None of the authors had a conflict of interest.

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Reply to G Lietz et al.

Dear Editor:

Thank you for allowing us to respond to Lietz et al. The authors used linear and compartmental models to evaluate measured changes in total body stores (TBSs) of vitamin A (VA) assuming inputs of TBSs, dietary intake, and fractional catabolic rate (FCR). These models yielded similar results because they are based on similar mathematics (first-order kinetics). We will discuss assumptions made, which are relevant to both models. The authors attribute discrepancy in our study to incorrect assumptions. We will address and clarify our assumptions followed by data and assumptions used for their models.

Our study was a randomized, positive- and placebo-controlled community-based trial (1). The study design used was paired retinol isotope dilution (RID), meaning that each child served as his or her own control to determine intervention response. Therefore, the findings with greatest confidence are relative treatment effects on VA status (VA+ group increased TBSs relative to VA−) and relative change within treatment group (VA− group maintained TBSs, whereas VA+ group increased TBSs). These conclusions are drawn because of the randomized, controlled, and paired design and hold regardless of assumptions as long as they are consistently applied (2). Absolute TBS quantification was more uncertain and reported with confidence intervals.

The challenged assumptions are as follows: serum:liver isotope enrichment ratio (SLR), dose absorption/retention, and inflammation/iron deficiency. We used SLR = 1, addressed in our Methods and Discussion (1). The challenge that it is lower is based on a study in rats fed VA during the mixing period, with a mean SLR = 0.65 (3). However, in their Discussion the authors claimed that, under other experimental conditions, they achieved an SLR >1 (3). They mention another rat study (4) that found an SLR of 1.2 in a treatment group fed VA during mixing. Data in monkeys measured a fasting SLR of 1.0 from days 7 to 28 postdose when ~6 µmol VA/d was consumed during mixing (5). We challenge the notion that SLR “cannot be equal to 1 under these circumstances” on the basis of data provided. The authors of the rat study (3) stated, “Although kinetic models may be devised to rationalize ratios both greater and less than unity, further analysis of this relationship should await the conduct of carefully defined kinetic studies.”

With regard to absorption/retention, Lietz et al. may have misunderstood assumptions applied, which we have clarified (2). We assumed absorption of 80–90% depending on elevated C-reactive protein (CRP). Retention at 14 d was further reduced by factor e^{-0.093} (0.93 at 14 d), yielding absorption/retention of 74–84%. Liver retention was further reduced assuming 80% TBSs in liver, yielding a range of 60–67%. Although these estimates are close to the study cited, we also considered other studies that measured labeled VA absorption, especially data regarding doses more closely resembling the size, enriched element, and enrichment amount used in Zambia (1, 5).

Lietz et al. state that infection and iron deficiency affect RID. To account for subclinical infection confirmed by measuring z̄-acid glycoprotein, dose absorption was reduced from ~99–100% (1, 5) to 90% for small, isotopically labeled doses. We further decreased absorption by 10% in children who had elevated CRP as recommended by a group of scientists, including coauthor Haskell. Low hemoglobin was an unused exclusion criterion, and we confirmed adequate serum zinc concentrations. The iron-deficient rats had hemoglobin concentrations of 37 g/L (6), whereas our children had concentrations of 116 g/L (1). Reduced SLR from infection was observed administering LPS or human IL-6 to rats, reducing serum retinol by ~47% and ~65%, respectively (6), indicating more severe inflammation than our 19% reduction from elevated CRP (1). We agree that infection, inflammation, and other micronutrient deficiencies affect RID, but the data presented were severe cases in animal models. Effects in our study would have less impact.

With regard to the models presented, we are concerned that some of our findings in which we have less confidence, such as dietary intake and FCR, are being overextrapolated. Lietz et al. state that for the models to agree with the observed increase in TBSs, FCR would have to be reduced or dietary intake increased. Likely there may be a combination of both. Our study was not kinetic; the FCR was estimated by using only 2 time points/child. Calculations assumed the dose was fully mixed by 14 d and kinetics had entered a terminal slope, which may not have been the case. Time to fully mix was estimated at 26 d in adults (7). The FCR would have been overestimated if the dose had not fully mixed by 14 d. The calculated FCR was used because it is an
improvement for our children compared with a 2.2% FCR used in infants (8). An overestimated FCR (underestimated half-life) mildly decreases TBS estimations given 14 d between dosing and sampling, whereas applying the FCR by Lietz et al. to a 90-d model would magnify any FCR overestimation. We are not the first to show that VA supplementation exaggerates RID response after intervention (9), which we acknowledged, and our statistical methods accounted for this (1).

Replicating the model presented by Lietz et al. included correspondence with the authors and revealed an additional factor of 0.76 for dietary VA retention. We are concerned that this factor is redundant with FCR, and this factor was not stated in their letter.

Although highly controlled, our study was not clinically but community based. Off-site VA intakes revealed large variation. Dietary VA content was calculated by using NutritionistPro (Axxya Systems; Redmond, Washington) with the best matching food item, which may inaccurately reflect VA content. For example, small dried fish were listed as 22 retinol activity equivalents (RAE)/100 g. When food samples were recently analyzed by HPLC in our laboratory, they actually had 460 ± 120 RAE/100 g. VA drying and preparation losses were overestimated. We assumed intakes were equal across groups due to randomization with site blocking, which minimized effects on study outcomes.

Finally, if we recalculate our data using alternative published assumptions of SLR = 0.8 (10), half-life = 154 d (7), and 90% TBSs in liver (8), values for liver concentration are only reduced by 8%; the prevalence of individuals with >1 μmol VA/g liver decreases from 59% to 47%, which should still raise eyebrows. Importantly, no matter which assumptions are made, it is very clear that Zambia has done a great job supporting VA status in this rural area. We look forward to collaborating with the VA community to tease out the effects of various factors on RID methodology to optimize the world’s VA status.

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