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Correspondence to Dr A Lucas, Medical Research Council Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge.

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Commentary

N F BUTTE AND C GARZA

Baylor College of Medicine, Houston, Texas, USA

Lucas *et al* have compared the measurement of formula intake by the deuterium dilution technique and by weighing formula bottles over a seven day period and have shown close agreement between the two methods.[1] They refer to our paper in which we concluded that the deuterium dilution technique overestimates milk intake,[2] and this requires some elaboration.

Our trial attempting to validate the method was designed according to the original protocol reported by Coward *et al*.[3] Differences in the way in which we used the method compared with that of Lucas *et al* require critical evaluation. The equilibration period of two hours that we used to calculate total body water was insufficient; in experiment 2, however, values of total body water predicted from samples taken two hours after the dose of isotope had been given were only 2·7% higher than those obtained by extrapolation from the exponential curve of decay of the isotope to the time of the start of the experiment. Because the half life of water in infants is about three days, assessment of milk intake by the deuterium dilution technique over 48 hours is probably too short, although agreement between methods was not improved when we extended the experiment to 120 hours. Deuterium enrichment analysis was performed at the Dunn Laboratory in both our study and theirs. In our study it was done on a Micromass 602 D spectrometer and an Aqua-sira mass spectrometer; possibly better precision and accuracy were achieved by Lucas *et al* in their study.

The short duration (48 hours) of the test weighing session was an important limitation of our study. We now know that the estimate resulting from a 48 hour test weighing session should be within about 28% (95% confidence interval) of an individual infant's intake.[4] Although this shortcoming led to a less precise estimate, it would not result in a systematic overestimation.

The way in which we calculated milk intake from the results obtained with the deuterium dilution technique was different from that used by Lucas *et al;* we made no adjustments for the fractionation of deuterium in water vapour. Lucas *et al* assumed that an equivalent of 13% of water intake was subject to fractionation. Although this proportion seems low, they are correct in stating that a 100% error in this figure would result in about a 1% error in water output. More importantly, they incorportated a correction factor of 5·7% and assumed this to be insensible water intake. This value was derived from a previous experiment with preterm infants and was the difference in intake observed between weighed records and results obtained by deuterium dilution. Although it may encompass insensible water influx, it also includes other possible sources of error.

Water influx through the skin and respiratory passages may be a potential, uncontrollable source of error in the 'dose to the infant' version of the deuterium dilution technique. The input of water vapour through the skin should be proportional to ambient humidity and the permeability of the skin to water.[5] Influx through the lungs should be related to respiratory minute volume and ambient humidity. If ambient water is depleted compared with the body's enrichment, water flux will be overestimated. The effects of unlabelled ambient water vapour at various relative humidities on water influxes measured using tritium were shown in kangaroo rats.[5] With absolute humidities ranging from 3·8 to 19·8 mg H_2O/l air water fluxes were overestimated by 7·7 to 44·2%, respectively. To account for this source of error variations in ambient humidity, skin permeability, surface:volume ratio, and minute volume would have to be considered in the calculation of water flux. Application of a constant correction factor may not be appropriate in environments where humidity varies daily, and the differences between indoor air conditioned and outdoor settings must be taken into account, as would be the case in Houston, Texas, where our own studies were conducted.

Recent preliminary findings from our laboratory, however, suggest that water influx may present only a minor source of error in the 'dose to the infant' deuterium dilution method. We have found that formula intakes predicted from deuterium dilution were only slightly higher than values obtained from direct weighing of formula bottles. Milk intakes of breast fed infants were consistently overestimated by the deuterium dilution technique when compared with values obtained by five day test weighings to the order of 10% more than were formula intakes. Because there is no obvious reason for the difference in water flux dynamics of breast fed and formula fed infants we must evaluate the test weighing procedure critically. Our measurements of

human milk intake were not corrected for insensible evaporative losses during feeding. We approximated, and possibly underestimated, the mean (SD) loss to be 3·0 (2·0)% of intake.[2] There is also the possibility that mothers did not weigh their infants at every feed. Although the diligence of these women and the consistency of milk intakes reported from several laboratories would suggest otherwise, we recognise it as a potential source of error. We find it even more perplexing that when we compared the 'dose to the mother' version of the deuterium dilution technique[6] and the test weighing method[7] we obtained good agreement.

At the time of writing we agree that the 'dose to the infant' version of the deuterium dilution technique is a promising method for measuring milk intake. The magnitude of the error due to water influx, however, requires further study, and the discrepancy in the extent of overestimation of milk intake seen between breast fed and formula fed infants requires explanation.

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