


Reply to SA Tanumihardjo and BA Underwood

Dear Sir:

Because of their role in the development of the relative-dose-response (RDR) and modified-relative-dose-response (MRDR) tests, Tanumihardjo and Underwood (1) are particularly well-placed to comment on the assumptions made in our article (2). They are incorrect, however, that we claimed that the results of the dose-response tests were due to random variation. Because the formulas used to compute the RDR and MRDR incorporate the serum retinol concentration, which is related to liver stores of retinol in individuals with vitamin A deficiency (3), we concluded that these tests may in fact be correlated with marginal or depleted liver stores. Our models served to illustrate, however, that any interpretation of the relation between dose-response values and serum retinol is necessarily compromised because of the occurrence of a mathematical artifact. Random variation in the serum retinol concentration contributes to this artifact.

The magnitude, but not the extent, of this artifact depends on the values of the variables used to calculate the dose response, e.g., serum retinol concentrations at baseline (R0) and 5 h after dosing (R5) with the RDR. In our models, these values were generated arbitrarily to be within ranges reported by Wieringa et al (4) and Tanumihardjo et al (5). Tanumihardjo and Underwood take issue with the frequency of RDR values <0 and >0.4 in our models. However, negative values have been reported repeatedly, which is incompatible with the concept of the RDR, because this was originally conceived as “the degree by which plasma vitamin A levels otherwise ‘normal’ to an individual are reduced by deficiencies in hepatic stores” (6) [sic]. More pertinent, the negative relation illustrated in our Figures 1 and 2 shows that the mathematical artifact exists across the range of RDR values that Tanumihardjo and Underwood appear to consider realistic, i.e., 0.0–0.4.

Our reasons for assuming R0 and R5 to be independent in our models 1 and 2 are outlined in our article (1) and in a letter by Verhoef (7) in this issue of the Journal. The claim by Tanumihardjo and Underwood that R5 values approximate the homeostatically regulated serum retinol concentration only in vitamin A-replete individuals, and not when liver stores are inadequate, is not substantiated by the report to which they refer (8), and contradicts the report by Lösch et al (6) and evidence put forward in my letter (7). However, if Tanumihardjo and Underwood are correct, then a model similar to ours could be used to examine the extent to which incorporation of this relation between the R0 and R5 would reduce the magnitude of the artifact.

Tanumihardjo and Underwood claim that several studies have shown that an improvement in the MRDR can co-exist with unchanged serum retinol concentrations. As we pointed out (1), however, a small change in serum retinol concentration, particularly in the low range toward zero, will necessarily result in a relatively large change in the MRDR because the serum retinol concentration constitutes the denominator in the formula to compute the MRDR. This can misleadingly provide the impression in population studies that the MRDR is more responsive to changes in the vitamin A status than in the serum retinol concentration; this greater responsiveness will necessarily be accompanied by a greater variance. Substitution of serum retinol concentration by its reciprocal, as is done when the MRDR is calculated, does not provide additional information about vitamin A status.

Tanumihardjo and Underwood state that false-positive results (i.e., individuals incorrectly classified as being vitamin A deficient) are rare when the MRDR is used in vitamin A–sufficient individuals. However, the cutoffs for MRDR used in various studies to indicate vitamin A deficiency were defined on the basis of normal values in populations believed to be vitamin A sufficient (1, 7) and not on published evidence about the relation between hepatic retinol stores (or a proxy indicator thereof) in humans and the MRDR. Thus, this claim appears to be the result of a circular argument.

Tanumihardjo and Underwood are also incorrect that we suggested the 3,4-didehydroretinol response was due to random variation. Because we wanted to show the effect of group differences in serum retinol concentration only, we assumed in our model that the distribution of 3,4-didehydroretinol was random and identical in both groups. As with the models used for the RDR, models similar to ours could be used to incorporate a possible relation between serum concentrations of retinol and 3,4-didehydroretinol to further explore the magnitude of the mathematical artifact that we described for the MRDR.

The author had no conflict of interest to disclose.

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REFERENCES


Validity of the dose-response tests for the determination of vitamin A status

Dear Sir:

We would like to respond to the article by Verhoef et al (1) on the validity of the dose-response tests for the determination of vitamin A status. Unfortunately, the basic assumptions of the authors are incorrect, which invalidate the main results of the article. For example, plasma retinol concentrations are equated with vitamin A status, even though plasma retinol concentrations give only a rough indication of vitamin A status. To illustrate this point, if a subject with a vitamin A status of X is sampled several times, plasma retinol concentrations will vary around a certain value (eg, 0.80 ± 0.10 μmol/L) and, similarly, modified-relative-dose-response (MRDR) values will vary around a certain value (eg, 0.09 ± 0.01 μmol/L). The questions are not how well these MRDR values correlate with plasma retinol concentrations, but how well the MRDR values correspond to the vitamin A status of X and whether, after supplementation, changes in plasma retinol or MRDR more closely relate to changes in vitamin A status X. Verhoef and West missed this point; therefore, their article addresses the wrong issue (the relation between the RDR and vitamin A status, in reality, and thus invalidates the simulation model and hence the results and conclusions).

Finally, Verhoef and West misinterpreted the results of our study (5). We reported lower plasma retinol concentrations, but also lower MRDR values (ie, better liver stores), in infants supplemented with iron. According to Verhoef and West, if mathematical artifacts underlie the relation between the MRDR and vitamin A status, lower plasma retinol concentrations should be associated with higher, not lower, MRDR values. Correlation coefficients were only added to show that there was no correlation between the MRDR and plasma retinol concentrations in infants supplemented with iron.

Furthermore, although Verhoef and West repeatedly referred to the article by Wieringa et al (5) as being from “our group,” only one author (CEW) of the first article is also a coauthor of the article by Verhoef et al (which consisted of 6 authors from 5 different departments); therefore, the 2 articles should not be considered to be from the same group. Verhoef and West had no access to the original data of Wieringa et al, and, therefore, have no basis for their statement that “the statistical analysis in the article was wrong because the MRDR was not normally distributed.” (In fact, the distribution was borderline normal.) More importantly, however, “heteroscedasticity of data does not produce any bias in the coefficient estimates, but will produce biased standard errors” (6). Hence, even if there is heteroscedasticity in the data, the correlation coefficients will still be reliable.

The discussion of normal distribution is beside the point, however. Although one does not need statistics to see the difference between the infants who received iron and the infants who did not receive iron (as depicted in Figure 3 of Wieringa et al’s article), robust, nonparametric chi-square testing identified a highly significant difference between the infants who did and did not receive iron.

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