

## COMMITTEE ON NUTRITION

# COMMENT ON METABOLIC BALANCE STUDIES AS A METHOD OF ESTIMATING BODY COMPOSITION OF INFANTS

## With Special Consideration of Nitrogen Balance Studies

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MANY REPORTS published during the past 60 years indicate the usefulness of metabolic balance studies in at least three areas of investigation: (1) comparison of nutritional properties of foods fed to comparable groups of human subjects or experimental animals under standardized conditions, (2) comparison of performance of normal subjects or experimental animals with those having certain metabolic abnormalities, and (3) comparison of the effects of two regimens of management with a single subject. The successful use of metabolic balance studies for these purposes has suggested to some individuals that results are precisely quantitative. Were this true, it would be possible on the basis of continuously or serially performed balance studies between two specified ages to make an accurate calculation of the change in one or another aspect of body composition. For example, the change in nitrogen or calcium content of the body could be calculated; from the change in nitrogen content, the change in protein content could be estimated, and from the change in calcium content, the change in skeletal mass could be estimated. The present communication will present the reasons for the failure of such calculations to provide valid estimates.

As an example, one might consider a normal infant with a birth weight of 3,200 gm. If protein comprised 12% of body weight at birth, and if nitrogen comprised 16% of protein, the nitrogen content of the body at birth would be 61 gm. The mean retention of nitrogen in 3-day metabolic balance stud-

ies performed biweekly from birth until 6 months of age, with the infant receiving a high-protein formula (3.5 gm protein in 100 ml) might be 1.25 gm/day,<sup>1</sup> or 228 gm in 182 days. The total content of nitrogen in the body at 6 months of age would be 228 gm + 61 gm = 289 gm. With a body weight of 7,000 gm at 6 months of age, protein would account for 25.8% of the body weight.

Because a protein content of the body equal to 25.8% of the body weight is far more than has been found by direct chemical analysis of the human body,<sup>2</sup> it is clear that the calculations given above are not justified. It is therefore of interest to examine the assumptions on which the above calculations are based.

### ASSUMPTIONS ON WHICH CALCULATIONS ARE BASED

*The first assumption* is that nitrogen is excreted only in urine and feces. It is likely that unmeasured losses of nitrogen, such as those from skin, nasal mucus, saliva, and vaginal discharge, may contribute to the falsely high estimate of nitrogen retention. Studies of cutaneous losses of nitrogen of resting adult subjects have been reviewed by Darke.<sup>3</sup> Such losses have ranged from 188 to 480 mg in 24 hours. Kuno<sup>4</sup> has reported that in temperate climates an average adult with occasional slight sweating may lose 300 mg of nitrogen or less daily through the skin.

Few studies of cutaneous losses of nitrogen of infants have been published. Cooke *et al.*<sup>5</sup> determined losses of nitrogen through

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the skin of three infants ranging from 7 to 16 months of age (8.1 to 9.7 kg). Fifteen metabolic balance studies were performed, some at 81° to 83° F (27.2° to 28.3°C) and some at 91°F (32.8°C). At 81 to 83°F cutaneous losses of nitrogen ranged from 53 to 173 mg/day, with a mean of 100 mg/day; losses tended to be somewhat greater at 91°F than at 81 to 83°F. There was no evidence that higher intakes of nitrogen were associated with greater cutaneous losses.

Presumably cutaneous nitrogen losses of the infant mentioned in the example above (apparent nitrogen retention: 1,250 mg/day) would be likely to be less than 100 mg/day because of the smaller mean surface area. However, if the value 100 mg/day is accepted and this correction introduced in the apparent nitrogen retention (i.e., 1,250 mg/day - 100 mg/day = 1,150 mg/day), it is clear that the calculated protein content of the body at age 6 months would still be unbelievably high.

The second assumption is that balance studies may be performed without significant systematic procedural errors. It has been emphasized by Wallace<sup>6</sup> that most procedural errors in metabolic balance studies summate to result in apparent retentions that are falsely high. Intakes tend to be over-estimated (a small amount of formula is lost from the infant's mouth; a small

amount adheres to the sides of the feeding bottle), and excretions tend to be underestimated (a small amount of feces adheres to the buttocks; a few drops of urine are lost).

Wallace<sup>6</sup> has pointed out that "when a high protein containing milk is fed an infant who is having the usual retention of nitrogen for growth," a small loss of intake and a similar loss of excreta will result in a relatively large increase in the determined balance. Calculations made with data from nitrogen balance studies with infants (Table I) indicate that the magnitude of the error will depend on age as well as protein intake. A 2% loss of both intake and excreta of a 31-to-60-day-old infant would result in an error of about 8% in retention of nitrogen if the feeding were human milk and an error of about 13% in retention of nitrogen with a high-protein feeding. Because of the smaller percentage of intake that is retained with increasing age, greater percentage errors in retention would result from 2% loss of both intake and excreta by older infants. Thus, an error of 20% in retention might occur with an infant 4 to 5 months of age receiving a high protein feeding (Table I). However, because procedural difficulties in performance of balance studies are less at this age, a loss of 2% of the intake or 2% of the excreta is much less

TABLE I  
ILLUSTRATION OF ERROR IN RETENTION OF NITROGEN INTRODUCED BY LOSS OF 2% OF INTAKE  
AND 2% OF EXCRETA DURING A METABOLIC BALANCE STUDY

Age (days)	Feeding	Intake (mg/kg/day)		Excretion (mg/kg/day)		Retention (mg/kg/day)		Error (%)
		Apparent*	Corrected*	Apparent	Corrected	Apparent	Corrected	
31-60	Human milk†	383	375	218	222	165	153	8
31-60	High protein formula‡	884	866	617	629	267	237	13
121-150	High protein formula‡	822	806	645	658	177	148	20

\* "Apparent" values for intake, excretion, and retention are based on the assumption that the intake and excretion of nitrogen recorded during the balance studies were the true values; "corrected" values are based on the assumption that a 2% loss of intake and a 2% loss of excreta occurred.

† "Apparent" values for infants fed human milk are mean values from study of Fomon and May.<sup>7</sup>

‡ "Apparent" values for infants fed the high protein feeding (O'ara) are mean values from the study of Fomon.<sup>1</sup>

likely. At a specified age the error will be proportional to the ratio of intake to retention.

*The third assumption* is that all the nitrogen in the body exists as protein, an assumption that probably does not introduce an important error. Rarely is the concentration of nonprotein nitrogen more than 45 mg/100 ml in blood of normal infants receiving high-protein diets. Even if one assumes that a similar concentration exists throughout the entire body, only a small fraction of the total nitrogen in the body would be accounted for. Thus, a 7,000-gm infant might have a body content of 3.1 gm of nonprotein nitrogen with a total nitrogen content of the body of 289 gm.

*The fourth assumption* is that retentions of nitrogen recorded during three-day metabolic balance studies are the same as retentions during the intervals between balance studies. Only if this assumption were correct would the amount of nitrogen accumulated during a specified interval be approximated by multiplying the mean retention recorded during balance studies, expressed in grams per day, by the number of elapsed days. Actually, the infant is in negative nitrogen balance during the first few days of life and may be in negative nitrogen balance during occasional episodes of acute infection of the upper respiratory tract. Minor indisposition, such as teething, reactions to routine immunizing injections, etc., may be associated with lesser retentions of nitrogen than those recorded during balance studies.

Metabolic balance studies are ordinarily performed only with apparently healthy infants. Rhinorrhea, looseness of stools, decrease in appetite, etc., are considered sufficient indications to defer study. Routine immunizations are ordinarily not given during or immediately before a balance study. Mean retentions of nitrogen may therefore be somewhat greater *during* balance studies than *between* balance studies.

Unfortunately, no data are available from continuous balance studies of an infant from shortly after birth until 4 or 6 months

of age. Swanson<sup>8</sup> performed almost continuous metabolic balance studies with one infant receiving a relatively high protein feeding. Balance studies were carried out during 6 of each 7 days between the ages of 14 and 90 days. The infant gained from approximately 3,550 gm to 5,500 gm and had a mean retention of nitrogen of approximately 923 mg/day. Assuming a protein content of the body equal to 12% of the body weight at 14 days of age, and assuming that each gram of retained nitrogen was equivalent to 6.25 gm of protein, the content of protein at 90 days of age would have been approximately 16% of the body weight. Such a percentage may be reasonable since the composition of lean body mass is probably changing most rapidly in the early months of life.

*The fifth assumption* concerns the suitability of the factor 6.25 used in conversion of content of nitrogen in the body to content of protein. Whether this factor is justified with respect to the human infant is unknown, and there is as yet no evidence to indicate whether the same factor is applicable for infants fed high protein diets and those fed diets with lower content of protein. One may speculate that the protein synthesized during periods of higher protein intake might be qualitatively different from that synthesized during periods of lower protein intake. However, in the hypothetical example given above in which an infant increased in body weight from 3,200 gm to 7,000 gm during the first 6 months of life while retaining 1.25 gm of nitrogen daily, it is clear that utilization of a factor of 6.00 or 6.50 instead of 6.25 would result in only minor alteration in the calculated protein content of the body at age 6 months.

### CONCLUSIONS

It seems probable that several factors must be considered jointly in explaining the falsely high body content of protein calculated on the basis of nitrogen balance studies. Of these, the most important are probably cumulative errors in balance techniques, nitrogen loss in sweat and desqua-

mated epithelium, and differences in retention of nitrogen during balance studies and in intervals between balance studies. Confirmation or refutation of the conclusion based on metabolic balance studies—that feeding of high-protein diets results in accumulation of relatively large amounts of protein in the body—must certainly come from assessments of body composition of normal infants made with techniques other than metabolic balance studies.

Similar errors are inherent in calculating body content of calcium, phosphorus, sodium, and other minerals from results of metabolic balance studies. However, in these instances the systematic procedural error will be relatively greater because a smaller percentage of the intake is retained. With some substances, e.g., sodium and potassium, failure to measure losses from skin will introduce a much greater error than is the case with nitrogen.

The unsuitability of metabolic balance studies as a means for estimation of body content of a substance indicates that the absolute values obtained with the methods are not precise. The methods are nevertheless useful when only relative results are sought, as in the nutritional evaluation of

two foods fed to comparable groups of infants at similar intakes of nitrogen or in comparison of performance of normal subjects with subjects having certain metabolic abnormalities. The usefulness of balance technique is also obvious in the study of patients in whom large retention or losses are to be anticipated.

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