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# Feasibility of Using the Stable Isotope $^{25}Mg$ To Study Mg Metabolism in Infants

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**ABSTRACT. The feasibility of using isotopic techniques to study Mg absorption and metabolism was explored in three full-term human infants. $^{25}Mg$ (98.8 atom %) was administered orally as an *in vivo* tracer. Fractional $^{25}Mg$ absorption, isotope retention, endogenous fecal Mg losses, and apparent Mg exchangeable pool size were then determined under three conditions of isotope administration: *1*) 20 mg $^{25}Mg$, with single feeding; *2*) 20 mg $^{25}Mg$, distributed over a 24-h period; and *3*) 60 mg $^{25}Mg$, over a 24-h period. Mg isotope ratios were determined by inductively coupled plasma mass spectrometry. Fractional absorption was increased in all three infants after distributed *versus* bolus administration at the 20 mg dose; mean (±SD) fractional absorption was 64.0 ± 3.9 *versus* 54.3 ± 5.9%, respectively. $^{25}Mg$ retention was also more in all three infants after distributed administration (55.8 ± 3.0 *versus* 44.3 ± 1.3% of dose). At the 60-mg $^{25}Mg$ dose, compared to 20 mg, fractional absorption was reduced but absolute isotope absorption more than doubled in all infants; urine isotope losses represented a similar fraction of the absorbed dose, thus, $^{25}Mg$ retention also more than doubled. Compared to the results of the isotope studies, net Mg absorption and balance were uninfluenced by total Mg intake. Isotope retention with distributed isotope administration resulted in measurable isotopic enrichment of plasma and erythrocytes at 72 h (*i.e.* plasma isotope enrichment was 6.3–10.2 and 19.2–23.5% for the 20- and 60-mg dose, respectively). With these doses, apparent Mg exchangeable pool size ranged from 5.5 to 7.6 mmol/kg body wt; these values showed a decrease with age both within and between infants. Our results indicate that Mg stable isotope studies may offer sufficient accuracy and reproducibility to permit meaningful investigations of Mg bioavailability and developmental changes in Mg metabolism in human infants. (*Pediatr Res* 27: 36–40, 1990)**

### Abbreviations

**ICP-MS, inductively coupled plasma mass spectrometry**
**MgEP, Mg exchangeable pool**

Limited information is available on the absorption and homeostasis of Mg in normal infants. A few studies of net Mg absorption and retention (1–4) and a single study of true absorption, utilizing the radiotracer $^{28}Mg$ (5), have been reported. Important aspects of Mg homeostasis such as developmental changes in Mg absorption and endogenous fecal excretion, source of urinary Mg losses (endogenous or dietary), body Mg pool sizes, etc. need to be explored if we are to understand the requirement for Mg during growth and developmental changes in Mg metabolism. Such studies require the use of isotopic probes. In our investigations we made use of our newly developed techniques for the precise measurement of Mg isotope ratios in biologic samples (6) to test the feasibility of using isotopic techniques to study Mg absorption and metabolism in full-term human infants. More specifically, we investigated the effect of size of isotope dose (20 or 60 mg) and mode of administration (bolus *versus* distributed) on isotope absorption and retention, on tissue isotope enrichment (plasma and erythrocytes), and on the relationship between plasma and urine isotope enrichment.

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## MATERIALS AND METHODS

*Subjects.* The subjects were normal Caucasian term infants (two males, one female) who lived at home and were each admitted to the Lora N. Thomas Metabolic Unit at the University of Iowa on three occasions. The protocol was approved by the University of Iowa Committee on Research Involving Human Subjects. The procedures were fully explained to one or both parents and written consent was obtained. The general care of these infants during the metabolic study was managed as described previously (7).

*Study design.* Three Mg absorption studies were performed in each of the three infants over a 5-mo period. A stable isotope of Mg, $^{25}Mg$, was administered in a single feeding (20 mg $^{25}Mg$) in the first study performed in each infant. In the other two studies, the isotope was fed over a 24-h period at a dose of 20 or 60 mg. The order of administration of the 20- or 60-mg $^{25}Mg$ dose was randomized such that two infants received 20 then 60 mg and one received 60 then 20 mg of isotope; all studies were performed at least 2 wk apart. Information on age, sex, body wt, and dietary Mg intake as well as $^{25}Mg$ dose and mode of administration is given in Table 1.

The 20-mg $^{25}Mg$ dose was estimated to result in a 10 to 20% increase in the $^{25}Mg$ content of the infants' exchangeable Mg pool based on data from the literature regarding the size of this pool in adults (8) and assuming 50% retention of the isotope dose. Five to 10% is the minimum *in vivo* $^{25}Mg$ enrichment required to permit accurate estimation of this body Mg pool by *in vivo* isotope dilution at our present level of measurement precision (9). A 60-mg $^{25}Mg$ dose was also administered because the size of the exchangeable Mg pool may well be several fold greater for infants than adults per kg body wt (10). However, the larger 60-mg $^{25}Mg$ dose was also estimated to nearly double the infants total Mg intake. Thus, this dose was only fed distributed over 24-h to enhance isotope absorption and reduce the possibility of any laxative effect of Mg.

Table 1. *Study population, Mg intake, and $^{25}$Mg dosing information*

| Infant | Sex | Age (d) | Body wt (kg) | Formula fed | Dietary Mg (formula and foods) (mg/d) | $^{25}$Mg dose (mg/mode) |
|---|---|---|---|---|---|---|
| 3898 | F | 192 | 7.13 | Enfamil w/Fe | 84.3 | 18.8/bolus |
| | | 248 | 7.87 | Enfamil w/Fe | 79.1 | 20.0/24 h |
| | | 262 | 8.10 | Enfamil w/Fe | 70.8 | 59.3/24 h |
| 3671 | M | 275 | 8.10 | Isomil 20 | 67.3 | 21.2/bolus |
| | | 289 | 8.14 | Isomil 20 | 70.3 | 61.4/24 h |
| | | 331 | 8.57 | Isomil 20 | 61.0 | 21.9/24 h |
| 3672 | M | 125 | 7.37 | Enfamil w/Fe | 62.8 | 19.1/bolus |
| | | 139 | 7.79 | Enfamil w/Fe | 82.6 | 18.7/24 h |
| | | 153 | 8.32 | Enfamil w/Fe | 76.1 | 59.3/24 h |

*Feedings.* The infants were fed ready-to-feed milk-based formula (Enfamil with iron, Mead Johnson Nutritionals, Evansville, IN) or a formula based on isolated soy protein (Isomil, Ross Laboratories, Columbus, OH) containing approximately 67 kcal/dL. The formula fed during the balance period of the study was fed for at least 11 d before the beginning of the absorption study. Infants over 140 d of age also received modest amounts of commercially prepared strained foods from one company (Gerber Products Co., Fremont, MI). During the balance studies, these foods supplied between 7 and 28% of dietary Mg intake.

*Magnesium absorption.* Metabolic balance studies of 72 h duration were carried out in the manner described previously (7). The stool collection was defined by the appearance of two doses of carmine red given 72 h apart. Collected stools were separated into approximately 24-h periods then homogenized. Urine was collected during the entire 72-h balance period. Urine was pooled into three 8-h collections for the first 24-h followed by two 24-h pools. Pre- and postbalance spot urine and fecal samples were also collected immediately before and after the balance period or carmine excretion, respectively. A single blood sample was taken 72-h after the beginning of $^{25}$Mg administration.

Intake of nutrients was calculated from the weighed intake of foods and the determined nutrient concentrations. Net absorption was calculated as intake minus fecal excretion, and net retention as intake minus total excretion. Food, urine, and fecal samples were prepared for total Mg analysis as described previously (11). Total Mg content was then determined by atomic absorption spectrophotometry (Perkin-Elmer model 5000 or 303, Perkin-Elmer Corp., Norwalk, CT).

*Administration$^{25}$ Mg label.* Highly enriched $^{25}$MgO was purchased from Oak Ridge National Laboratory (Oak Ridge, TN). The isotopic composition of this preparation was (wt%) $^{25}$Mg, 98.84; $^{24}$Mg, 0.93; $^{26}$Mg, 0.23; the natural isotopic abundance of $^{25}$Mg is 10.28 (wt%, 12).

The $^{25}$MgO powder was dissolved in 1 N $HNO_3$, pH adjusted slowly to 6.0, and the solution brought to a known volume with deionized water. Accurately weighed aliquots of this $^{25}$Mg solution were then mixed with infant formula 2–3 h before feeding; the isotope plus formula were fed to the infant quantitatively with several rinses. The amount of $^{25}$Mg solution added resulted in the ingestion of approximately 20 to 60 mg $^{25}$Mg. The 20-mg $^{25}$Mg dose was administered on one occasion as a single feed and on a second over a 24-h period. The 60-mg $^{25}$Mg dose was always administered over a 24-h period. The carmine marker was given with the $^{25}$Mg-enriched formula in the case of bolus administration or with the first $^{25}$Mg-enriched feeding in the case of 24-h administration.

*Isotopic analyses and calculations.* Serum, urine, and fecal samples were prepared for isotopic analysis as described previously (6). Isotope ratio measurements were obtained with ICP-MS using an Elan Model 250 system (SCIEX/Perkin Elmer, Norwalk, CT). We have recently demonstrated that measurement precision (%CV) for the isotope pairs $^{25}$Mg/$^{24}$Mg and $^{26}$Mg/$^{24}$Mg with this method is in the range of 0.1–1% for a number of different biologic matrices (6).

The general concepts and analytical considerations applicable to stable isotope absorption methods, especially the isotope balance approach, have been described previously (13). A detailed description of the calculations specific to the stable isotope studies of this investigation is given in Appendix 1. Briefly the expression for calculating the fractional absorption (F) of the $^{25}$Mg dose is given by the following equation:

$$F = [^{25}Mg^*_{intake} - {}^{25}Mg^*_{feces}]/[^{25}Mg^*_{intake}]$$

Where $^{25}$Mg* designates $^{25}$Mg present in excess of its natural isotopic abundance; therefore, $^{25}Mg^*_{feces}$ represents the portion of the oral $^{25}$Mg dose recovered in the fecal pool. The error in estimation of fraction absorption by this method related to reentry of the absorbed $^{25}$Mg* dose was calculated to represent less than 2%. The total amount of $^{25}$Mg* absorbed was simply calculated as ($^{25}Mg^*_{intake}$-$^{25}Mg^*_{feces}$). Assuming that absorption of dietary Mg was equal to that of the $^{25}$Mg label, the true amount of dietary Mg absorbed was calculated by multiplying the dietary Mg intake by F. Fecal Mg of endogenous origin was then estimated as the difference between total fecal Mg content and unabsorbed Mg of dietary origin (14).

In addition, using the concept of *in vivo* isotope dilution (15), the magnitude of the apparent MgEP at 72 h after isotope dose was calculated for each test period. The size of this exchangable pool is simply the mass of body Mg with which the exogenous $^{25}$Mg* mixed during the 72-h period. Unlike body water or K, not all of body Mg is available for exchange (*i.e.* especially bone Mg) so that the calculated pool (mmol/kg body wt) is less than the value for total body Mg.

## RESULTS

*Isotope studies.* The $^{25}$Mg* absorption, urinary excretion, and retention data as well as estimates of endogenous fecal losses are shown in Table 2 for each of the test periods for all three infants. For ease of comparison, the data for each infant are given in the order 20 mg/bolus, 20 mg/24 h, 60 mg/24 h regardless of the actual order in which the absorption tests were performed; the latter can be discerned from the age of the infant. For each infant, $^{25}$Mg* absorption, expressed as fractional absorption or as total mg absorbed, was more when the 20-mg dose of $^{25}$Mg* was administered over a 24-h period than when it was administered as a bolus. As a result, fractional absorption averaged 64.0 ± 3.9% (mean ± SD) when the 20 mg of $^{25}$Mg* was administered over 24 h *versus* 54.3 ± 5.9% with bolus administration. The greater isotope absorption with 24-h administration did not result in greater urinary isotope losses. Consequently, $^{25}$Mg* retention was also more in each infant when the isotope dose was fed over 24-h. Mean isotope retention (±SD) was 11.27 ± 1.11 *versus* 8.73 ± 0.60 mg or 55.8 ± 3.0 *versus* 44.3 ± 1.3% of administered dose for the 24-h and bolus administration protocols, respectively.

In this series of studies, absorption from a bolus dose of isotope was always tested before absorption from a distributed dose. As such, the infants were always somewhat older and larger for the latter absorption studies. However, the conclusions regarding $^{25}$Mg* absorption for the two modes of administration are changed if the data are corrected for differences in body wt. In addition, no effect of age on Mg absorption was discernible in this feasibility study.

When 60 mg of $^{25}$Mg* was administered over 24-h, fractional absorption was slightly reduced compared to the 20-mg dose

given in the same fashion, but total isotope absorption more than doubled. Urine isotope loss at the higher dose level was absolutely greater (total mg $^{25}$Mg*) but represented a similar fraction of the absorbed dose. As a result, $^{25}$Mg* retention also more than doubled.

An important aspect of this feasibility study was to examine the suitability of the different dose/administration regimes and the 72-h balance period for isotopic studies of Mg absorption. Sufficient isotopic enrichment of the 24-h fecal pools occurred at all $^{25}$Mg* doses for accurate estimation of $^{25}$Mg* excretion (and absorption). With the 20-mg dose, peak $^{25}$Mg enrichment of the 24-h pools ranged from 75 to 220% and all 24-h fecal pools showed some degree of isotopic enrichment. With the larger 60-mg $^{25}$Mg* dose, fecal pool isotope enrichments were much greater with peak values ranging from 370 to 560% $^{25}$Mg enrichment.

In six of the nine absorption studies performed, the isotope ratio ($^{25}$Mg/$^{24}$Mg) of stool samples collected after completion of the 72-h balance period was greater than the natural ratio; percent isotope enrichment was 3% or more. The latter was true for the studies performed in all three infants when 60 mg of $^{25}$Mg* had been administered over 24 h. Isotope enrichment of these stools collected after 72 h could be due to reentry of absorbed $^{25}$Mg* into the intestinal tract, to continued excretion of unabsorbed $^{25}$Mg*, or both. In the case of the 60-mg isotope dose administered over 24 h, only 48 h had elapsed since the end of isotope administration. Excretion of a small amount of unabsorbed $^{25}$Mg* at this time is likely (14, 16). Regardless of the mechanism by which the $^{25}$Mg* reached the feces, the resulting error in estimated fractional absorption was small. In the worst case, isotope enrichment of the postcarmine fecal sample was only 8.4%. Assuming all of this fecal $^{25}$Mg* was from unabsorbed dietary $^{25}$Mg*, for this infant (no. 3898, 59.3 mg/24 h) excreting about 37 mg total Mg/day, $^{25}$Mg* excretion was underestimated by about 0.3 mg and fractional absorption was overestimated by about 0.5% (64.5 *versus* 65.0% absorption).

In addition, with the feeding protocol used in these studies–in which isotope administration coincided with carmine administration–in only one study was any increase in isotope enrichment noted in fecal samples excreted before appearance of the carmine marker. In that single study (no. 3672, 19.1 mg/bolus), fecal $^{25}$Mg* excretion during the balance period was underestimated by about 0.3 mg and, in this case, fractional absorption was overestimated by 1.2% (49.1 *versus* 47.9% absorption).

The use of $^{25}$Mg isotope together with measurement of net Mg balance also allowed estimation of endogenous fecal Mg losses; these values are also shown in Table 2. Values for endogenous fecal losses ranged from 0 to 47.9 and averaged 17.3 mg (or 5.8 mg/day). If we consider only the estimates from the balance periods in which 20 mg of $^{25}$Mg* was administered over 24 h, the situation in which fractional isotope absorption was most likely to reflect true dietary Mg absorption, endogenous losses ranged from 20.3 to 47.9 mg or from 7.9 to 18.0% of intake.

*Comparison of isotope and net balance data.* Data on total Mg intake, net Mg absorption (mg and % of intake), and apparent Mg balance are shown in Table 3 for comparison. Net absorption measured by the balance technique appears unrelated to total Mg intake (food plus $^{25}$Mg*), infant age, body wt, or formula fed in this limited data set. Expressed as percent of intake, net absorption values were lower than observed for the isotope studies due to endogenous losses of Mg. Surprisingly, two of the three infants showed at least one balance period in which little net Mg retention occurred. However, measured nitrogen balances for these same periods were positive (mean of 91 mg N/kg body wt/day in all cases, data not shown).

*Apparent MgEP size.* Data on the $^{25}$Mg enrichment of plasma, erythrocytes, and urine, as well as the calculated size of the apparent MgEP at 72 h, are shown in Table 4 for the two balance periods in which isotope was administered over 24 h. Inasmuch as isotope retention was greatest during these periods, isotope enrichment of plasma and urine at 72 h was >5%, which allowed for more accurate estimates of apparent MgEP size. Both the 20- and 60-mg dose of $^{25}$Mg* resulted in significant isotopic enrichment of plasma and urine that was roughly proportional to the administered dose. The relative enrichments of plasma and urine were similar in all cases and warrant our use of a mean isotope ratio value (plasma and urine) in the estimation of MgEP size. Erythrocytes also showed measurable isotopic enrichment proportional to dose; however, enrichment of erythrocytes at 72 h was less than plasma. The calculated value for the apparent

Table 2. *$^{25}$Mg absorption, urinary excretion, and retention data plus endogenous losses*

| Infant | Age (d) | $^{25}$Mg dose (mg/mode) | $^{25}$Mg absorption (%) | $^{25}$Mg absorption (mg) | Urine $^{25}$Mg (mg) | $^{25}$Mg retention (mg) | Fecal endogenous Mg (mg) |
|---|---|---|---|---|---|---|---|
| 3898 | 192 | 18.8/bolus | 60.8 | 11.5 | 2.87 | 8.58 | 25.2 |
| | 248 | 20.0/24 h | 68.4 | 13.7 | 1.83 | 11.8 | 20.3 |
| | 262 | 59.3/24 h | 65.0 | 38.6 | 6.10 | 32.5 | 16.5 |
| 3671 | 275 | 21.2/bolus | 53.1 | 11.2 | 1.85 | 9.40 | 0 |
| | 331 | 21.9/24 h | 62.5 | 13.7 | 1.73 | 12.0 | 21.0 |
| | 289 | 61.4/24 h | 43.6 | 26.8 | 3.37 | 23.4 | 6.6 |
| 3672 | 125 | 19.1/bolus | 49.1 | 9.4 | 1.17 | 8.22 | 12.9 |
| | 139 | 18.7/24 h | 61.1 | 11.4 | 1.44 | 10.0 | 47.9 |
| | 153 | 59.3/24 h | 55.0 | 32.6 | 4.10 | 28.5 | 5.6 |

Table 3. *Mg intake, net absorption, and apparent Mg balance for 72-h balance period**

| Infant | Age (d) | $^{25}$Mg dose (mg/mode) | Dietary Mg (foods and formula) (mg) | Net absorption (mg) | Net absorption (% of intake) | Net retention (mg) |
|---|---|---|---|---|---|---|
| 3898 | 192 | 18.8/bolus | 253 | 140 | 51.5 | 39 |
| | 248 | 20.00/24 h | 237 | 155 | 60.3 | 44 |
| | 262 | 59.3/24 h | 212 | 160 | 59.0 | 37 |
| 3671 | 275 | 21.2/bolus | 202 | 119 | 53.3 | 23 |
| | 331 | 21.9/24 h | 183 | 104 | 52.2 | 1 |
| | 289 | 61.4/24 h | 211 | 112 | 41.1 | 15 |
| 3672 | 125 | 19.1/bolus | 188 | 89 | 42.9 | 10 |
| | 139 | 18.7/24 h | 248 | 115 | 43.1 | 0 |
| | 153 | 59.3/24 h | 228 | 153 | 53.3 | 41 |

* Total Mg intake from all sources can be calculated as the sum of the amount from the $^{25}$Mg dose plus dietary Mg intake. Net absorption data are given both in terms of the total mg of Mg absorbed during the 72-h balance period and as a fraction of total Mg intake.

Table 4. *$^{25}$Mg enrichment of plasma and erythrocytes and apparent MgEP size*

| Infant | Age (d) | $^{25}$Mg dose (mg/mode) | $^{25}$Mg enrichment (%) Plasma | Erythrocytes | Urine | Apparent MgEP (mmol/kg body wt) |
|---|---|---|---|---|---|---|
| 3898 | 248 | 20.0/24 h | 8.7 | 4.2 | 8.8 | 6.8 |
| | 262 | 59.3/24 h | 23.5 | 10.1 | 28.4 | 6.0 |
| 3671 | 331 | 21.9/24 h | 10.2 | 3.8 | 9.0 | 5.5 |
| | 289 | 61.4/24 h | 19.2 | 7.9 | 20.1 | 5.6 |
| 3672 | 139 | 18.7/24 h | 6.3 | 2.2 | 6.8 | 7.6 |
| | 153 | 59.3/24 h | 20.8 | 7.7 | 23.2 | 6.0 |

MgEP of these infants ranged from 5.5 to 7.6 mmol/kg body wt. In addition, as shown in Figure 1, the size of the apparent MgEP appeared to decrease with age. This was true for both the longitudinal studies performed with single infants as well as between the infants studied.

## DISCUSSION

The major objective of this investigation was to determine the feasibility of using stable isotopes of Mg as *in vivo* tracers to study Mg absorption and metabolism in human infants. Schwartz *et al.* (17) demonstrated that true Mg absorption can be estimated in adult humans using $^{26}$Mg*. More recently, Liu *et al.* (16) reported measurement of true Mg absorption of very low birth wt infants from fortified human milk using both an intrinsic ($^{25}$Mg*) and extrinsic ($^{26}$Mg*) stable isotope tag. We have shown that stable isotopes of Mg can be used as tracers to study Mg absorption, retention, and tissue isotope incorporation in human infants and have examined some of the isotope dosage and sample collection requirements.

The 20-mg $^{25}$Mg* dose, which increased total Mg intake of these infants by 22 to 36%, was absorbed and retained best when administered to the infant with its normal feedings throughout the day. This same dose administered as a bolus was consistently less well absorbed and resulted in greater urinary losses in two of the three infants. Thus, it is likely that distributed administration of the isotope resulted in fractional absorption rates that more closely approximate true dietary Mg absorption. This is an important consideration for studies of endogenous Mg losses that are based on this assumption. Thus, it is likely that endogenous Mg losses were somewhat underestimated during the absorption studies in which bolus administration of isotope was used. The same can be said of the studies performed with the 60-mg $^{25}$Mg* dose in which the isotope dose increased Mg intake on that day by 78 to 87%. It is also worth noting that the fecal pool isotope enrichments were great enough after the 20-mg $^{25}$Mg* dose to suggest that less isotope (*i.e.* 10-mg $^{25}$Mg* dose) could be used for studies of Mg absorption and endogenous secretion.

Greater isotope retention was also observed with the distributed *versus* bolus administration of isotope. This observation is important for future studies of tissue isotope incorporation and estimation of apparent MgEP size. For such studies, achievement of sufficient isotope enrichment *in vivo* is likely to be the limiting factor for human studies. In this regard, the distributed 20-mg $^{25}$Mg* dose resulted in plasma enrichments ranging from 6.3 to 10.2% at 72 h. This represents a level of enrichment 12- to 20-fold greater than measurement precision and should be sufficient to allow accurate estimation of apparent MgEP size (9).

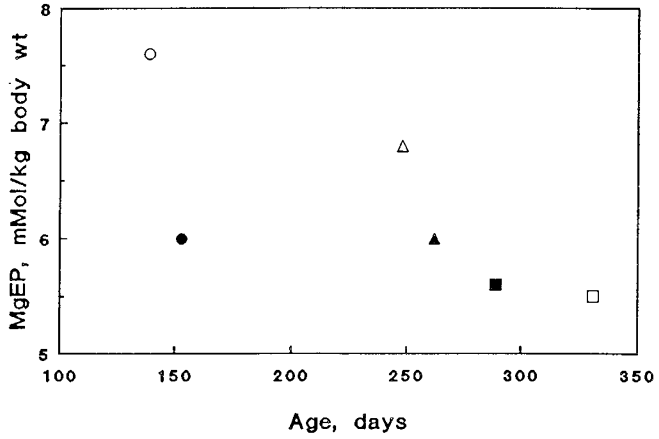


Fig. 1. The relationship between apparent MgEP size (mmol/kg body wt) and infant age (d). For all three infants exchangeable pool size estimates are shown as *open* or *solid symbols* for the 20- and 60-mg $^{25}$Mg doses, respectively. Infants are designated as 3898 (△,▲), 3672 (○,●) and 3671 (□,■).

The 60-mg $^{25}$Mg* dose resulted in proportionately greater plasma and erythrocyte $^{25}$Mg enrichment at 72 h (19.2 to 23.5 and 7.7 to 10.1% for plasma and erythrocytes, respectively). Achievement of isotope enrichment *in vivo* of such magnitude suggests that kinetic studies of Mg absorption and body distribution should be feasible with ICP-MS methodology in the human infant. The 60-mg $^{25}$Mg* dose in these studies did represent about an 80% increase in total Mg intake of these infants, which may not be desirable for such studies. However, it should be possible to circumvent this problem by starting with infant formula reduced in total Mg content and to supplement this formula with $^{25}$Mg* or $^{26}$Mg*.

Perhaps the most encouraging aspects of these feasibility studies were the consistency of the responses seen and the fact that the observations made "biologic sense." Fractional absorption was consistently less after bolus *versus* distributed administration of the same dose of isotope. Similarly, fractional absorption was less after the 60- *versus* 20-mg $^{25}$Mg* dose in all three infants. This is expected if Mg absorption occurs in part by a carrier-mediated process (18). It is recognized that the fecal isotope balance approach suffers from some of the same nonrandom errors that occur in standard metabolic balance studies. However, our results demonstrate that this approach offers a degree of consistency that should permit studies of Mg bioavailability and developmental changes in Mg absorption that would not be possible with standard balance methodology.

$^{25}$Mg* retention data were also consistent between infants relative to effect of mode of administration and dose received; retention was always greater with distributed *versus* bolus administration of isotope at the same dosage and more than doubled in each infant when $^{25}$Mg* intake increased from 20 to 60 mg. Apparent Mg absorption and balance for the same collection periods varied considerably within and between infants; a consistent increase in the amount of Mg absorbed or retained was not observed even with the 60-mg $^{25}$Mg* supplement.

Such variability in net absorption and balance data is not unique to our study. For example, Dauncey *et al.* (3) studied Mg absorption and retention by low birth wt infants and reported that mean apparent absorption was 43% and mean retention 25% of intake; however, the range in absorption was -15 to 48%. Given that these infants ranged in postconceptional age from 27 to 38 wk, it is difficult to know whether the variability in absorption and retention observed was due to developmental changes or the balance technique or both. There was a tendency for mean Mg retention to increase with age but the variability was large and no statistical evaluation was presented. Similarly, Ziegler and Fomon reported (11), in a study of the effect of lactose on mineral utilization in term infants, that Mg retention was (mean ± SD, $n$ = 11) 15 ± 8 or 13 ± 8% of intake for formula containing or not containing lactose, respectively.

Finally, the calculated magnitude of the apparent MgEP size per kg body wt decreased with age both within and between the three infants. Although the biologic significance of changes in this parameter are unknown at present, such a change is compatible with known changes in body composition and bone metabolism during infancy. The percent of body wt as water, as well as intracellular water content, declines from the age of 3 mo to 1 y, the age range of the infants in this investigation (19). Inasmuch as soft tissue Mg is primarily intracellular in distribution, this change in composition may be associated with decreased exchangeability of soft tissue Mg. In addition, the rate of bone turnover and remodeling also decreases as the infant ages (20). The effect of age on the exchangeability of bone Mg has not been studied in humans, but in rats it shows a marked age dependence of more than 5-fold greater in rats 30 d old than in rats 60 d old (10).

In conclusion, this investigation represents the first attempt to study true Mg absorption and endogenous fecal losses in full-

term human infants. Our results demonstrate the feasibility of such studies and indicate that Mg stable isotope studies may offer sufficient accuracy and reproducibility to permit meaningful investigations of Mg bioavailability and developmental changes in Mg homeostasis. Because these were feasibility studies involving only three infants, few conclusions can be drawn about the absolute fractional absorption or endogenous secretion rates observed. However, it is worth mentioning that the mean 64.0% fractional absorption (20 mg $^{25}$Mg*/24 h) is higher than observed for adults (44.3%, 21; 46.6%, 22) and lower than observed for premature infants (86%, 16) under roughly comparable experimental conditions. Little information on endogenous Mg losses is available even for adults. Avioli and Berman (23) reported losses ranging from 6 to 36 mg/d in 15 adult subjects. The values observed in these infants (20 mg $^{25}$Mg*/24 h) of from 6.8 to 16.0 mg/d are similar in absolute amount to the adult values and much greater if expressed per kg body wt or of % total Mg intake. This latter observation, however, needs further confirmation because of the small number of infants studied and the relative imprecision of this method for estimating endogenous losses.

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## APPENDIX 1

The calculations involved in the estimation of fractional absorption, isotope retention, and apparent Mg exchangeable pool size are described below. For these experiments, isotope ratio measurement, determined by ICP-MS, was combined with elemental Mg analysis, measured by atomic absorption spectrophotometry. In the following equations, R designates the isotope ratio expressed on a weight basis for the isotope pair $^{25}$Mg/$^{24}$Mg and R° designates the natural ratio of this same isotope pair (0.1319, 12).

The formula for calculation of $^{25}$Mg* (excess above natural level) present in the fecal pool is derived as follows:

$$R = R° + (^{25}Mg^*/^{24}Mg) \quad (1)$$

and

$$Mg = (1.2829)^{24}Mg + {}^{25}Mg^* \quad (2)$$

Where: R is the isotope ratio ($^{25}$Mg/$^{24}$Mg) of the *in vivo*-enriched fecal sample; $^{25}$Mg* is the amount of $^{25}$Mg originating from the oral $^{25}$Mg* dose that is recovered in the stool collection; $^{24}$Mg is the $^{24}$Mg content of the stool collection, and Mg is the total elemental Mg content of the sample determined by atomic absorption spectrophotometry. Inasmuch as $^{24}$Mg is 77.95% of natural Mg (12), then $(1.2829)^{24}$Mg equals the total amount of Mg in the stool collection of natural isotopic composition.

Solving equations (1) and (2) yields:

$$^{25}Mg^* = [Mg(R - R°)]/[1.2829 + (R - R°)]$$

The small contribution of $^{24}$Mg from the $^{25}$Mg dose was ignored in this calculation after determining that this simplification introduced no significant source of error.

The same expression was used to calculate the $^{25}$Mg* content of urine samples, with the following modification. If the prebalance spot urine isotope ratio ($R_{baseline}$) was greater than R° (*i.e.* for second or third experiment in a single infant), then the value for urinary $^{25}$Mg* due to the recently administered dose had to be corrected for baseline isotope of enrichment by subtraction $^{25}Mg^*_{baseline}$. Once urinary $^{25}$Mg* excretion was determined, then $^{25}$Mg* retention was calculated as the difference between $^{25}$Mg* dose absorbed and that excreted in the urine. In addition, the increase in the $^{25}$Mg* content of fecal, urine, or blood samples was also expressed as %$^{25}$Mg enrichment in some instances. Percent $^{25}$Mg enrichment was calculated using the following expression:

$$\%^{25}Mg \text{ enrichment} = [(R - R_{baseline})/R_{baseline}] \times 100$$

Where R = isotope ratio of the enriched sample and $R_{baseline}$ = isotope ratio of the baseline sample.

Lastly, the magnitude of the apparent MgEP at 72 h after isotope dose was calculated as follows:

$$\text{apparent MgEP} = (^{25}Mg^*_r)/[1.2829(R_{P72} - R_{PO})]$$

Where: $^{25}Mg^*_r$ = $^{25}$Mg* dose retained at 72 h; $RP_{72}$ and $R_{PO}$ = R of plasma (or urine) at 72 and O h, respectively, after isotope dose. For these studies, the $R_{PO}$ value used was the isotope ratio from a spot urine taken just before isotope administration; $RP_{72}$ was the average isotope ratio of a plasma sample taken at 72 h and a spot urine obtained at about the same time.