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Dual Tracer Stable Isotopic Assessment of Calcium Absorption and Endogenous Fecal Excretion in Low Birth Weight Infants

STEVEN A. ABRAMS, NORA V. ESTEBAN, NANCY E. VIEIRA, AND ALFRED L. YERGEY

Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, Bethesda, Maryland 20892; Holy Cross Hospital, Silver Spring, Maryland 20510; and Children's National Medical Center, Washington, District of Columbia 20010

ABSTRACT. Using a dual tracer (^{44}Ca orally and ^{46}Ca i.v.) stable isotope technique, true dietary Ca absorption, endogenous fecal Ca excretion, and net Ca retention were measured in 12 low birth weight (1426 ± 260 g) infants fed a high Ca-containing formula. Endogenous fecal Ca excretion averaged 7.2 ± 4.1% of intake, and exceeded 10% of intake in three infants. Net Ca retention, 103 ± 38 mg/kg/d, was consistent with previous studies of Ca retention obtained using mass balance techniques and correlated closely ($r = 0.98$, $p < 0.001$) with true Ca absorption but not with endogenous fecal excretion ($r = -0.40$, $p = 0.19$). Although endogenous fecal excretion may represent a significant source of Ca loss for some low birth weight infants, these data suggest that net Ca retention in low birth weight infants fed a high Ca-containing formula is primarily determined by the total dietary Ca absorbed. (*Pediatr Res* 29: 615–618, 1991)

Abbreviations

LBW, low birth weight
V_f, endogenous fecal Ca excretion
V_i, Ca intake
V_a, true dietary Ca absorption
V_u, urinary Ca excretion
V_o, net Ca retention (bone accretion)

Numerous recent studies in LBW infants have assessed the bioavailability of Ca in special formulas containing high concentrations of Ca relative to human milk. However, these studies have yielded extremely variable results regarding the fractional and total Ca absorbed and retained (1–8). For example, when different investigators studied essentially the same formula, the net V_o reported ranged from approximately 80 to 170 mg/kg/d (1, 2, 4–7).

The etiology of the reported variability in Ca retention remains unclear. Minor differences in patient characteristics are likely to be only partly responsible. Differences between investigators in details of the mass balance method do not explain the relatively high coefficients of variation for absorption and retention seen within many studies.

We hypothesized that secretory Ca losses in the feces, V_f, may be substantial in LBW infants (4, 9, 10) and might explain a significant portion of the variability in reported Ca balance data. The effect of V_f on V_o has not been assessed in most previous studies of Ca balance in infants because the mass balance technique does not differentiate between unabsorbed and endogenously excreted fecal Ca.

The use of stable isotopes of Ca allows for the direct measurement of V_f without requiring exposure to ionizing radiation. V_a and V_f can be determined from the simultaneous administration of both i.v. and oral isotopic tracers with subsequent monitoring of fecal and urinary tracer excretion (11–17). In this study, we used ^{44}Ca and ^{46}Ca to measure V_a and V_f in a group of healthy, growing, LBW infants. The objectives of our study were to measure the rates of V_a and V_f in LBW infants on a fixed, high Ca-containing diet and to determine their relationship to V_o.

MATERIALS AND METHODS

Patient population. Infants admitted to the neonatal intensive care units at Holy Cross Hospital, Silver Spring, MD, and Children's National Medical Center, Washington, DC, who weighed between 750–1750 g at birth, and whose mothers chose not to breast feed, were eligible for enrollment in this study. A dual tracer stable isotope study, with subsequent 24-h urine and 96-h stool collections, was conducted when the infants were receiving complete enteral nutrition of the designated formula for a minimum of 1 wk and were free of medical illnesses. At the time of the study, all patients were free of ventilatory support, diuretics, and antibiotic therapy. Three infants (CB, BH, and RM) were receiving caffeine. No patient had received steroids postnatally. The study was approved by the Institutional Review Committee of the National Institute of Child Health and Human Development and by the review committees of the respective hospitals. Informed written consent was obtained from the parents prior to enrollment in the study.

Initial feedings were begun at 4 ± 3 d (mean ± SD) with feedings of dilute Similac Special Care (Ross Laboratories, Columbus, OH) and advanced over a 5- to 10-d period to full-strength formula at approximately 120 kcal/kg/d. Formula was provided from ready-to-feed bottles, 24 kcal per fluid ounce in 10 patients and 20 kcal per fluid ounce in two patients. All infants were fed by bottle or intermittent orogastric gavage. Vitamin D 200 IU/d was given in the form of a multivitamin supplement. Total vitamin D intake of formula plus supplement was approximately 400 IU/d.

Intake and output measurements. Details of the urine and stool collections have been described previously (14–17). Eighteen h before the beginning of the study, one-half volume of a single feeding was mixed with 1.5 mg/kg of ^{44}Ca and refrigerated at −4°C overnight. The following morning, the infants were given 0.015 mg/kg of ^{46}Ca i.v. over 5–10 min. Subsequently, the premixed feeding with the added ^{44}Ca was fed orally or via orogastric tube. The remainder of the volume of that feeding was added to the syringe (for tube feedings), or bottle, and given to

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Correspondence: Steven A. Abrams, M.D., 9000 Rockville Pike, LTPB/NICHD, Bldg. 10, Rm. 6C-101, Bethesda, MD 20892.

the infants. Samples of the formula and the formula mixed with tracer were collected before feeding. After the single feeding, any residual formula in the bottle or syringe/tubing was collected. The formula samples were analyzed for total Ca and Ca isotope ratios. From these measurements, the total tracer remaining in the bottle, or syringe/tubing was calculated to determine the actual doses of tracer administered (16).

Immediately after the ^{46}Ca infusion, the infants were placed unrestrained on a mesh hammock suspended over a stainless steel metabolic bed within an incubator. Urine was collected in 4-h aliquots in sterile, Ca-free, polypropylene containers for a total of 24 h. Stools were collected individually using sterile polyethylene bags adhered to the buttocks. After 24 h, the infants were removed from the metabolic bed and stools collected in polyethylene bags for an additional 72 h.

Nutrient intake was calculated from the volume of formula ingested and the concentration of Ca measured in the formula. Any spillage was noted and subtracted from intake. Body weight, length, head circumference, and serum Ca, phosphorus, and alkaline phosphatase activity were measured at the beginning of the study.

Isotope preparation and analytical techniques. Sterile solutions of ^{46}Ca, 0.37–0.50 mmol/L (0.015–0.02 mg/mL) and ^{44}Ca, 87.33–162.18 mmol/L (3.5–6.5 mg/mL) were prepared by the National Institutes of Health pharmacy and tested for pyrogenicity and sterility before use. Ca isotopes were obtained from Oak Ridge National Laboratory, Oak Ridge, TN.

Total Ca was measured on all urine samples by flame atomic absorption spectrophotometry. Stool and formula samples were individually homogenized and an aliquot was ashed using a CEM Microwave Digestion System, model MDS-81D (CEM Corp., Indian Trail, NC) before analysis.

Isotope enrichment was determined using a Finnigan MAT Thermoquad (Bremen, Germany) mass spectrometer. After precipitation of the Ca with ammonium oxalate, each sample was analyzed for the ratio of $^{44}Ca/^{48}Ca$ and $^{46}Ca/^{48}Ca$. A total of four blocks of 10 scans each were obtained and the median enrichment of the four blocks determined (14–16). The relative SD of this median for enriched samples was <1%. For nonenriched samples, this technique yields results for the naturally occurring ratios within 1.5% of the accepted ratio as determined by the National Institute of Standards and Technology (18).

Calculations. The mathematical methods for the calculation of V_a using a dual tracer stable isotope technique have been described previously (14–16). The dynamics of the system are depicted in Figure 1. The true fractional absorption of Ca (alpha) is calculated as the ratio of the accumulated oral *versus* i.v. tracer in urine during the 24 h after tracer administration.

$$\text{alpha} = \frac{\int_0^t \text{oral dose in urine}}{\int_0^t \text{i.v. dose in urine}}$$

V_a is then calculated from alpha and V_i as:

$$V_a = \text{alpha} \cdot V_i$$

The method for the direct calculation of V_f after bolus administration of an i.v. tracer is adapted from the description of Aubert *et al.* (11, 12) from studies using radiotracers. We have recently described the application of this method to studies in children (3 to 14 y of age) using Ca-stable isotopes (17). In this technique, V_f and V_u are assumed to occur from a single central pool. This pool is believed to consist of plasma and some components of extracellular fluid and surface bone. Ca flow to stable bone from the central pool may involve intermediate, kinetically determined bone pools; however, these do not affect the calculation of V_f or V_u (19–21). With this assumption, the ratio V_f/V_u is equal to the ratio of the accumulated tracer

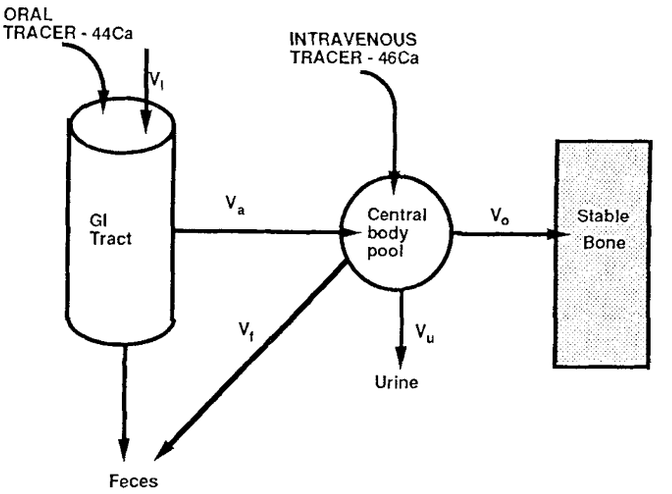

Fig. 1. Model for Ca distribution in LBW infants. The true fraction of dietary Ca (V_i) absorbed is represented by V_a. Ca secretion into the gastrointestinal tract may occur at separate sites from absorption. V_f represents secretory losses into the stool. Urinary Ca excretion is shown as V_u. Net Ca retention with transfer to stable bone from a central rapidly exchanging pool (presumed to consist of plasma, extracellular fluid, and surface bone) (21) is represented by V_o. Ca resorption from stable bone in LBW infants is assumed to be negligible during the time course of the study.

recovered in the stool compared to the tracer recovered in the urine. This can be expressed as follows:

$$V_f = \frac{\int_0^t \text{i.v. dose in stool}}{\int_0^t \text{i.v. dose in urine}} \cdot V_u$$

A period of time (t) is chosen such that the recovery of the tracer has neared "completeness," that is, the curve of accumulated tracer recovery has achieved an asymptotic value. From Figure 1, it can be seen that the net Ca retained and subsequently transferred to deep bone, V_o, is equal to the difference between V_a and the sum of all losses. Assuming negligible dermal losses, this leads to:

$$V_o = V_a - (V_u + V_f)$$

Relationships among variables were compared by linear regression analysis. All data are expressed as mean ± SD. Coefficients of variation were calculated as the SD divided by the mean (22).

RESULTS

Thirteen patients were enrolled in the study. A single infant developed evidence of sepsis during the study and was excluded. Gestational ages, birth weights, body weights, and ages at the time of the study of the remaining 12 patients are shown in Table 1. One infant (MC) was small for gestational age, weighing 1250 g at birth, 37 wk gestation.

Calcium-stable isotope data for the study infants are shown in Table 2. The results for the small-for-gestational-age infant were similar to the data of the other infants and were included in the analyses. For the 12 study infants, V_a, V_f, V_u, and V_o represented 56 ± 16, 7 ± 4, 2 ± 1, and 48 ± 18%, respectively, of dietary V_i.

V_o was highly correlated to true fractional and total Ca absorption ($r = 0.94$ and 0.98, respectively, $p < 0.001$ for each). V_o was not significantly correlated to V_f ($r = -0.40$, $p = 0.19$). There was no significant relationship between V_o or V_a and birth weight, gestational age, body weight, V_i, body length, head circumference, age at study, age at first feed, feeding method, serum Ca,

Table 1. *Study population*

Patient	Gestational age (wk)	Birth wt (g)	Postnatal age (d)	Body wt (g)
CB	27	776	41	1400
MC	37	1250	15	1563
SC	33	1710	13	2000
JE	33	1615	16	1815
BH	31	1525	19	1659
RH	34	1640	13	1835
NL	30	1365	25	1790
RL	34	1490	13	1528
KM	30	1404	17	1696
RM	31	1417	22	1488
DP	34	1690	8	1703
PP	30	1228	35	1841
Mean	32	1426	20	1693
SD	3	260	10	174

Table 2. *Ca balance in low birth weight infants (mg/kg/d)*

Patient	V_i	V_a	V_f	V_u	V_o
CB	189	141	7	3	131
MC	215	120	9	7	104
SC	216	138	13	7	118
JE	216	62	28	2	32
BH	212	133	17	7	109
RH	242	117	8	1	108
NL	209	134	15	6	113
RL	204	124	33	2	89
KM	226	182	7	2	173
RM	226	83	19	2	61
DP	225	160	25	1	134
PP	216	71	7	4	60
Mean	216	122	15	4	103
SD	13	35	9	2	38
CV*	6	29	60	50	37

* CV = coefficient of variation, expressed as %.

serum phosphorus, alkaline phosphatase activity, or V_u ($r < 0.3$, $p > 0.2$ for each).

DISCUSSION

The dual tracer technique has been demonstrated to be an accurate and reproducible method for evaluating Ca balance in adults and children (14, 15, 21). It allows for the calculation of fractional absorption directly from a 24-h urine collection. The use of stable isotopes also eliminates the need for any radiation exposure to the patient. Furthermore, the use of Ca isotopes allows for kinetic measurements of Ca dynamics (18, 19, 23).

Our results indicate that V_o is primarily related to V_a, not V_f. V_f represented approximately 7% of the infants' dietary V_i and was much greater than their V_u (Table 2). This pattern is different from that observed in adults in whom V_f is approximately 2 mg/kg/d and is usually lower than V_u (24). The reasons for these higher rates of V_f in premature infants are unknown but may be related to gastrointestinal immaturity (9, 21).

That V_o might be related to V_f was suggested by the study of Barltrop *et al.* (9, 10), in which V_f varied from 4–150 mg/kg/d in premature infants (mean 86 mg/kg/d). However, that study used a single orally administered Ca isotope, and V_f was calculated indirectly based on mass balance and fecal isotope recovery data. Using a similar technique, Senterre (25) found much lower rates (17 mg/kg/d) for V_f in LBW infants, as did Moore *et al.* (20) in a small study ($n = 2$) using an i.v. administered Ca isotope to calculate V_f directly. The rate of V_f in LBW infants in this study, 15.5 ± 8.9 mg/kg/d, is similar to the results obtained by Senterre and by Moore *et al.* and much lower than the rate reported by Barltrop *et al.*

The three patients in whom V_f exceeded 10% of intake suggest that some LBW infants may have relatively large secretory losses. Because all patients received comparable V_i, this study did not assess the possible effect of dietary V_i on V_f. However, studies in adults have not shown a close relationship between dietary V_i and V_f (24).

The V_o for patients in this study, 103 ± 38 mg/kg/d, meets the lower estimates of the *in utero* Ca retention (100–130 mg/kg/d) during the 3rd trimester (10). It is below the reports of 130–170 mg/kg/d Ca retention (1, 2, 5–7), but it is somewhat higher than a recent report of approximately 80 mg/kg/d retention with this formula (or a similar earlier formulation) (4).

The coefficients of variation for V_a, 29%, and for V_o, 37% (Table 2), are similar to previous reports (2, 4) and suggest significant intersubject variability in Ca bioavailability. Neither V_a nor V_o were closely correlated to V_f, V_u, anthropometric parameters, or biochemical indices. The r value of −0.40 for the relationship between V_f and V_o suggests that only 16% of the variability in V_o was accounted for by variability in endogenous excretion. These relationships have not been assessed in previous studies.

Isotope methods using an extrinsically added tracer require the assumption that the tracer is adequately equilibrated *in vivo* with the native Ca. Although this appears not to be completely the case *in vitro* for casein-bound Ca (26), this equilibration may occur *in vivo* within the gastrointestinal tract. Two recent studies have provided evidence that this may occur. Eastell *et al.* (15) demonstrated that Ca absorption in adults measured by dual tracer stable isotope studies is comparable to that obtained from mass balance. Liu *et al.* (27) showed that an extrinsic Ca tracer was absorbed similarly to an intrinsic Ca tracer in fortified human milk-fed premature infants.

Although our results support the use of high Ca-containing formulas to achieve *in utero* rates of Ca retention in LBW infants, they do not explain the etiology of the large intersubject variability in Ca retention. We suggest, however, that these variations represent true differences in the ability of LBW infants to absorb Ca, the etiology of which may relate to as yet unidentified physiologic or hormonal factors. Although reports of fractures in LBW infants fed high mineral-containing formulas are rare (28), those caring for such infants must be aware that some infants (Table 1) will retain Ca well below *in utero* rates and may remain at risk for inadequate bone mineralization.

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