Effect of Mg nutriture on the dynamics of administered ^{25}Mg exchange in vivo in the rat

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The effect of Mg nutriture on Mg exchange and interorgan distribution was studied in adult rats ten days after a single I.P. dose of ^{25}Mg (~5 mg). First the effects of level of Mg intake (0.25, 0.05, or 0.01% Mg) on standard measures of Mg nutriture were studied for 62d to fully document the Mg status of the adult rats. The Mg-deficient diet led to a reduction in plasma, erythrocyte and urine Mg concentration but the only tissues affected were kidney and bone; no outward signs of deficiency were observed. At this point, the 4 remaining rats from each diet group received a single dose of ^{25}Mg and were killed 10d later. Unlike measures of total Mg content, Mg restriction was observed to significantly alter the distribution of isotope within the soft tissue compartment. The proportion of retained isotope accumulated by soft tissues other than skeletal muscle increased. Because this was not true for skeletal muscle, exogenous ^{25}Mg label was diverted to more metabolically active tissues during Mg restriction. The apparent Mg exchangeable pool (MgEP) size, determined by in vivo stable isotope dilution, reflected this difference in skeletal muscle ^{25}Mg accumulation; MgEP size was 39% lower in Mg restricted (0.01% Mg) compared to control (0.05% Mg) rats. The pool of exchangeable Mg in bone was also reduced by Mg restriction but, unlike the soft tissue compartment, the reduction in bone exchangeable Mg was quantitatively similar to the reduction in total Mg content.

Keywords: Mg status; Mg deficiency; stable isotope ^{25}Mg

Introduction

Despite the importance of Mg to human health, our knowledge of its homeostasis and methods for assessment of status are inadequate.[1] Investigations into the regulation of Mg metabolism have been hampered, in part, by the lack of suitable tracer methodology. Radioactive ^{28}Mg first became available in the late 1950s and its introduction led to a short flurry of interest in studies of Mg homeostasis. However, the use of ^{28}Mg as a radiotracer has two practical limitations: availability and short half-life and issues of internal radiation exposure. Its half-life (21.3h) is much too short for studies requiring complete isotope exchange with soft tissue Mg. For example, if achievement of isotope equilibrium required at least 120h,[2] a 30-fold reduction in activity of the radiotracer would take place over this time period. In addition, the use of radiotracers in studies with humans, especially infants and children, is contraindicated. These two shortcomings impose serious limitations.

Natural Mg consists of three stable isotopes (^{24}Mg, 79%; ^{25}Mg, 10%; and ^{26}Mg, 11%) and both minor isotopes are available as highly enriched preparations. Thus, if methods for precise measurement of isotope ratios ($^{25}Mg/^{24}Mg$ and $^{26}Mg/^{24}Mg$) in biological materials were available, these stable isotopes could be used as biological tracers for Mg. Schwartz and co-workers have demonstrated this usefulness of ^{26}Mg for studies of Mg absorption.[3] In addition, they have reported estimates of the biologic half-life of Mg in normal rats based on ^{26}Mg and ^{28}Mg excretion kinetics. These latter studies, however, were limited in time frame by the relative imprecision of neutron activation for measurement of ^{26}Mg at low isotopic enrichments.[4] We have recently developed a new method for measurement of the stable isotope ratios $^{25}Mg/^{24}Mg$ and $^{26}Mg/^{24}Mg$ in

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biologic material with precision (coefficient of variation, CV) in the range of 0.1–1.0%.[5] The purpose of the present investigation was to study the effect of level of Mg nutriture on ^{25}Mg exchange and interorgan distribution in the rat model and to determine the quantitative correspondence between changes in organ pools of exchangeable Mg and total Mg content.

Materials and methods

Adult male F344 rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) with initial weights of 224 ± 11 g were utilized in this investigation. Animals were fed a purified Mg-deficient diet (*Table 1*; .0085% Mg, wt/wt) to which 5% non-nutritive fiber was added along with sufficient MgO to bring the total Mg content of the diet to the levels indicated below. This diet met or exceeded the nutrient requirements of the rat as designated by the NAS-NRC,[6] based on calculations from the manufacturers information; in addition, the diet contained 1.2% Ca and 0.8% P (by analysis). Rats were housed in groups and fed diet and deionized water ad libitum until shortly before isotope injection. Three days prior to isotope injection, rats were transferred to individual cages for adaptation.

This experiment was conducted in two phases:

Phase I: The purpose of this portion of the experiment was to document the effect of level of Mg intake on conventional measures of Mg status in adult rats. Considerable controversy exists in the literature over the Mg requirements of adult rats.[6] As a result, it was necessary to document the effect of level of Mg intake on tissue Mg content, urine Mg and several hematologic parameters in adult F344 rats prior to administration of Mg isotope (Phase II). A total of 81 adult rats were utilized for the entire experiment (Phase I & II). Five rats were killed and blood and tissue samples were taken for baseline measurements at the beginning of the experiment (day 0). The remaining 76 animals were assigned to one of three dietary treatments: a high (H) Mg diet (0.245 ± 0.004% Mg), a control (C) Mg diet (0.051 ± .001% Mg) or a Mg deficient (D) diet (0.011 ± .001% Mg). Rats were housed in groups and 24h food intakes for each cage of rats (up to 5 rats/cage) were determined every 3 to 5 days. Individual rats were identified by ear punching and individual body weights tracked on the same days as food intake.

Rats from each dietary treatment group were killed on day 10, 20, 30, 45, and 62 of the experiment. The number of animals assigned to each diet and killed at each time point are shown in *Table 2*. At each time point, blood was collected by aortic catheterization into a test tube containing heparin with the rat under anesthesia. The rats were then killed with an overdose of anesthesia and tissue samples were taken for analysis. Tissue samples (heart, right kidney, right femur and thigh muscle) as well as packed red cells were weighed fresh and then frozen for analysis. In addition, hematocrit (%) and red cell counts (# cell × 10^6/mL) were performed on fresh blood samples. For 12h prior to tissue collection, each rat to be killed was housed individually in a plastic metabolic cage and fasted to allow collection of a clean 12h urine sample.

Phase II: The purpose of this portion of the experiment was to determine the whole body retention and interorgan distribution of administered ^{25}Mg isotope in adult rats at various levels of Mg nutriture. At the end of the 62 days of Phase I, the remaining 4 rats from each group were utilized for this portion of the investigation. These animals were placed in individual cages and continued to receive their respective diets and deionized water ad libitum. After three days of adaptation, each animal received 4.66 mg ^{25}Mg (4.71 mg total Mg) by I.P. injection. The rats were returned to their cages and given free access to food and water for the remainder of the experiment. All twelve animals were killed, in random order, 10 days after isotope administration. This time was chosen based on the results from a preliminary experiment.*

Table 1 Composition of Basic Mg deficient diet[1]

Ingredients	Dry weight (%)
Casein, high nitrogen	25.0
Gelatin	5.0
DL-Methionine	0.3
Dextrose	56.7
Vegetable oil	5.0
Vitamin mix[2]	2.2
Mineral mix[3]	6.0

[1] United States Biochemical, Cleveland, OH.
[2] Contained (as g/kg mix): DL-α-tocopherol,5; L-ascorbic acid, 45; choline chloride, 75; D-calcium pantothenate, 3; inositol, 5; menadione, 2.25; niacin, 4.5; PABA, 5; pyridoxine HCl, 1; riboflavin, 1; thiamine HCl, 1; vitamin A acetate, 900,000 units; cholecalciferol, 100,000 units; biotin, .02; folic acid .09, vitamin B_{12}, 0.00135; dextrose, 852.
[3] This supplement provided the following levels of nutrients (g/kg diet): calcium carbonate, 27.63; monobasic calcium phosphate, 6.92; copper sulfate, 0.03; ferric citrate, 2.58; manganous sulfate, 0.37; potassium iodide, 0.08; dibasic potassium phosphate, 6.92; sodium chloride, 15.46; zinc carbonate, 0.023.

Table 2 Diet assignment and number of animals killed at each time point

Diet	Rats per diet group	Time Course: Day 0[1]	10	20	30	45	62	75[2]
			No. of rats killed					
D	34	—	4	4	6	8	8	4
C	24	—	2	2	4	6	6	4
H	18	—	2	2	2	2	6	4

[1] Five rats were sacrificed on this day for baseline measurements.
[2] These animals were fed the indicated diet throughout but then received a single dose of ^{25}Mg isotope on day 65 and were sacrificed 10 days later.

*Unpublished data. Twenty rats were fed an Mg deficient diet (.0085% Mg) for 3d, then 4 were killed for baseline measurements.

At sacrifice, heart, kidney, liver, brain, skeletal muscle, diaphragm and blood samples were taken for analysis of total Mg content and isotope ratio ($^{25}Mg/^{24}Mg$) as described above. In addition, we collected gastrocnemius (white) and soleus (red; 7) muscle samples from the right leg of each animal; isotope ratio measurements only were made for these latter samples. Intestinal contents were rinsed with 0.9% NaCl and the remaining carcass also saved for total Mg and isotopic analysis.

Analytical details

Mg and Ca contents of diets were determined by atomic absorption spectrometry (Model 5000, Perkin-Elmer Corp, Norwalk, CT, USA) after digestion with $HNO_3/HClO_4$[8]; the P content of the same digests was measured colorimetrically.[9] The analyzed values for Ca, Mg, and P in non-fat milk powder (NBS reference material 1549), by these same methods, compared to the certified values were (mg/g): 12.5 ± 0.1 vs. 13.0 ± 0.5, 1.17 ± 0.01 vs. 1.20 ± 0.03 and 10.6 ± 0.1 vs. 10.6 ± 0.2, respectively.

Tissue samples were prepared for measurement of isotope ratios and total Mg content as described previously.[5] Erythrocytes were separated from plasma by centrifugation. After removal of plasma and layer containing white blood cells, a weighed sample of erythrocytes was taken for digestion. Rat carcasses were separated into hair and skin, soft tissue and skeletal components, by careful dissection with forceps and scalpel after partial cooking in a microwave oven. Carcass components were first pre-digested with concentrated HNO_3 at room temperature for 7 to 14 days. Aliquots of the predigested samples were further wet ashed with HNO_3, H_2SO_4 (not bone) and H_2O_2. The skin and soft tissue components contained significant lipid. As a result, quantitative analysis of total Mg content of these components was performed by in vitro isotope dilution as described previously[5]; ^{26}Mg (enough to achieve at least a 100% enrichment) was added as the in vitro spike prior to the initial room temperature digestion with HNO_3.

Isotope ratio measurements were made by inductively coupled plasma mass spectrometry (ICP-MS) using an Elan Model 250 system (SCIEX, Thornhill, Ontario, Canada). The measured ion beam intensity ratios were converted to the expected true isotope ratios (wt/wt) using a calibration procedure with stable isotope standard solutions as described previously.[5] This method is capable of measurement of the ratios $^{26}Mg/^{24}Mg$ ($R_{26/24}$) and $^{25}Mg/^{24}Mg$ ($R_{25/24}$) in biological samples with analytical precision (± 1CV) in the range of 0.1–1.0% without a measureable matrix effect.

In tissue or urine samples enriched in vivo with ^{25}Mg, isotope excess ($^{25}Mg^*$) was calculated as follows:

$$^{25}Mg^* = Mg[a(R_{25/24}) - b]/[a(R_{25/24}) + (1\text{-}b)]$$

where Mg = the total Mg content of the sample; a = the proportionality constant relating the natural weight abundance of ^{24}Mg to total Mg; b = the proportionality constant relating the natural weight abundance of ^{25}Mg to total Mg.[10]

All chemicals used in this work were of analytical-reagent grade, purchased from various supply houses. De-ionized water was obtained from a Milli-Q system (Millipore, Bedford, MA) operating at above 10 mΩ.

Statistical Analysis

For parameters that were determined throughout the experiment (Phases I & II, *Table 3*), data were first analyzed by 2-Way ANOVA (diet and time) using the procedure described by Armitage for non-orthogonal two-way tables[11]; individual means were then compared using Sheffe's or Tukey's multiple comparisons test[12] and the overall MS_{error} term. For comparisons made between groups within the isotope portion of the experiment only, the data were analyzed by 1-Way ANOVA followed by mean comparisons made by use of Tukey's multiple comparisons test (*Tables 4* and *5*). Unless otherwise stated all data are presented as mean ± SD.

Results

Growth rate and food intake of the adult rats used in this investigation were unaffected by dietary treatment; final body weight (g) and average food intake (g/day) for the H, C and D treatment groups were (mean ± SEM) 327 ± 5 and 21.5 ± 1.2, 322 ± 4 and 21.5 ± 1.4, and 325 ± 1, and 22.0 ± 1.5, respectively. In addition, the animals fed the D diet showed no outward signs of Mg deficiency (such as priapism, hyperemia of extremities or skin lesions).

The effect of level of Mg intake on a number of commonly monitored parameters of Mg nutriture over the time course of the study was determined. Plasma and fasting urine Mg concentrations were affected most by dietary Mg intake (*Table 3*). In rats fed the D diet plasma Mg levels declined throughout the study to a final level of 0.30 ± 0.02 mmol/L; a value significantly less than observed for the rats fed the C or H diets. Fasting urinary Mg excretion paralleled dietary intake throughout and was approximately 5 and 25-fold less in the rats fed the D diet compared to the C and H diets, respectively. In contrast to these results, hematocrit values, erythrocyte counts and plasma Ca levels (data not shown) were unaffected by Mg intake. Erythrocyte Mg content decreased over time only in those animals fed the D diet; compared to the baseline value of 58.4 ± 4.1, erythrocyte Mg content was 36.9

Sixteen rats received 3.62 ± 0.08 mg ^{25}Mg by I.P. injection; 4 rats were killed 48, 72, 96, and 120 hours after dosing. At 120 hours, the isotope ratio ($^{25}Mg/^{24}Mg$) of heart, liver, kidney, brain, and RBCs were similar and all slightly (5 to 8%) greater than plasma, but that of skeletal was still significantly less than plasma (~6%). Extrapolation of tissue isotope ratio versus time curves from this experiment suggested that 10d should be sufficient time for all soft tissues to reach isotopic equilibrium with plasma.

Table 3 Effect of level of Mg intake on various parameters of Mg nutriture[1,2]

Parameter	Dietary	Day 0[1]	10	20	30	45	62	75
Plasma Mg	D		0.62 ± 0.06[a]	0.48 ± 0.06[a]	0.46 ± 0.14[a]	0.37 ± 0.03[a]	0.37 ± 0.05[a]	0.30 ± 0.02[a]
(mmol/L)	C	0.74 ± 0.06[3]	0.97(1)[4,b]	0.91(1)[b]	0.73 ±0.07[b]	0.64 ± 0.05[b]	0.63 ± 0.04[b]	0.66 ± 0.05[b]
	H		0.88(1)[b]	0.82(1)[b]	0.92(1)[b]	0.84 ± 0.02[c]	0.81 ± 0.03[c]	0.75 ± 0.03[b]
Urine Mg	D		0.02 ± 0.02[a]	0.17 ± 0.05[a]	0.11 ± 0.04[a]	0.15 ± 0.04[a]	0.16 ± 0.06[a]	0.07 ± 0.02[a]
(mg/12h)	C	2.26 ± 0.45	0.40 ± 0.22[a]	0.77 ± 0.32[a]	0.48 ± 0.19[a]	0.45 ± 0.02[a]	0.77 ± 0.13[b]	0.62 ± 0.19[b]
	H		1.47 ± 0.11[b]	2.96 ± 0.06[b]	2.48 ± 0.58[b]	2.28 ± 0.58[b]	1.99 ± 0.65[c]	2.38 ± 0.48[c]
Heart Mg	D		227 ± 8	—[5]	226 ± 10	—	225 ± 8	218 ± 13
(ug/g wet wt)	C	212 ± 12	227 ± 8	—	218 ± 4	—	226 ± 9	220 ± 9
	H		234 ± 4	—	219 ± 14	—	230 ± 10	225 ± 5
Kidney Mg	D		209 ± 4[a]	—	194 ± 10[a]	—	198 ± 7[a]	199 ± 7[a]
(ug/g wet wt)	C	217 ± 16	223 ± 7[a,b]	—	204 ± 4[a,b]	—	205 ± 9[a,b]	207 ± 6[a,b]
	H		233 ± 13[b]	—	212 ± 10[b]	—	216 ± 9[b]	216 ± 4[b]
Skeletal Muscle Mg	D		282 ± 19	—	300 ± 17	—	286 ± 5	290 ± 4
(ug/g wet wt)	C	278 ± 8	282 ± 1	—	297 ± 9	—	289 ± 14	290 ± 6
	H		292 ± 31	—	323 ± 11	—	280 ± 12	303 ± 9
Right femur Mg	D		2.79 ± 0.06[a]	—	2.19 ± 0.13[a]	—	2.17 ± 0.11[a]	2.03 ± 0.07[a]
(mg/g tiss)	C	3.21 ± 0.11	2.95 ± 0.37[a]	—	2.73 ± 0.14[b]	—	2.87 ±0.25[b]	2.86 ± 0.08[b]
	H		3.33 ± 0.19[a]	—	3.03 ± 0.05[b]	—	3.09 ± 0.16[b]	3.11 ± 0.15[b]
Right femur Mg	D		1.45 ± 0.04[a]	—	1.50 ± 0.03[a]	—	1.52 ± 0.11[a]	1.53 ± 0.06[a]
(total mg)	C	1.41 ± 0.08	1.52 ± 0.11[a]	—	1.73 ± 0.14[b]	—	1.93 ± 0.22[b]	2.03 ± 0.17[b]
	H		1.66 ± 0.16[a]	—	1.78 ± 0.11[b]	—	2.12 ± 0.10[c]	2.26 ± 0.06[c]

[1] Data are expressed as mean ± SD.
[2] Data were analyzed by 2-Way ANOVA. For only those parameters with a significant diet effect, the mean values were compared using Scheffe's or Tukey's multiple comparisions test. Means in any column with different superscripts are statistically different ($P < 0.05$).
[3] Baseline values for 5 rats killed prior to initiation of dietary treatment are shown in this column.
[4] Indicates the number of animals from which this value was derived if different from the number of animals killed on that day (*Table 2*). Hemolyzed blood samples were not used for plasma Mg determinations.
[5] Analysis not performed on animals killed on this day.

± 1.0, 52.7 ± 2.8 and 53.9 ± 1.5 ug/g packed cells in those rats fed the D, C, and H Mg diets, respectively, for 75 days.

In spite of the low plasma Mg level in the rats fed the D diet, of the soft tissues examined (heart, skeletal muscle, and kidney) only kidney showed a significant reduction in total Mg concentration. This slight reduction in Mg content, however, was not accompanied by an increase in kidney Ca content; for example, right kidney Ca content averaged 33 ± 2 ug/g wet weight for all rats killed on day 62 of the experiment. Total Mg content of liver, brain, and diaphragm taken from animals killed at the end of the isotope portion of the experiment (Phase II, day 75) were also determined and found to be unaffected by dietary treatment; Mg content averaged 239 ± 8, 157 ± 5, and 244 ± 15 ug/g wet weight for liver, brain and diaphragm, respectively. These results are unaltered by the expression of organ Mg content on a per whole organ basis (where appropriate) since there were no effects of dietary treatment on organ weight.

Femur Mg content, expressed as mg/g tissue or total mg, was influenced by Mg intake. By either measure, femure Mg content of rats fed the D diet was

Table 4 Effect of level of Mg intake on carcass Mg content[1]

Diet	Non-Skeleton[2] Soft Tissue	Skin + Hair	Sum	Skeleton[3]	Total Carcass
D	12.2[a,4]	2.3[a]	14.5[a]	11.6[a]	26.1[a]
	(0.3)	(0.4)	(0.2)	(0.1)	(0.2)
C	13.5[b]	2.4[a]	15.9[b]	14.5[b]	30.4[b]
	(0.2)	(0.4)	(0.3)	(0.2)	(0.4)
H	13.8[b]	2.9[a]	16.7[c]	16.3[c]	33.0[c]
	(0.7)	(0.3)	(0.4)	(0.8)	(0.7)

Note: Data are given as mg/100g body wt.
[1] Values are expressed as the mean (± SD) for 4 rats/diet group.
[2] Determined by in vitro isotope dilution (5) plus the sum of the mg Mg present in all organs analyzed separately.
[3] Determined by atomic absorption spectrophotometry.
[4] Within a column, means with different superscripts are statistically different ($P < 0.05$).

Table 5 Calculated size of the MgEP and total carcass Mg content[1]

Diet	MgEP[2]	Carcass Mg
D	16.6[3,a]	26.1[a]
	(0.6)	(0.2)
C	27.2[b]	30.5[b]
	(1.7)	(0.4)
H	44.4[c]	33.0[c]
	(8.9)	(0.7)

Note: Data are given as mg/100g body weight.
[1] Data shown as mean (± SD) for 4 rats/diet group.
[2] MgEP = apparent Mg exchangeable pool size, calculated by in vivo isotope dilution using the plasma $R_{25/24}$ at 10d and total μg $^{25}Mg^*$ label retained.
[3] Within a column, means with different superscripts are statistically different ($P < 0.05$).

significantly less than that of rats fed the C or H diets. Total femur Mg (mg) was also slightly greater in the rats fed the H compared to C diet. The reduced femur Mg content of rats fed the D diet did not result in altered mineralization, at least as measured by femur Ca content, since Ca content was the same between all diet groups; femur Ca content (mg Ca/g and total mg) was 182 ± 8 and 133 ± 3, 190 ± 2 and 135 ± 8, and 185 ± 11 and 139 ± 6 for the H, C, and D treatment groups, respectively.

The effect of level of Mg intake on the components of total carcass Mg content—soft tissue, skin and hair, and skeleton—are shown in *Table 4* (determined on rats from Phase II only). Total soft tissue Mg content was reduced in rats fed the D diet compared to rats fed either the C or H diet. When the Mg content of skin and hair was added to this value a small, but statistically significant, difference in total non-skeletal Mg content was also observed between the H and C dietary treatment groups. As seen for individual tissue Mg concentrations, skeletal total Mg content was most affected by level of Mg intake. Compared to the rats fed the H diet, the Mg content of the total skeleton of rats fed the C or D diets was reduced by 11 and 29%, respectively. Skeletal Mg content, thus, made up 49, 48, and 44% of total carcass Mg in the rats fed the H, C, and D diets, respectively.

Data on the tissue retention of ^{25}Mg label ($^{25}Mg^*$) 10 days after isotope dose (Phase II) are shown in *Figures 1* and *2*. Total $^{25}Mg^*$ retention (% of administered dose) increased with decreasing Mg intake from 13.9 ± 1.7 to 26.9 ± 0.9 to 39.7 ± 0.3% for the rats fed the H, C, and D diets, respectively. The distribution of retained $^{25}Mg^*$ among the various body tissue compo-

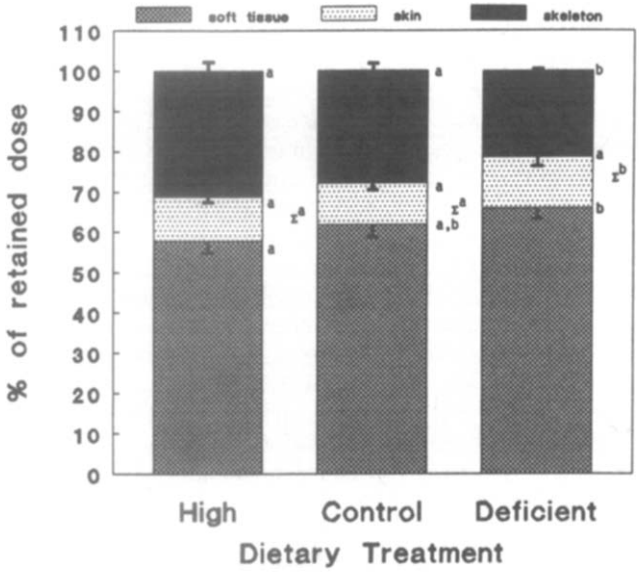

Figure 1 Distribution of retained $^{25}Mg^*$ among the major carcass components (mean ± SD). The Σ sign indicates the combined value for skin plus soft tissue. Statistical comparisons were made between dietary treatment groups for each carcass component; values with different superscripts are significantly different ($P < 0.05$).

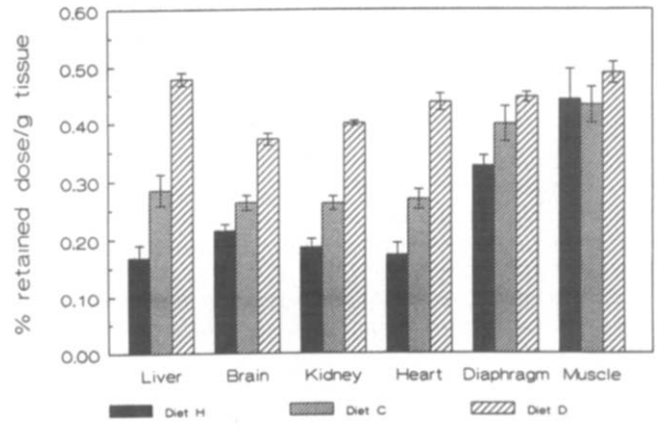

Figure 2 Soft tissue $^{25}Mg^*$ accumulation (% retained dose/g tissue) as influenced by level of dietary Mg; values are shown as mean ± SD. All tissues except skeletal muscle showed a significant diet effect ($P < 0.001$) with the means for all three treatments being significantly different ($P < 0.05$).

nents was also influenced by the level of Mg intake. On the whole animal scale (*Figure 1*), retention of $^{25}Mg^*$ by non-skeletal tissue made up a similar proportion of the total dose retained for those animals fed the H and C diets (69 and 72%, respectively). In the rats fed the D diet, this proportion increased significantly (to 79%). Conversely, the proportion of $^{25}Mg^*$ retained by the skeleton was significantly reduced in those rats fed the D diet (21 vs. 28 and 31%, respectively, for the D, C, and H diets).

Even within the soft tissue compartment, not all organs responded similarly to level of Mg intake. As shown in *Figure 2*, the proportion of retained $^{25}Mg^*$ accumulated by liver, brain, kidney, and heart (per g tissue) was quantitatively similar in control animals (Diet C). In addition, accumulation by these tissues responded similarly to alterations in Mg intake; i.e., accumulation decreased in these tissues from rats fed the H diet and increased in rats fed the D diet. In contrast to these tissues, skeletal muscle accumulated proportionately more $^{25}Mg^*$ in animals fed the C diet but accumulation was not enhanced by Mg restriction. This was true whether we analyzed a mixed muscle type sample (thigh muscle) or more specifically white and red muscle. In fact, the ratio of skeletal muscle to plasma specific activity ($^{25}Mg^*/^{24}Mg$) was lower in Mg deficient compared to control animals (0.89 ± 0.04 vs. 1.26 ± .05, $P < .05$) whereas this ratio tended to increase in Mg deficient animals for other soft tissues. As a result, the proportion of $^{25}Mg^*$ accumulated by non-skeletal muscle soft tissue was much greater in animals fed the D diet than under the other dietary conditions.

Using the concept of in vivo isotope dilution,[13] a value for the apparent Mg exchangeable pool (MgEP) at 10d was calculated for each animal based on the increase in plasma isotope ratio ($R_{25/24}$) from baseline and the total μg of $^{25}Mg^*$ label retained.[14] The comparison between the calculated value of the apparent MgEP and total carcass Mg content is shown in *Table*

5. The calculated value of the apparent MgEP for the control rats was 89% of measured total carcass Mg. Compared to control rats, the value of the apparent MgEP was reduced by 39% in rats fed the D diet whereas total carcass Mg was reduced by only 14%. The large reduction in the calculated MgEPS was due to the proportionately lower retention of $^{25}Mg^*$ by skeletal muscle and bone in deficient rats. If we utilize values for $^{25}Mg^*$ retention by the soft tissue versus skeletal components of the carcass to determine Mg exchangeability, the role of altered skeletal muscle $^{25}Mg^*$ accumulation discussed in the previous paragraph is clarified. The calculated exchangeable pool of Mg in bone decreased by 3.6 mg/100g body weight (average for deficient compared to control rats), a value quantitatively similar to the measured decrease in total Mg content of 3.0 mg. In contrast, the calculated soft tissue exchangeable pool of Mg decreased by 6.5 mg/100g body weight, but total Mg content decreased by only 1.4 mg.

The calculated size of the apparent MgEP for the rats fed the H diet was greater than measured total carcass Mg and had a large coefficient of variation (20%); the latter was considerably smaller for the other two dietary treatments (6.3 and 3.6%, respectively, for the C and D treatments).

Discussion

In the adult rats utilized in this investigation, level of Mg intake had no effect on growth or food intake and the feeding of a Mg deficient diet did not result in overt signs of Mg deficiency. Rats fed the D diet did have significantly reduced plasma and erythrocyte Mg levels as well as very low urinary Mg losses. However, the only tissues to show significant decreases in Mg concentration were kidney and bone. A small decrease in the Mg content of other soft tissues must have existed, however, since a decrease in total carcass soft tissue Mg content (mg/100g body weight) was observed. Although some controversy exists in the literature over the specific effects Mg deficiency produces in adult rats,[15] our findings on blood, urine and tissue Mg content are in keeping with those of several previous investigations.[16-18]

In contrast to measures of Mg status based on total Mg content, the results of the isotope studies suggest that the dynamics of Mg metabolism were altered by dietary Mg restriction. Soft tissues such as heart, liver and brain of those rats fed the D diet accumulated a larger proportion of the exogenous ^{25}Mg label than was observed for control rats; whereas the proportion accumulated by skeletal muscle did not increase with Mg restriction. Because skeletal muscle Mg is such a large component of the total soft tissue Mg pool in vivo,[19] diversion of newly administered Mg to more metabolically active tissues during Mg restriction probably has important adaptive benefit to the animal. That this might be the case was also suggested by earlier in vivo and in vitro studies which utilized ^{28}Mg radiotracer.[20-22] In these older investigations, however, only very short time intervals were studied (less than 24 hours) and the data were not corrected for differences in total Mg content between various tissues.

To the best of our knowledge, this investigation, represents the first attempt to use in vivo stable isotope dilution to investigate the effect of level of Mg intake on whole body Mg status. The data presented give insight into the potential usefulness of this approach as well as the possible limitations. Compared to control rats, the calculated size of the MgEP of rats fed the D diet was reduced by 39%—a reduction much larger than the actual measured deficit in total carcass Mg content. This large decrease in the size of the apparent MgEP compared to total Mg content was due primarily to the lack of increase in accumulation of Mg isotope by skeletal muscle of these animals. It was also true that a measurable decrease in soft tissue Mg concentration (ug/g wet weight) was only observed for kidney. Thus, it can be argued that the large decrease in the size of the MgEP was a more sensitive indicator of altered soft tissue Mg metabolism in these rats than soft tissue Mg content. Aikawa et al.[22] also reported a discrepancy between the decrease in the size of the apparent MgEP, based on in vivo ^{28}Mg dilution 24 hours after isotope dose, and lack of change in total Mg content of soft tissue samples from rabbits fed a Mg deficient diet. A quantitative comparison could not be made in this latter experiment because of the short time allowed for isotope equilibration and the fact that carcass Mg content was not determined.

In this animal model of dietary-induced Mg deficiency, plasma and erythrocyte Mg levels were also greatly reduced and indicative of altered Mg metabolism. However, in the human clinical setting numerous examples exist of altered cellular Mg metabolism in the face of normal extracellular Mg concentrations.[23-25] A few investigators have made estimates of the size of the apparent MgEP in man utilizing ^{28}Mg radiotracer and have attempted to distinguish between altered and normal Mg metabolism on the basis of exchangeable pool size.[26-29] The results of these few studies are encouraging and suggest that a reduction in the size of the apparent MgEP may also be indicative of altered cellular Mg metabolism in the face of normal serum Mg levels.

Degree of isotopic enrichment of biologic samples relative to measurement precision represents the major limitation to the use of stable isotopes as biologic tracers. In the rats fed a high level of dietary Mg, the calculated size of the MgEP was a greater than measured carcass Mg content in all 4 rats, and the estimate had a large coefficient of variation. In these animals, plasma enrichment 10d after isotope dose was approximately 5% and, thus, only 5-to 10-fold greater than measurement precision. The calculation of apparent MgEP size is largely dependent on the difference in isotopic enrichment of plasma taken at baseline and at day 10. The absolute error in this calculated value increases exponentially as the difference in isotope ratios decreases. Thus, in these animals any small error in the measurement of $R_{25/24}$ would result in a large

change in the calculated value of the MgEP. It should be noted, however, that isotope retention as well as plasma and tissue isotopic enrichment would have been considerably higher in these animals if determined at an earlier time point.[30] Isotopic equilibrium between tissues and plasma in these animals likely occurred by 24-72h.[20,21,30]

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References

1 Elin, R.J. (1987). Assessment of magnesium status. *Clin. Chem.* **33,** 1965–1970
2 Aviolli, L.V. and Berman, M. (1966). ^{28}Mg kinetics in man. *J. Appl. Physiol.* **216,** 1688–1694
3 Schwartz, R. (1984). Stable ^{26}Mg for dietary magnesium availability measurements. In: *Stable Isotopes in Nutrition* (J.R. Turnland and P.E. Johnson, eds.) pp. 77–99, American Chemical Society, Washington, DC
4 Currie, V.E., Lengemann, F.W., Wentworth, R.A., and Schwartz, R. (1975). Stable ^{26}Mg as an in vivo tracer in investigation of magnesium utilization. *Intl. J. Nuclear Med. Biol.* **2,** 159–164
5 Schuette, S., Vereault D., Ting, B.T.G., and Janghorbani, M. (1988). Accurate measurement of stable isotopes of magnesium in biological materials with inductively coupled plasma mass spectrometry. *Analyst* **113,** 1837–1842
6 National Research Council. (1978) *Nutrient Requirements of Laboratory Animals, 3rd ed.* Report of Subcommittee on Laboratory Animal Nutrition, pp. 17–18, National Academy of Sciences, Washington, DC
7 Chutkow, J.G. (1979). Lability of skeletal muscle magnesium in vivo. *Mayo Clin. Proc.* **49,** 448–454
8 Analytical Methods Committee. (1963). *Official Standardized and Recommended Methods of Analysis.* S.C. Jolly, ed., W. Heffer & Sons, LTD, Cambridge, UK
9 Fiske, C.H. and Subbarow, Y. (1925). The colorimetric determination of phosphorus. *J. Biol. Chem.* **66,** 375–382
10 *CRC Handbook of Chemistry and Physics, 58 ed.* (1977). R.C. Weast, ed., CRC Press, Cleveland, OH
11 Armitage, P. (1971). *Statistical Methods in Medical Research,* pp. 262–268 and 331–332, Blackwell Scientific, Oxford, UK
12 Winer, B.J. (1962) *Statistical Principles in Experimental Design,* pp. 196–201, McGraw-Hill, New York
13 Moore, F.D., Olesen, K.H., McMurrey, J.D., Parker, H.V., Ball, M.R., and Boyden, C.M. (1963). *The Body Cell Mass and Its Supporting Environment,* pp. 3–42, W.B. Saunders Company, Philadelphia
14 Janghorbani, M. and Ting, B.T.G. (1990). Stable isotope methods for studies of mineral/trace element metabolism. *J. Nutr. Biochem.* **1,** 4–19
15 Wallach, S. (1987). Magnesium exchangeability and bioavailability in magnesium deficiency. In: *Magnesium in Cellular Processes and Medicine* (B.M. Altura, J. Durlach, and M.S. Seelig, eds.) pp. 27–49, Karger, Basel
16 Whang, R. and Welt, L.G. (1963). Observations in experimental magnesium depletion. *J. Clin. Invest.* **42,** 305–313
17 Heroux, O., Peter, D, and Heggtveit, A. (1977). Long-term effect of suboptimal dietary magnesium on magnesium and calcium contents of organs, on cold tolerance and on life-span and its pathological consequences in rats. *J. Nutr.* **107,** 1640–1652
18 Hunt, B.J. (1971). Age and magnesium deficiency in the rat with emphasis on bone and muscle magnesium. *Am. J. Physiol.* **221,** 1809–1817
19 Aikawa, J.K. (1981) *Magnesium: Its Biologic Significance,* pp. 43–49, CRC Press, Boca Raton, FL
20 Field, A.C. and Smith, B.S.W. (1964). Effect of magnesium deficiency on the uptake of ^{28}Mg by the tissues in mature rats. *Brit. J. Nutr.* **18,** 103–113
21 Page, E. and Polemeni, P.I. (1972). Magnesium exchange in rat ventricle. *J. Physiol.* **224,** 121–139
22 Aikawa, J.K., Reardon, J.Z., and Harms, D.R. (1962). Effect of a magnesium-deficient diet on magnesium metabolism in rabbits: A study with ^{28}Mg. *J. Nutr.* **76,** 90–93
23 Whang, R. (1987). Magnesium deficiency: Pathogenesis, prevalence, and clinical implications. *Am. J. Med.* **82** (suppl 3A), 24–29
24 Rasmussen, H.S., McNair, P., Goransson, L., Balslov, S., Larsen, O.G., and Aurup, P. (1988). Magnesium deficiency in patients with ischemic heart disease with and without acute myocardial infarction uncovered by an intravenous loading test. *Arch. Intern. Med.* **148,** 329–332
25 Sjogren, A., Floren, C.-H., and Nilsson, A. (1988). Evaluation of magnesium status in Crohn's disease as assessed by intracellular analysis and intravenous magnesium infusion. *Scand. J. Gastroenterol.* **23,** 555–561
26 Mendelson, J.H., Barnes, B., Mayman, C., and Victor, M. (1965). The determination of exchangeable magnesium in alcoholic patients. *Metabolism* **14,** 88–97
27 Boddy, K., Robertson, I., Mahaffy, M.E., Katoch, D.S., Murray, R.G., Dunn, F.G., and Hutton, I. (1978). Magnesium metabolism in patients with coronary heart disease. *Eur. J. Clin. Invest.* **8,** 87–91.
28 Wallach, S., Rizek, J.E., Dimich, A., Prasad, N., and Siler, W. (1966). Magnesium transport in normal and uremic patients. *J. Clin. Endocrinol. Metab.* **26,** 1069–1080
29 Dimich, A., Rizek, J.E., Wallach, S., and Siler, W. (1966). Magnesium transport in patients with thyroid disease. *J. Clin. Endocrinol. Metab.* **26,** 1081–1092
30 Schuette, S., Vereault, D. Ting, B., and Janghorbani, M. (1989). In vivo studies of Mg exchange utilizing the stable isotope ^{25}Mg in the rat. *Mag. Res.* **2,** 51–52