which may misclassify status and negatively affect public health programs. We encourage that more-sensitive methods of VA assessment be used.

None of the authors had a conflict of interest.

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Reply to SA Tanumihardjo et al.

Dear Editor:

We thank Tanumihardjo et al. for their interest in our study and for raising important discussion points. They suggest that the serum retinol (SR) concentration found in our study population may reflect adequate liver vitamin A (VA) and therefore does not respond to the intervention. Their arguments are based on the following: 1) a suggested absence of VA deficiency reflected by a low prevalence of SR <0.70 μmol/L; 2) a high percentage of circulating SR, based on an SR:RBP molar ratio of 1.27; and 3) a misinterpretation in our study of the Olson theoretical relation between SR concentrations and liver reserves. We respectfully disagree with their views.

First, contrary to their suggestion, our study population was clearly in the subadequate to deficient range. At baseline, 91% of the children without inflammation had SR concentrations <1.05 μmol/L (the cutoff for adequacy) and 23% (not 8% as stated by Tanumihardjo et al.) had SR concentrations <0.70 μmol/L, indicating deficiency. The dietary intake of preformed VA, measured at the study midpoint, was very low (22 μg retinol activity equivalents/d in the control group). Voluntary VA fortification of sugar and oil had just been introduced in Kenya and had not yet penetrated our study area at the time the study was conducted.

We did not use the prevalence of SR concentrations <0.70 μmol/L as the primary outcome because dichotomization of continuous outcomes can lead to a serious reduction in statistical precision (1, 2). This reduced precision also explains why we failed to find an intervention effect on the prevalence of SR concentrations <0.70 μmol/L despite a difference in SR concentrations between groups.

Concerning the second point raised, our estimate of 1.27 for the molar concentrations of SR to retinol-binding protein (RBP) is likely related to the type of assay that was applied. RBP values can vary between types of assays (3), and the specific kit applied by us has not been validated against a gold standard. In some laboratories, it is common practice to artificially correct RBP values derived by ELISA by measuring a control serum sample with a known retinol concentration and assuming a 1:1 molar ratio (J Erhardt, personal communication, 2014), which we did not do because there is no standardized material available to do so.

With regard to the third argument, in our article we assumed that the SR concentration reflects liver VA stores <0.07 μmol/g liver (20 μg/g), which corresponds to an SR concentration <1.75 μmol/L (50 μg/dL). In fact, as we show below, this range is a conservative estimate, based on the reported relation between plasma VA concentrations and liver VA stores [Olson's (4) Figure 3, reproduced here as **Figure 1**A]. Tanumihardjo et al. contend, however, that 1) this reported relation is not based on real data, 2) values >1 μmol/g (>286 μg/g) liver include predicted serum retinyl esters, and 3) Olson's Figure 5 (4) is based on real data and does not entirely support the hypothetical SR–liver VA relation.

On the basis of his Figure 5, Olsen [4; originally from Suthutvoravoot and Olson (5), reproduced here as Figure 1B] failed to find an association between plasma VA concentrations and liver VA stores. The original data, however, clearly show that liver VA concentration is subject to multiplicative variation and rather follows a log-normal distribution (Figure 1 in reference 5), suggesting that the relation between SR concentration and liver VA stores is better described by a lin-log plot (i.e., with liver VA stores on a logarithmic scale on the x axis and plasma VA concentrations on a linear scale on the y axis). We used a ruler to estimate concentrations from the individual data points from an enlarged version of Olson's Figure 5 (4); the resulting lin-log scatterplot (Figure 1C) suggests that plasma VA concentrations increase monotonically with liver stores. Linear regression analysis indeed confirms that a 10-fold increase in liver VA stores is associated with an increase in plasma VA concentrations by 0.32 μmol/L (95% CI: 0.03, 0.60 μmol/L; P = 0.03; Figure 1C, solid line). When this analysis was limited to liver VA stores ≤ 286 μg/g (i.e., excluding the range that may include predicted serum retinyl esters), the corresponding increase was 0.39 μmol/L (95% CI: 0.02, 0.76 μmol/L; P = 0.04; Figure 1C, dashed line). Thus, we conclude that SR concentrations in the range observed at the end of our intervention (minimum–maximum:
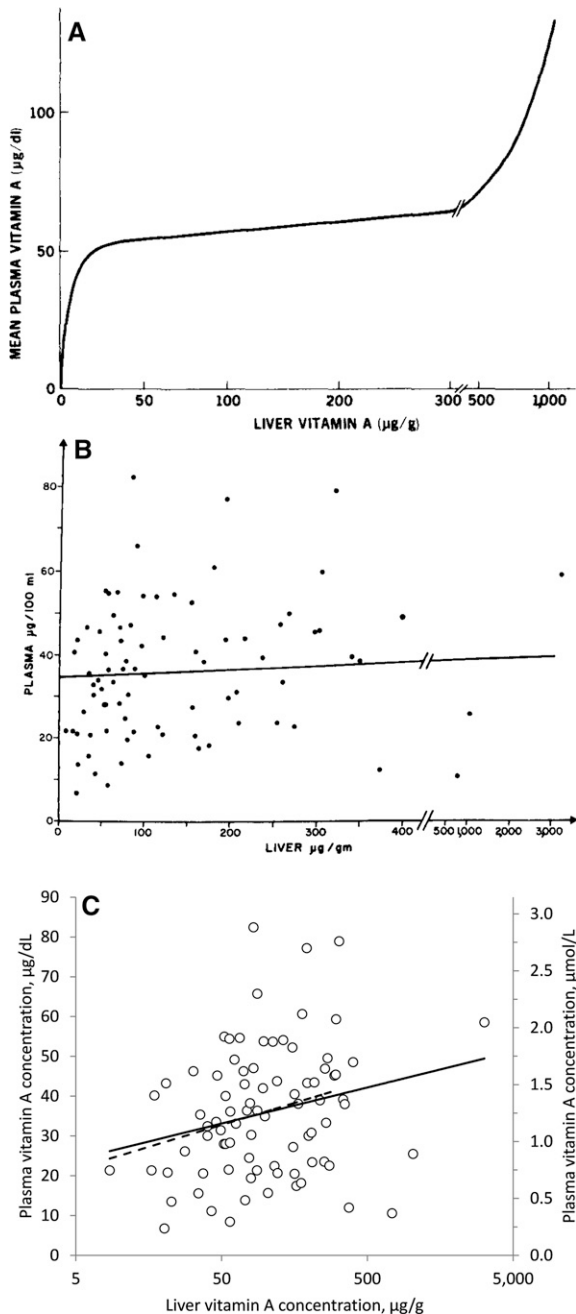
intervention groups that we reported reflects intake from the intervention during preceding days, as seen in other dietary intervention studies, and can be interpreted as an indicator of compliance with the intervention. It neither proves nor disproves that VA status improved. Tanumihardjo et al. are also correct that van Jaarsveld et al. showed an increase in SR concentration over time within each of the intervention groups; a comparison between intervention groups, however, showed that the absence of an intervention effect on SR concentration could not be excluded (difference in change: 0.034 μmol/L; 95% CI: -0.013, 0.081 μmol/L).

In summary, we show that SR concentration can well be used as an indicator to assess the impact of dietary interventions in settings with marginal or deficient status. The use of more-sensitive measures are only required when measuring such effects against background concentrations in the adequate range.

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FIGURE 1 Relations between plasma vitamin A concentrations and liver vitamin A stores as reported by Olson (4) in his Figure 3 (A) and his Figure 5 (originally from Suthutvoravoot and Olson (5) (B). (C) Data derived from panel B, with liver vitamin A stores displayed on a logarithmic scale, are shown (see text). Solid line: association by linear regression analysis on the basis of all data; dashed line: association by linear regression analysis, with restriction to liver vitamin A stores ≤ 286 μg/g. Panels A and B are reproduced from references 4 and 5, respectively, with permission.

0.30–1.52 μmol/L; 25th–75th percentiles: 0.68–0.91 μmol/L) reflect liver VA stores. This also explains why biofortified cassava and supplementation with β-carotene resulted in a small but compelling increase in SR concentrations; such an increase would be undetectable if SR concentrations were within the range that is under homeostatic control.

Tanumihardjo et al. are correct to point out that van Jaarsveld et al. (6) did not present data on serum β-carotene concentrations, as we incorrectly reported. The high concentration of β-carotene in the

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