4. National Nutrition Monitoring Bureau (NNMB). Prevalence of Vitamin A Deficiency among Rural Preschool Children. Hyderabad, India: National Institute of Nutrition, Indian Council of Medical Research. Report No 23;2006.
5. Craft NE. Retinol analysis in dried blood spots by HPLC. J Nutr. 2000;130:882-5.
6. Filteau SM. Vitamin A and the acute-phase response. Nutrition. 1999;15:326–328.
7. Christian P, Schulze K, Stoltzfus RJ, West KP Jr. Hyporetinolemia, illness symptoms, and acute phase protein response in pregnant women with and without night blindness. Am J Clin Nutr. 1998;67:1237-43.
8. Filteau SM, Tomkins AM. Micronutrients and tropical infections. Trans R Soc Trop Med Hyg. 1994;88:1-3.

Evaluation of Phototherapy Devices

The recent paper by Subramanian, *et al*. [1] evaluating phototherapy devices used for treating neonatal hyperbilirubinemia contains several flaws that limit the practical relevance and interpretation of the measurements.

1. The authors fail to distinguish adequately between photodegradation, in which bilirubin is degraded to substances of lower molecular weight, and photoisomerization, in which the pigment's structure changes but no degradation occurs. Both processes contribute to the effects of phototherapy in humans, but their relative contributions are presently unknown. However, in the Gun rat animal model photoisomerization is more important than photodegradation and the same is likely to hold for humans. In the article the authors focus largely on photodegradation, the pathway of least importance.

2. The relative effectiveness of the different lights tested was determined by comparing their effects on methanolic solutions of bilirubin *in vitro*. Residual bilirubin was measured after 15-120 min of exposure, by which time a substantial fraction of the original bilirubin had been bleached and degraded. Preparation of the methanolic solutions was not described. Aside from the fact that bilirubin is insoluble in methanol, the photochemistry of bilirubin in organic solvents is rather different from that in serum or aqueous albumin solutions. Therefore, methanolic solutions of bilirubin are inappropriate for comparative testing of phototherapy lights.

3. The test solutions were clearly over-irradiated and not sampled early enough to detect the relative rates of formation of the physiologically important photoisomers of bilirubin. Bleaching of bilirubin has been used in previous studies of the relative efficacy of phototherapy lights, but there is no evidence that this is a valid method, especially when bleaching is allowed to continue to a point where secondary reactions of no relevance to phototherapy may be taking place. Measuring initial rates of formation of bilirubin photoisomers would probably provide a more valid and clinically relevant method.

4. The authors claim to have developed a novel high precision HPLC/MS technique for measuring bilirubin photoisomers, yet presented no examples of typical separations. Samples were extracted with a solvent containing formic acid. Configurational photoisomers of bilirubin, the most rapidly formed isomers in humans, are highly sensitive to acids, undergoing reversion to unisomerized bilirubin. Therefore, it seems unlikely that these important photoproducts would have survived the HPLC conditions. Reference standards were prepared by irradiating bilirubin in methanolic solution, but no details were provided of how specific photoisomers or other photoproducts were identified and characterized.

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REFERENCE

1. Subramanian S, Sankar MJ, Deorari AK, Velpandian T*, Kannan P, Prakash GV, *et al*. Evaluation of phototherapy devices used for neonatal hyperbilirubinemia. Indian Pediatr. 2010; Nov 30 (E-pub ahead of print).

REPLY

We thank the reader for the interest shown in our article published recently in the Journal (Epub). The questions raised by the reader are indeed valid. However, they

would probably not have arisen if all our original figures and the text were retained in the final published article (the figures have to be removed because of space constraints). Nevertheless, we have tried to address the concerns here:

1. The major outcome variable of our study was the amount of native bilirubin left over after exposure to light. We consciously avoided using the amount of isomers formed as the primary outcome variable as we did not characterize them. We therefore used the term photoconversion rather than photodegradation or photoisomerization. On the other hand, we would also like to point out that the technique used by us (LC-MS/MS in a highly efficient Multiple Reaction Monitoring mode [MRM] along with hydrophilic interaction chromatography) separates bilirubin from its isomers having similar molecular weights. So, we do not agree with the reader's comment that we 'focused largely on photodegradation and not photoisomerization'.

2. We agree with the reader that the photochemistry of bilirubin in organic solvents could be different from serum/aqueous albumin solutions. Still for the comparative evaluation of different light sources under controlled experimental conditions, we opted for the methanolic solution of bilirubin at the concentration of 1μg/ml because of the following factors: (*a*) lack of aqueous solubility of bilirubin (*b*) concerns over availability of unbound fraction of bilirubin from plasma for photoreactions and (*c*) the risk of interferences in estimation by the biomatrix. Usage of organic solvents for water insoluble drugs for photodegradation analysis is not uncommon. For the preparation of stock concentration of bilirubin, dilute ammonia solution of methanol was used and it was serially diluted to reach the concentration of 1μg/mL with methanol.

3. It is true that over-irradiated samples are capable of producing more and more photoconversion products. However, the method adopted by us for determination of bilirubin concentration (LC-MS/MS) is the gold standard for measuring compounds with higher precision. As it is quantifying the compounds based on their molecular weight, color of the compound is immaterial. The standard methanolic bilirubin appearing at 1.23 min and the formation of a photoisomer product at 1.9 min can very well be seen in the accompanying **web figure.** Moreover, we have used more time points for quantification.

4. We have shown the separation of peaks within the period of 3 min in LC-MS/MS using the method reported in the manuscript (Figure available on request). For *in vivo* quantification process (ongoing study), the method was optimized to include the extraction solvent with an internal standard in the composition of 70% acetonitrile containing 0.1% formic acid. Therefore, the same method was adopted for this in vitro study. From the observed data using the method, it is convincing that the photoisomers formed and survived the experimental conditions. However, we did not isolate any photoisomer for further characterization. Further studies are in progress to isolate and characterize the photoisomers for their quantification *in vivo* conditions.

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Short course of Antibiotics in Neonatal Sepsis

It is appreciable effort on part of Saini, *et al*. [1], to cut short the usage of antibiotics in case of culture negative "sepsis" but we have some observations regarding the study.

A complete sepsis screen score should have been taken into consideration before deciding to start the antibiotics. CRP alone with clinical suspicion will lead to falsely high number of neonates getting enrolled which will affect the primary outcome as these 'false positive' cases are less likely to present with' treatment failure [2]. Sepsis score not relying upon 'CRP alone' would have been more useful as this costly test is not universally available, as mentioned by the authors also.

Babies falling sick within fifteen day period are presumed to be the continuum of initial episode while a re-infection or sepsis caused by different organism cannot be ruled out completely. The number of neonates who were labeled as 'treatment failure' will be inflated falsely because of re-infection/fresh sepsis. It is not possible to ensure equal distribution of these fresh cases in both groups as sample size is very small. So the