Author's response to reviews

Title: Proxy markers of serum retinol concentration, used alone and in combination, to assess population vitamin A status in Kenyan children: a cross-sectional study

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Proxy markers of serum retinol concentration, used alone and in combination, to assess population vitamin A status in Kenyan children: a cross-sectional study

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We thank the reviewers for their thoughtful and constructive comments, which we found very helpful to improve our paper.

REVIEWER 1:

Major compulsory revisions - NONE

No comment required.

Minor essential revisions

Question 1) Figure 5 represents the most important and potentially revolutionary principle in this manuscript. In order for this principle to be understood by readers, there needs to be more explanation than is currently provided in the body of the text or in the figure’s legend. The explanation given in “Online supplemental material 2” needs to be adapted and moved to the main body of this manuscript. This reviewer understands journal-imposed constraints on manuscript length, but for such an important idea, surely the editors can be flexible on this issue. We have revised ‘Supplementary material 2’ and moved it to Figure 1. As an alternative, if the Editor so wishes, we could be willing to move this material to the body of the text.

Question 2) The prevalence of inflammation in the study sample is quite low. Although this reviewer was initially not familiar with other studies measuring inflammatory markers in school-age children, a quick online search reveals several articles demonstrating much higher prevalence rates of inflammation in this age group as measured by several different markers. Perhaps the authors wish to offer some explanation for the low prevalence of inflammation found in their study and discuss the possible implications of this on generalizing their results to other populations.

The prevalence of inflammation is relatively low, most likely because the transmission of malaria is low and has declined much over the last decade in this area. As a consequence, the prevalence of Plasmodium infection is low, particularly in the age range studied. We agree that inflammation often plays a much more prominent role in other areas or in younger children (L. 259-261). We note, however, that C-reactive protein concentration is included as an inflammation marker in our prediction rule that forms the basis for estimating the prevalence of vitamin A deficiency (L. 221-223). We also already noted in our discussion that additional studies are required to confirm whether our linear predictor yields valid results in different populations (L. 300-301).

Question 3) For those readers less familiar with Bland-Altmann plots, the authors may wish to explain what the dotted lines in figure 3 indicate. This could be done either in the title or a label on the graph itself. The text calls these lines “limits of agreement”, but many readers will not know what this means.

We have added more details to the figure and also added an explanation in the legend to Figure 4 (Page 20).

Question 4) In line 212, the authors should substitute the word “diverged” for “diverted”. To divert means to be turned aside or turn from one course or from one usage to another. To diverge means to grow farther apart.

This has been changed as suggested (L. 219).
Question 1) I am not a laboratorian, but is 10.5% interplate CV and 6.0% intra-assay CV for RBP measurement excessive? Compared to the CVs for transthyretin, CRP, and AGP, the RBP CVs seem quite high. In addition, several publications report lower CVs for RPB analyzed by ELISA. Does this warrant some explanation in the manuscript or is this normal for the assay used in this study? The authors may also wish to address the impact of these apparently elevated CVs on their analysis.

The CVs for RBP are as expected and within normally acceptable range for ELISA assays but higher than those for transthyretin, CRP, and AGP because RBP was done by manual assay whereas the other markers were done by automated assay, which resulted in more precise results.

Question 2) In the table, the authors may wish to explain what the digits in (parentheses) and [brackets] are. It seems to have escaped the Reviewer’s attention that the footnote to Table 1 already explained that ‘values indicate mean (SD) or n [%] unless indicated otherwise’.

Question 3) Many of the specific results presented in Table 1 are repeated in the first paragraph of the Results section. This may not be necessary. The first paragraph of the Results section has been condensed.

Question 4) Perhaps the authors could give some more detail of their use of logistic regression modeling and the results thereof presented in figure 4 for less statistically sophisticated readers. We have now elaborated on this part of the statistical analysis (L. 172-198).
vitamin A deficiency as a public health problem (sic)\(^{2}\); and b) WHO recommends that the prevalence in the population with low serum retinol (0.70 µmol/l or below) can be used to assess the severity of vitamin A deficiency in most age groups as a public health problem (sic)\(^{2}\). WHO. Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations. Geneva, World Health Organization, 2011. [http://www.who.int/vmnis/indicators/retinol.pdf]. Thus we believe that our title is appropriate and propose to leave it as it is.


We believe it would be too much of a good thing to use all references suggested by the Reviewer but we have now included a reference to the paper of Futterman et al. (1995; L. 67) in addition to our reference #10.

Remark 3: The manuscript states that HPLC is “expensive, technically demanding and rarely available in developing countries.” I believe that this is no longer true: HPLC analyses of serum retinol concentration are routinely carried out in laboratories in a number of developing countries. The requirements for HPLC (reliable electric power, reliable sources of reagents, maintenance of equipment, etc.) are the same as for the immunoassays used in this report. ELISA assays (especially the sandwich method of Erhardt et al., J. Nutr. 134:3127, 2004) do have the advantage in terms of cost and sample throughput. The authors are invited to respond.

The Reviewer may be correct that there is a growing number of developing countries where HPLC analysis of serum retinol concentration is routinely carried out. In our experience, however, this number is still very low. In many laboratories where HPLC equipment is available, analyses are done only sporadically, quality control procedures are lacking, and staff is poorly trained. Even in Kenya, in which one of the more advanced countries in Africa, we could not find a laboratory with a track record in conducting HPLC measurements. Of the four co-authors, none has had experience in assessing the proficiency of 16 selected laboratories, particularly in Africa, in measuring retinol in serum. Although all laboratories claimed to have the capacity to measure retinol in serum, the results were poor (Hulsloot PJ, Brouwer JJ, Burema J. Estimating the prevalence of vitamin A deficiency in countries with populations with mild to severe vitamin A deficiency. Clin Chem 2002;48:2061-63).

We agree that ELISA assays have the advantage in terms of cost and sample throughput, which is the reason why we evaluated the use of such assays as a possible alternative to HPLC.

Specific comments (Minor Issues):

L. 57: Most studies have found that the molar ratio of retinol:RBP is approximately 0.9, not 1.0 (Smith FR, Raz A, and Goodman DS (1970). Radioimmunoassay of human plasma retinol-binding protein. J Clin Invest. 49:754-8761, 1970). Reference 6 found a similar molar ratio (i.e., RBP is not fully saturated with retinol even in well-nourished subjects). Reference 6 (and others) found that this value did not depend on plasma retinol concentration.

We agree and changed the sentence about this in the MS accordingly (L. 58).

L. 71-72: Since serum retinol concentration < 0.7 uM was used as criterion for vitamin A deficiency, it would be good to give a reference for use of this value.

This has been done as suggested (L. 74).
In addition to BM I, were there any other measures of general nutritional status? Were there dietary estimates of vitamin A intake that led to selection of this study group? Because serum retinol concentration is the major criterion used by these authors, it would be useful to have a frequency plot of serum retinol concentrations.

We selected the students randomly within a school and the schools themselves were selected based on the criteria stated in the MS; no other criteria were used. All measures of general nutritional status are given in Table 1. We agree with the Reviewer that it is important to understand the distribution of serum retinol concentration and we added a sentence to the Results section (L. 202-203) and a frequency plot of serum retinol concentration is now added as online supplementary material.

Was the word “diverged” intended instead of “diverted”? This has now been changed (L. 219).

The greatest divergence between retinol concentrations as measured by HPLC and those measured by fluorescence are at high concentrations. This suggests that retinyl esters (not measured by this HPLC method), perhaps from postprandial samples, were affecting the fluorescence results (a well-known phenomenon).

In contrast to the one reference cited, most studies have found the RDR to be a useful measure of vitamin A status. I suggest that the sentence be re-phrased. We respectfully disagree and consider the RDR to be flawed for reasons given in the reference. We have been careful not to make unnecessary “inflammatory” remarks (which earlier has been questioned as valid indicator of vitamin A status). Thus we propose to leave the sentence as it is.

Table 1, Substudy: Superscript on “Inflammation” should be “2” instead of “1”. This has now been changed.