Feasibility of measuring organ magnesium turnover in vivo by continuous feeding of a stable isotope

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The feasibility of measuring organ endogenous magnesium (Mg_{en}) turnover in vivo by continuous feeding of a single stable isotope of Mg was demonstrated in this investigation. Adult CD-1 mice were fed a Mg deficient diet and deionized water with (+Mg) or without (−Mg) added ^{24}Mg (290 μg/mL) for 16 days. The change in organ ^{25}Mg content over time was then accurately determined by in vitro isotope dilution with ^{26}Mg as spike. Organ endogenous Mg content was then calculated as $^{25}Mg_{en}$/0.1028 and exogenous Mg (Mg_{ex}) content from the expression $Mg_{ex} = Mg_{total} - Mg_{en}$. All soft tissues examined in the +Mg group showed significant turnover of Mg_{en} and accumulation of Mg_{ex}. The rate at which this occurred was organ specific. Apparent half-lives for Mg_{en} turnover were 3.83, 4.13, 5.87, and 8.77 days for liver, heart, brain, and skeletal muscle, respectively. Mg restriction resulted in a dramatic decrease in the rate of Mg_{en} turnover with apparent half-lives ranging from 60.3 to 146 days. Brain showed the smallest decrease in Mg_{en} turnover with Mg restriction, and was the only tissue observed to lose a significant amount of total Mg.

Keywords: Mg turnover; Mg deficiency; stable Mg isotopes

Introduction

Previous studies of in vivo Mg exchange and turnover following bolus administration of a Mg tracer have been limited by a number of problems. Investigations employing radioactive ^{28}Mg[1-3] have been restricted in time frame by the short half-life of this isotope (~21.3 hr). Whereas, those performed with stable isotopes of Mg were hindered by the high natural abundance of the minor isotopic forms (^{25}Mg and ^{26}Mg) relative to the currently achievable measurement precision.[4,5] We now report on the application of a new approach which permits long-term studies of Mg turnover in animals without these limitations.

Our new approach makes use of the fact that Mg exists naturally in tissues as a combination of three stable isotopes (^{24}Mg, ^{25}Mg, and ^{26}Mg) occurring in a known ratio. Thus, all organs and cellular compartments are naturally labeled at this ratio. If all dietary Mg is then replaced with a single isotope, theoretically the quantitative disappearance of either of the remaining isotopic forms of Mg from an organ could be followed over time as a measure of Mg turnover. The study reported here was devised primarily to demonstrate the feasibility of this approach.

Materials and methods

Measurement of endogenous Mg turnover—The concept

In the environment and body tissues, Mg naturally exists as three stable isotopes (wt%)[6]: ^{24}Mg (77.95%), ^{25}Mg (10.28%) and ^{26}Mg (11.77%). All three isotopic forms are available as highly enriched (> 98% pure) preparations (Oak Ridge National Laboratories, Oak Ridge, TN, USA).

Theoretically, if all dietary Mg of natural isotopic composition is replaced (at t = 0) with a single isotopic form, isotopic analysis of the whole animal, specific organ, or even subcellular components at times after t = 0 should permit evaluation of the rate at which Mg originally present in the animal (endogenous Mg: Mg_{en}) disappears from various organs and simultaneously the rate at which dietary Mg (exogenous Mg: Mg_{ex}) accumulates. With this approach it should be possible to study the effects of dietary Mg content as well as manipulation of other dietary constituents or host factors on tissue Mg turnover in vivo.

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In the present experiment, we made use of all three isotopic forms of Mg as follows: ^{24}Mg (99.92%) was substituted for dietary Mg of natural isotopic composition; the change in organ ^{25}Mg content was determined over time as our measure of Mg_{en} turnover; and ^{26}Mg (99.72%) was used as an in vitro spike during isotopic analyses to aid in accurate quantification of ^{25}Mg content of samples.

Animal protocol

Forty-five adult male outbred CD-1 mice (Charles River, Raleigh, NC, USA) were used in this experiment. All were fed a semisynthetic Mg-deficient diet (cat #TD8744, Teklad, Madison, WI, USA) supplemented with $MgCl_2$ (360 μg/mL Mg) of natural abundance in their drinking water for 2 weeks; the Mg deficient diet contained 20 μg/g Mg (by analysis). On day 0 of the experiment, three mice were killed and tissue samples taken for baseline isotope ratio determinations. The remaining 42 mice were randomly assigned to either a control group (+Mg) or a Mg restricted group (−Mg). The +Mg group continued to receive the Mg-deficient diet, but was switched to a supplement containing only $^{24}MgCl_2$ (290 μg/mL ^{24}Mg). The −Mg group was fed Mg deficient diet and only deionized water.

Three mice from each diet group were killed on days 1, 2, 3, 5, 8, 12, and 16 of the experiment and selected tissues (liver, heart, thigh muscle, brain, and right femur) were taken for isotopic analyses.* Whole blood was also collected for separation into plasma and packed red cells from the first several mice. This procedure was abandoned when it became clear that it was not possible to collect blood free from significant hemolysis. The latter precluded meaningful measurement of plasma Mg isotope ratios.

The mice were housed in groups (up to 5 mice/cage) and received diet and $MgCl_2$ supplement or deionized water ad libitum throughout. Twenty-four hour food and water (or supplement) intake was determined on days, 1, 4, and 11 of the experiment for each cage of mice. Body weights of individual mice were determined on day 0 of the experiment and just prior to the time each mouse was to be killed. In order to reduce the amount of Mg contamination from environmental sources, we used polyester batting instead of wood chips for the animal's bedding material. Little Mg could be extracted from this material with dilute acid and the mice did not appear to chew on the batting.

Analytical procedures

The semisynthetic diet and all tissue samples (except heart) were prepared for quantitative isotopic analysis as previously described.[7] Briefly, each sample was placed in a beaker and spiked with a known amount of ^{26}Mg and digested with concentrated HNO_3 and H_2O_2. The Mg in an aliquot of digest was then precipitated as $Mg(NH_4)PO_4$ and the precipitate redissolved in dilute acid. In this manner, total Mg content of the diet and ^{25}Mg content of all tissues were determined by in vitro isotope dilution.[7] Because of the small weight of the mouse hearts (~100–120 mg wet wt), samples were digested in conical test tubes using 400 μL HNO_3, 50 μL H_2O_2 and a hot air blower (~125° C). The entire Mg content of the digests was then precipitated in the same test tube. Tissue Mg content results are expressed per g wet wt.

Subcellular fractions (cytosol and particulate) were also prepared from liver samples obtained from one +Mg mouse per time point using standard fractionation techniques.[8,9] A 10% homogenate (wt/vol) was prepared with ice-cold 0.25 M sucrose buffered with 5 mmol/L HEPES (pH 7.4) using a loose fitting Potter-Elvehjem homogenizer. A slow speed centrifugation (770*g* for 10 min) and wash was used to remove nuclei, unbroken cells, and debris. The supernatant was then subjected to high speed centrifugation (100,000*g* for 60 min) to yield a cytosolic fraction and pellet fraction containing a mixture of organelles. Protein content of each fraction was determined by Lowry protein assay[10] using bovine serum albumin (cat# 690-10, Sigma Chemical Co., St. Louis, MO, USA) as standard. Fractions were prepared for quantitative isotopic analysis in a manner similar to that used for whole tissue. ^{25}Mg content of the fractions was expressed as μg ^{25}Mg/fraction after normalization for protein content.

Isotopic analyses were performed by inductively coupled plasma mass spectrometry (Elan Model 250, SCIEX, Thornhill, Ontario, Canada) as described previously.[7] Ion beam intensity ratios were converted to the expected true isotope ratio (wt/wt) by comparison to isotope standards whose ratios covered the range observed for the samples. Measurement precision of this method was typically better than 0.5% for each matrix of interest. Interorgan variability was also generally < 0.5%, with the largest deviation in measured ratio and $MgCl_2$ standard being 1.2%. Thus, the overall accuracy of the isotope ratio measurement was ~1.0%.

All chemicals used in this work were of analytical-reagent grade purchased from various supply houses. Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA) operated at above 10 m Ω.

Calculations and terminology

Two isotope ratios were determined for each sample (wt/wt): $^{24}Mg/^{25}Mg$ ($R_{24/25}$) and $^{26}Mg/^{25}Mg$ ($R_{26/25}$). Organ content of ^{25}Mg (in μg) was then calculated according to Equation 1.

$$^{25}Mg = [a - b(R_{26/25})]/(R_{26/25} - R^{\circ}_{26/25}) \quad (1)$$

Where a = μg of ^{26}Mg in the in vitro spike; b = μg of ^{25}Mg in the in vitro ^{26}Mg spike (0.09% of a); $R_{26/25}$ = isotope ratio of sample containing in vitro ^{26}Mg spike; $R^{\circ}_{26/25}$ = baseline isotope ratio of sample prior to in vitro spiking. For this calculation the assumption made was that $R^{\circ}_{26/25}$ was no different in tissue samples taken on days 1–16 than on day 0. This assumption was tested for liver. The $R^{\circ}_{26/25}$ of unspiked liver samples from +Mg mice sacrificed on day 0 was 1.148 ± 0.005 (compared with 1.145 calculated from natural abundance values)[6] versus 1.144 ± 0.008 for liver samples from +Mg mice killed on day 16.

For each organ, if ^{25}Mg contributions from extra-organ sources were negligible during the course of the experiment, then the measured value for ^{25}Mg (Equation 1) would represent ^{25}Mg of endogenous origin. However, in vivo there were three potential sources from which ^{25}Mg could enter plasma and, thus, be available for uptake by an organ after initiation of ^{24}Mg feeding: the ^{24}Mg supplement, the Mg deficient diet, and organ-organ exchange. The ^{24}Mg supplement was 99.92% pure. The potential contribution to tissue ^{25}Mg content from this source was < 2% of the ^{25}Mg content calculated using Equation 1 for all tissues after 16 days of ^{24}Mg feeding. Tissue ^{25}Mg values were not corrected for this contribution.

On the other hand, the Mg deficient diet contained 20 μg/g

*Unpublished data. In a pre-test of feeding this Mg-deficient diet to adult CD-1 mice, no effect of Mg deficiency on food intake, body weight, or outward appearance was observed for up to 10 days. However, during the actual experiment, one mouse from the −Mg group died on day 12 at sacrifice, but just prior to receiving anesthesia, and another died on day 13. Thus, on day 16 only 2 mice from the −Mg group were killed for tissue samples.

natural Mg, representing a potentially significant source of ^{25}Mg. The contribution of ^{25}Mg from this source was determined as follows for each tissue. First the amount of ^{24}Mg present in a tissue sample in excess of its natural abundance ($^{24}Mg^*$) was calculated, i.e., that originating from the $^{24}MgCl_2$ supplement (see Equation 2).

$$^{24}Mg^* = (R_{24/25} - R^\circ_{24/25})^{25}Mg \quad (2)$$

Where $R_{24/25}$ = isotope ratio of tissue sample; $R^\circ_{24/25}$ = isotope ratio of tissue sample from animals killed on day 0 (prior to ^{24}Mg feeding); ^{25}Mg = organ ^{25}Mg content (Equation 1). This amount of $^{24}Mg^*$ was then expressed as a fraction of the total amount of ^{24}Mg ingested (from supplement intake records) to obtain a retention factor. Using the food intake records and this retention factor, the μg of ^{25}Mg/g tissue originating from retained dietary ^{25}Mg was then calculated.

For this experiment, we used group $^{24}MgCl_2$ supplement and food intake records. As a result, an average correction factor was calculated and applied to data from all mice. However, it would be possible in future experiments to calculate this correction factor for individual animals if individual food and supplement intakes were measured. Application of this correction factor to ^{25}Mg values from tissue samples taken on day 16 of the experiment resulted in corrected ^{25}Mg values that were lower by 2% (femur) to 18% (heart). The effect of this correction factor on the shape of the ^{25}Mg disappearance curve is shown in *Figure 1* for heart. Heart data had the largest correction of all soft tissues. In this first experiment, we applied the same correction factor to tissue ^{25}Mg values from + Mg and − Mg mice. In future experiments it would also be possible to determine specific correction factors for Mg-deficient animals if a known amount, albeit a lesser amount, of ^{24}Mg was fed to these animals as well. After a corrected ^{25}Mg value was obtained, total Mg of endogenous origin could be calculated from the expression $Mg_{en} = {}^{25}Mg_{en}/0.1028$.

The potential magnitude of the effect of organ-organ ^{25}Mg exchange on the observed results is addressed in detail in the next section. Because this was a feasibility experiment, we chose to use mice as our animal model. This had the advantage of reducing isotope costs, but the disadvantage of not allowing the time course of plasma ^{24}Mg enrichment to be accurately determined due to consistent hemolysis in collected blood samples. If plasma isotopic data were available, both efflux and influx rates between plasma and the organs of interest could be determined explicitly as has been recently described by Levenson et al.[11] for Cu using a similar experimental approach. Because this cannot be done for the present experiment, we will discuss the results in terms of apparent turnover of Mg_{en} from the organ of interest.

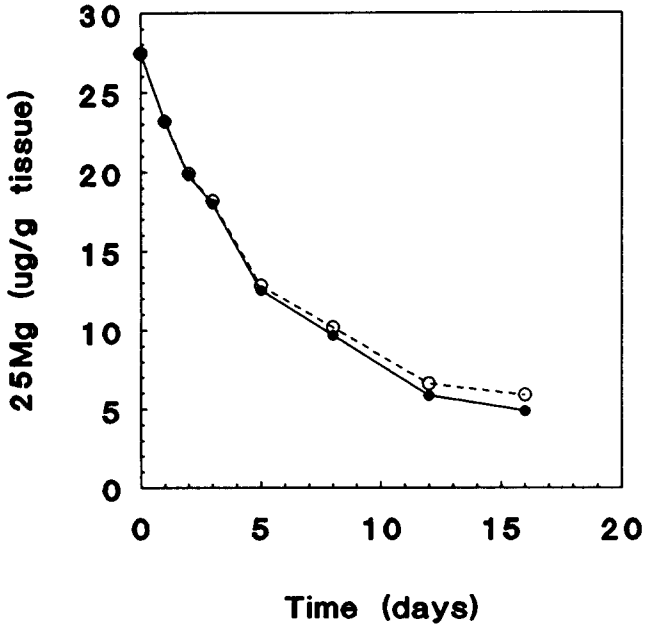

Figure 1 Effect of correcting heart ^{25}Mg content for ^{25}Mg intake from the Mg deficient diet in +Mg mice (○ uncorrected, ● corrected).

Kinetic analysis of apparent Mg_{en} turnover curves was performed by computer-assisted nonlinear regression analysis.[12] Best curve fit was defined as that which gave the lowest Akaike Information Criteria;[13] this definition takes into account the residual sum of squares, number of data points, and the number of parameters estimated.

Knowledge of tissue ^{25}Mg content as well as tissue $R_{24/25}$ for each animal allowed calculation of total Mg content (Mg_{en} + Mg_{ex}) as indicated in Equations 3 and 4. For Mg replete mice:

$$Mg_{total} = {}^{25}Mg + {}^{26}Mg + {}^{24}Mg \quad (3)$$

Where ^{25}Mg = ^{25}Mg content (μg/g) uncorrected for dietary contribution; $^{26}Mg = (^{25}Mg)(1.1449)$; $^{24}Mg = (^{25}Mg)(R_{24/25})$.

For Mg restricted mice

$$Mg_{total} = {}^{25}Mg/0.1028 \quad (4)$$

All data are shown as mean ± SD unless otherwise indicated.

Results and discussion

Over the time course of this experiment, feeding of the Mg-deficient diet without supplementation did not significantly alter body wt or food intake of the adult mice. Body weights of +Mg and −Mg mice averaged 30.8 ± 2.1 and 30.3 ± 2.0 g, respectively. At no time were the body weights between the groups significantly different. Similarly, 24-hr food intakes averaged 5.1 ± 1.2 g/mouse for +Mg animals and 4.7 ± 0.9 g/mouse for −Mg animals and were not different between groups. The lack of effect of Mg deficiency on the body weight of adult rodents is similar to what we observed previously[5] for adult F344 rats that were clearly hypomagnesemic. The CD-1 mice used in the present experiment were ~60 days of age and had reached 90% of their final body weight. Thus, growth suppression was not an effect of Mg deficiency. In addition, no outward signs of Mg deficiency were observed in these adult mice, in keeping with the earlier report of Alcock and Shils.[14] However, the fact that the Mg-deficient diet was very low in Mg (20 μg/g) and that two mice from the −Mg group died between days 12 and 16* indicates that the −Mg mice were Mg deficient. Mg deficiency in mice had been shown to result in convulsive seizures, almost always resulting in death.[14]

The primary purpose of this investigation was to establish whether the approach described in this manuscript can be used to measure Mg turnover of various tissues in vivo. The decrease in Mg_{en} content of liver and muscle with time for mice fed ^{24}Mg (+Mg)

* See asterisked note, p. 39 herein.

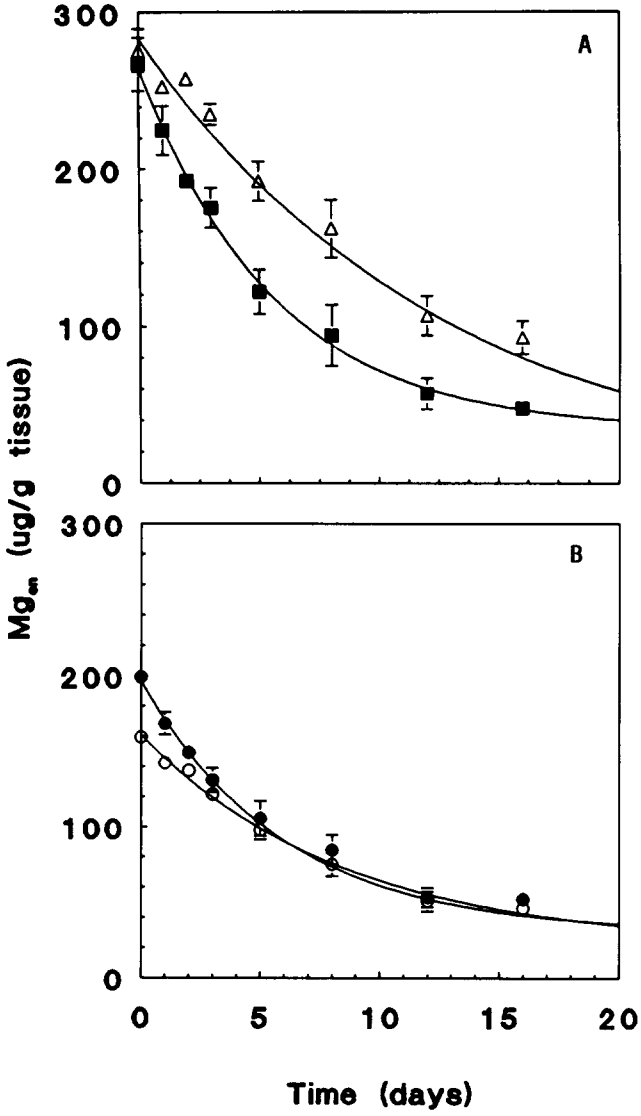

Figure 2a & b (a) Time course of the change in liver (■) and muscle (△) Mg_{en} content (μg Mg_{en}/g tissue) in +Mg mice. (b) Time course of the change in heart (●) and brain (○) Mg_{en} content in the same +Mg animals. All data are shown as mean ± SEM. Expressions for curves fit to the data are those listed in *Table 1*.

is shown in *Figure 2a*. Both tissues showed a nonlinear decrease in Mg_{en} content with time such that at the end of day 16 only 20% to 30% of Mg_{en} remained. The overall rate of decrease in Mg_{en} content was significantly greater for liver than for muscle ($P < 0.01$) suggesting that some component of the apparent endogenous liver Mg turnover was more rapid than that for endogenous muscle Mg. Apparent Mg_{en} turnover data for heart and brain tissue from these same control mice are shown in *Figure 2b*. The first-order kinetic parameters derived from the disappearance curves for all four tissues are summarized in *Table 1*. It is evident from these data that the major portion of intracellular Mg in liver and heart turned over with a half-life of ~4 days. Both tissues, however, also apparently contain a small portion of Mg that was observed not to exchange, at least not over the time course of this experiment. Brain also appears to contain a small portion of nonexchangeable Mg, but the remainder turned over with a half-life of 5.87 days; significantly longer ($P < 0.01$) than observed for liver or heart tissue. In contrast, all of skeletal muscle Mg_{en} was exchangeable, but at a slower rate (8.77 days).

Table 1 Kinetic expressions describing the time course of organ Mg_{en} in +Mg mice

Organ	Expression†	$t_{1/2}$
Liver	$Mg_{en} = 231.6e^{-0.181t} + 33.89$	3.83[a]‡
Heart	$Mg_{en} = 166.5e^{-0.168t} + 30.14$	4.13[a]
Brain	$Mg_{en} = 139.3e^{-0.118t} + 21.67$	5.87[b]
Muscle	$Mg_{en} = 283.5e^{-0.079t}$	8.77[c]

† Mg_{en} is in μg/g tissue and t is in days.
‡ Values within a column with different superscripts are significantly different. Significance was determined by Students *t* test with each individual comparison considered significant if $P < 0.001$, resulting in an overall experimental error level of $P < 0.01$.

Accompanying the decrease in ^{25}Mg content of all the tissues was a concomitant increase in the $R_{24/25}$ ratio observed; this ratio increased from a baseline value of 7.49 ± 0.09 (compared with 7.58 calculated from natural abundance values),[6] to values from day 16 of the experiment of 35.6, 36.6, 26.4, and 26.0 for liver, heart, brain, and muscle, respectively. The resultant changes in tissue endogenous and exogenous Mg content with time are shown in *Figure 3*, expressed as the ratio of Mg_{ex}/Mg_{en}. Clearly endogenous organ Mg was being replaced by dietary ^{24}Mg.

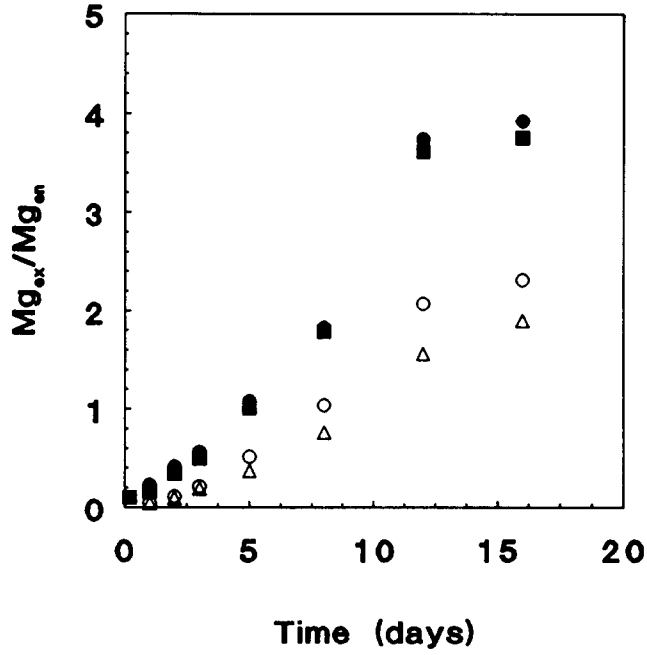

Figure 3 Time course of the change in Mg_{en} and Mg_{ex} content of liver (■), heart (●), brain (○), and muscle (△). Data are expressed as the ratio of Mg_{ex}/Mg_{en} where $Mg_{ex} = Mg_{total} - Mg_{en}$. Mean data only are shown for clarity.

The differences in Mg exchangeability and turnover rate between the soft tissues examined are in keeping with the results of earlier studies which examined tissue isotope accumulation after bolus administration of ^{28}Mg or ^{25}Mg.[1-3,15,16] Liver and heart have been observed to accumulate isotope quickly from the plasma pool achieving specific activities greater than plasma by 12 hr. As evidenced by our results, these tissues have significant pools of rapidly exchangeable Mg. Brain has been observed to take up Mg label somewhat more slowly than liver or heart, but the specific activity of brain was also observed to reach a value greater than plasma by 24 hr. Muscle accumulates Mg isotope from the plasma pool much more slowly and has not been observed to achieve isotope enrichment in excess of the specific activity of plasma for up to 48 hr.

No previous studies of Mg efflux in vivo exist with which to compare the results of the present experiment. Mg efflux from cardiac and liver tissues has been investigated in vitro. Page and Polimeni[16] calculated that the half-life for ^{28}Mg efflux from rat heart was 3 hr based on a 90 min perfusion period; this value was in good agreement with their calculated influx rate, also observed in vitro. Wallach et al.[17] observed in vitro Mg efflux from liver slices to occur with a half-life of approximately 26 min during a 180 min experiment. These calculated half-lives are significantly shorter than observed in the present in vivo experiment.

It is likely that the true half-life for endogenous Mg efflux lies somewhere between the values derived from the two approaches. The ^{28}Mg efflux studies were predicated on the assumption that all relevant cellular Mg pools had equilibrated with the exogenous ^{28}Mg label prior to initiation of the efflux studies (after 20–22 hr in vivo for the studies of Page and Polimeni;[16] 30 min in vitro for the studies of Wallach et al.[17]). Based on the results of the recent in vitro studies of Grubbs et al.[18-20] this assumption may not be correct. The method proposed in the present investigation is not dependent on this assumption since all cellular pools are naturally labeled at a known ratio at the beginning of the experiment. On the other hand, the estimated rates of Mg_{en} efflux determined from the decline in tissue ^{25}Mg content are likely to be underestimates, especially at early time points, due to simultaneous uptake of ^{25}Mg from plasma. The extent of the latter could not be determined in the present experiment due to the lack of plasma data. Plasma isotopic composition would not reflect that of the diet immediately after initiation of ^{24}Mg feeding. In addition, it is possible that there are small pools of intracellular Mg that exchange with more rapid time course than can be observed with this in vivo approach.

We also attempted to determine the apparent Mg_{en} turnover of subcellular fractions prepared from liver tissues. These data are shown in *Figure 4*. Because this aspect of the work was exploratory, a sample of liver from only one mouse per time point was fractionated. The fractionation procedure used yielded a cytosolic fraction and a particulate fraction that was made up of all organelles except nuclei. Both cellular fractions showed significant Mg_{en} turnover apparently with a nonzero equilibrium value similar to the curve observed for whole liver. While there was clearly the potential for Mg isotopes to move between cellular compartments during the isolation procedure, these data suggest that in vivo the turnover of Mg_{en} occurs as rapidly in pools of intracellular Mg contained in organelles as in the cytoplasmic compartment.

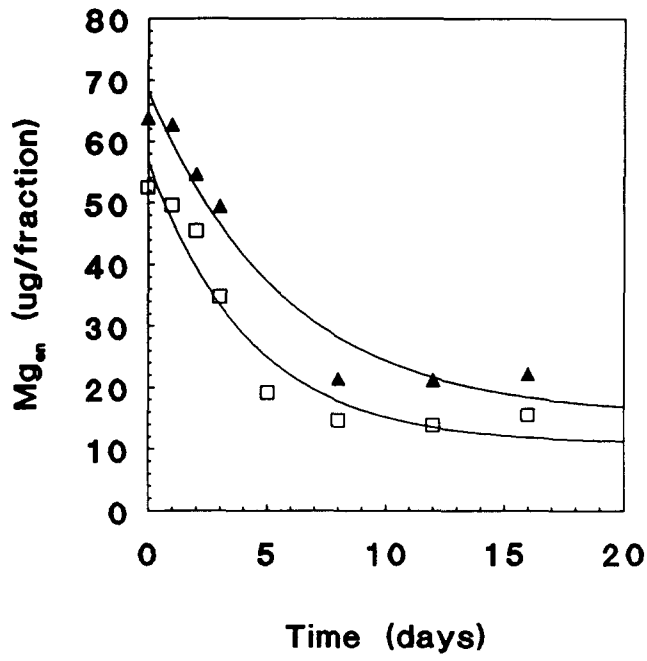

Figure 4 Time course of the change in the Mg_{en} content of cytosolic (▲) and pellet (□) fractions isolated from liver. Data from a single fractionation of liver from one mouse per time point are shown. Data are expressed as total μg Mg_{en}/fraction normalized for total protein content.

Grubbs and Maguire have reported on the intracellular compartmentalization and isotopic exchange of Mg in vitro using S49 lymphoma cells and radioactive ^{28}Mg.[18,19] In contrast to our results, they observed that under nonproliferative conditions only a small fraction of cell Mg (2%–3%) can be exchanged with extracellular Mg at incubation times as long as 16 hr. The size of this exchangeable pool can be increased up to a maximum of 7% by stimulation of Mg influx with 4-β-phorbol 12-myristate 13-acetate (PMA) or by exposure to high levels of extracellular Mg. Under proliferating conditions, up to 24% of cellular Mg was found to exchange with extracellular Mg unrelated to the increase in cell number. Under both conditions (proliferating and nonproliferating), newly transported Mg appeared to remain in the cytoplasmic compartment and to exchange with only a small subcompartment of total cytoplasmic Mg for as long as 40–44 hr. The reason for the apparent discrepancy in observations concerning the exchangeability of Mg contained in organelles between these in vitro results and our in vivo observations remains to be determined, but are likely due,

in part, to differences in the time courses involved. Clearly, however, our preliminary subcellular fractionation results need further refinement and confirmation.

The effect of Mg deficiency on apparent Mg_{en} turnover is shown in *Figure 5* for two soft tissues, heart and muscle. In both tissues, the effect of Mg restriction was to reduce the apparent turnover of Mg_{en} to a very low rate. Similarly, apparent Mg_{en} turnover in liver and brain tissue from −Mg mice decreased dramatically with the half-life increasing from 3.83 to 146 and 5.87 to 60.3 days, respectively.

The very low rate of net Mg_{en} turnover in the tissues of −Mg mice is not unexpected given that Mg deficiency in adult animals generally results in little change in the total Mg content of most soft tissues.[5] In our experiment, brain was the only soft tissue examined to show a slight but statistically significant decrease in total Mg content with time in the −Mg animals. Total Mg content of brain decreased from 157 ± 4 to 140 ± 8 μg/g wet wt over the course of the experiment ($P < 0.001$). Brain tissue was also observed to have the shortest half-life for apparent Mg_{en} turnover in the −Mg animals ($t_{1/2}$ = 60.3 days for brain compared with values ≥130 days for other soft tissues).

What could not be anticipated from the results of previous investigations was the magnitude of the change in apparent turnover of tissue Mg_{en} that occurs during Mg restriction. Theoretically, cellular Mg content could be maintained during the Mg restriction by an increase in influx rate, a decrease in efflux rate, or both. Previous studies of in vitro ^{28}Mg flux have suggested that both Mg influx and efflux rates are influenced by extracellular Mg concentration. Page and Polimeni[16] observed a three-fold decrease in ^{28}Mg efflux from perfused rat heart when the perfusate Mg concentration was changed from 2.8 to 0 mmol/L and a similar 2.6-fold increase in calculated influx. Wallach et al.[17] observed a change in ^{28}Mg influx and efflux from prelabeled slices of liver of similar magnitude when Mg concentration was decreased from 2.5 to 0.5 mmol/L. Our data suggest that, in vivo, the change in flux rates may be considerably greater (10- to 30-fold).

In contrast to what was observed for the various soft tissues, bone (femur) Mg_{en} content decreased significantly ($P < 0.01$) in both +Mg and −Mg mice over the time course of the experiment. Femur Mg_{en} decreased from 2826 ± 74 to 2201 ± 316 μg/g femur in +Mg mice, whereas, total Mg content did not change over the course of the experiment (x = 2893 ± 226 μg/g). In +Mg mice, the decrease in Mg_{en} content was apparently the result of normal bone modeling and surface isotope exchange in which ^{25}Mg was replaced with ^{24}Mg from the diet. Femur Mg_{en} turnover in these mice occurred at a rate of approximately 1.1%/day. In −Mg animals, both Mg_{en} and total Mg content decreased significantly with time (from 2826 ± 74 to 2198 ± 68 and 2826 ± 74 to 2238 ± 64 μg/g, respectively).

In the −Mg mice it was possible to make an estimate of the magnitude of the effect that organ-organ isotope exchange may have had on our estimates of apparent Mg_{en} turnover. For example, femur ^{25}Mg content in the −Mg mice decreased by 22%. In these −Mg animals, if all the ^{25}Mg released from bone (~100 μg*) was taken up by the soft tissues, a turnover rate of approximately 1.3%/day could have been supported by transfer of ^{25}Mg from bone to soft tissue without detection by our methodology. A turnover rate of 1.3%/day for soft tissue from −Mg mice would still represent a reduction of at least 70% to 75% from that observed in +Mg animals. Thus, our results clearly emphasize the dramatic effect Mg restriction had on apparent Mg_{en} turnover.

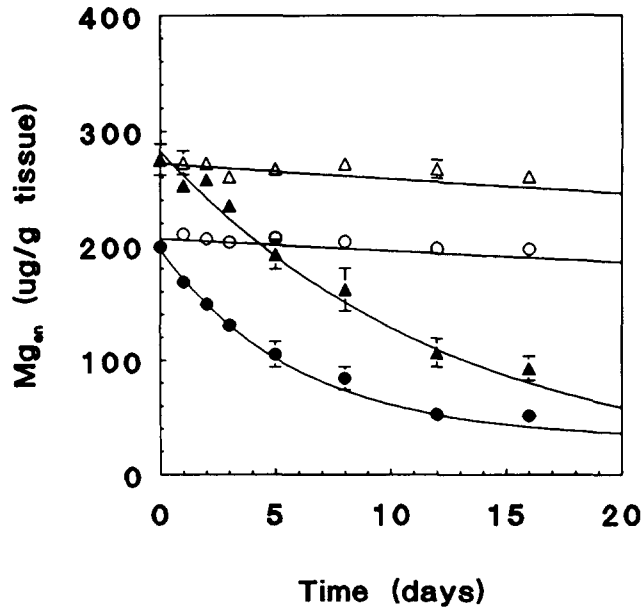

Figure 5 Time course of the change in Mg_{en} content of heart (●, ○) and skeletal muscle (▲, △) from +Mg (closed symbols) and −Mg (open symbols) mice. Data (μg Mg_{en}/g tissue) are shown as mean ± SEM. The increase in half-life of apparent Mg_{en} turnover with Mg restriction was from 4.13 to 131 and 8.77 to 134 days for heart and muscle, respectively.

In conclusion, we have demonstrated for the first time the feasibility of measuring the in vivo kinetics of organ Mg_{en} turnover using the stable isotope approach. Based on the feeding of a single isotopic form of Mg (^{24}Mg), we observed a rapid decline in the ^{25}Mg content of soft tissues concomitant with a rapid increase in ^{24}Mg content. With this methodology it should be possible to follow the effects of level of dietary Mg as well as manipulation of other dietary constituents or host factors on organ Mg fluxes for periods as long as several weeks. In addition, direct measure-

*For the calculation of the total amount of ^{25}Mg released from bone we assumed that femur Mg loss was representative of the whole skeleton and that the quantity of Mg (mg/100g body), as well as the distribution between soft tissue and bone, was the same in mice as had been determined for rats.[5]

ment of tissue Mg_{en} turnover with this approach demonstated that there are tissue specific differences in Mg turnover under normal (Mg replete) conditions and in response to Mg restriction. All soft tissues examined reduced apparent Mg_{en} turnover during Mg restriction by 10- to 30-fold. Brain tissue showed the smallest decrease in Mg_{en} turnover during Mg restriction and was the only soft tissue observed to lose a significant amount of total Mg.

Acknowledgments

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