBP, which has one high-affinity binding site for retinol.[3,8] An important limitation is that the same QC samples were not used across all three platforms, and only one lab reported participating in an external quality assurance program. Potential selection bias is also an issue as we lost 10 out of the 49 random children/mother pairs we expected. This analysis was not the primary purpose of the evaluation and as such includes small sample sizes and a limited number of DBS filter spots originally prepared for each participant, which limited the ability for duplicate analyses in some cases. However, the median biomarker concentrations for the larger analytic samples for DBS-RBP and serum RBP comparisons ($n = 162$ children and $n = 157$ women) found similar results to the smaller primary analytic samples ($n = 39$ children and $n = 28$ mothers). The R^2 values were higher in the larger samples; however, they were still poor (children 0.25 vs. 0.09; mothers 0.20 vs. 0.09).

Despite numerous potential logistical and financial benefits to assessing vitamin A status using the DBS-RBP in large-scale population-based surveys in low- and middle-income countries, our findings in a population of children and mothers in Uganda suggest Scimedx DBS-RBP was a poor surrogate for retinol and was not reliable. More research is needed to better understand the relationships between vitamin A indicators across contexts and population groups.

AUTHORS' CONTRIBUTIONS

The authors' responsibilities were as follows: RDW and NDF carried out analysis and wrote the first draft of the manuscript; JS analyzed samples and assisted with generation of the figures; SAT provided vitamin A_2 and technical support, oversaw MRDR analyses, and revised the manuscript; MZ and RLS assisted with secondary analysis data checks, interpreted data, and revised the manuscript; and MEDJ designed the study, interpreted data, and wrote the first draft of the manuscript. SN, SH, MA, and AL designed the study, oversaw data collection, interpreted data, and revised the manuscript. CM designed the study, interpreted data, and revised the manuscript. LJR interpreted data and revised the manuscript. All authors have read and approve the final manuscript.

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DECLARATION OF CONFLICTING INTERESTS

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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