Compartmental Analysis of Magnesium Kinetics in Mg-Sufficient and Mg-Deficient Rats

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In this study, we determined magnesium kinetic values in normal rats using stable-isotope techniques. Additionally, we calculated the mass of the exchangeable pools of Mg in Mg-deficient rats to determine whether it can be used as a marker of Mg status. Rats were fed either a control diet (1,000 mg Mg/kg) or a Mg-deficient diet (60 mg Mg/kg). After 2 weeks on the experimental diets, each rat received an intravenous injection of ^{26}Mg. The plasma Mg disappearance curve over the next 7 days was used to measure the mass and fractional transport rate of 3 rapidly exchanging Mg metabolic pools. In control rats, the mass of pool 1 (1.37 mg) was half that of pool 2 (2.46 mg), and pool 3 (47.7 mg) accounted for greater than 90% of exchangeable Mg. In Mg-deficient rats, we observed a significant decrease in the size of the 3 exchangeable pools of Mg (0.36, 0.72, and 20.2 mg, respectively) relative to the control rats. Furthermore, the fractional transport rate of Mg from pool 1 to pool 3 in Mg-deficient rats was 3 times the rate in the control rats, and the rate of irreversible loss from pool 1 was lower in Mg-deficient rats. In summary, this study allows us to establish Mg kinetic data in Mg-sufficient and Mg-deficient rats. The present experiment supports the conclusion that the isotopic test identifies animals with severe Mg deficiency.

MAGNESIUM is a cation involved in many enzymatic reactions, and it is critical in ion transport systems. Thus, Mg plays an essential role in a wide range of fundamental cellular reactions, and a deficiency of this mineral may lead to serious biochemical and symptomatic changes.[1,2] In developed countries, marginal Mg intake may induce a high prevalence of marginal Mg deficiency.[3,4] Mg depletion also may occur due to dysregulation of the control mechanisms of Mg metabolism. There is emerging evidence that a poor Mg status is associated with etiologic factors in various metabolic diseases.

Radioactive isotopes and, less commonly, stable isotopes have been used as research tools in assessing mineral metabolism. Multiple approaches have been used in an attempt to determine true Mg status,[5] but a dilemma exists in choosing the most appropriate method because of uncertainty as to which tissue best represents the body stores. The size of the exchangeable pools of a mineral has been used previously to evaluate mineral status; however, this concept was never used for Mg. The purpose of this study was to establish Mg kinetics data using stable isotopes in Mg-sufficient and Mg-deficient rats to explore the tissue Mg status.

MATERIALS AND METHODS

Animals and Diets

Male Wistar rats (IFFA-CREDO, L'Arbresle, France) weighing approximately 150 g were used. The rats were housed under conditions of constant temperature (20° to 22°C) and humidity (45% to 50%) with a standard dark cycle (8 PM to 8 AM). The animal care procedures followed our institutional laboratory animal care guidelines.

The rats were randomized into 2 groups of 16 animals. For 2 weeks, they were fed either the control diet (control group) or a similar very–low-Mg diet (Mg-deficient group). The semipurified diets contained the following (g/kg): casein 200, sucrose 650, maize oil 50, alphacel (cellulose) 50, DL-methionine 3, choline bitartrate 2, modified AIN-76 mineral mix 35, and AIN-76A vitamin mix 10 (ICN Biomedicals, Orsay, France). The Mg concentration of the diets as determined by flame atomic absorption spectrometric analysis (model 400; Perkin Elmer, Norwalk, CT) was 60 and 1,000 mg/kg for the Mg-deficient diet and control diet, respectively. Distilled water and food were provided ad libitum.

Isotope Injection and Sampling

After the 2-week period on the experimental diets, each rat received an intravenous injection of 1.37 mg ^{26}Mg in 1 mL isotonic solution (8.9 mg/mL NaCl and 2.7 mg/mL $HNaCO_3$, pH-7). Because only a limited phlebotomy could be performed on the rats, it was necessary to use 2 groups of animals fed the control diet and 2 groups fed the Mg-deficient diet. In each case, 1 group was used for ''short-term'' phlebotomy, in which repeated samples were obtained in the first 6 hours after injection, while a second group was used for ''long-term'' phlebotomy, in which repeated samples were obtained from 1 to 7 days after isotope infusion. Specifically, 8 rats in each group (Mg-sufficient and Mg-deficient diets) were studied at 5, 30, 60, and 90 minutes and 2, 3, 4, and 6 hours after ^{26}Mg injection (short-term kinetics). The rats were anesthetized, and at each time point, blood was collected via a catheter in the left carotid artery. Eight additional rats in each group were studied at 1, 2, 3, 4, 5, and 7 days after ^{26}Mg injection (long-term kinetics). At each time point, rats were anesthetized and blood was collected at the retro-orbital sinus. Blood samples were centrifuged, and the plasma was separated. Erythrocytes were collected at 6 hours and 7 days, washed with saline solution, and then hemolyzed.

The anesthetized rats were then euthanized and their tibias were collected.

Analysis

The ^{26}Mg content of plasma samples was determined by inductively coupled plasma mass spectrometry (ICP/MS) (PlasmaQuad II Systems; Fisons Instruments, Manchester, UK).[6] Prior to analysis, the plasma was diluted in 1% HNO_3 and natural Mg and beryllium were added as external and internal standards, respectively. The total Mg concentration in the analyzed samples was about 50 μg/L.

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Submitted December 30, 1999; accepted February 28, 2000.

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0026-0495/00/4910-0025$10.00/0
doi:10.1053/meta.2000.8600

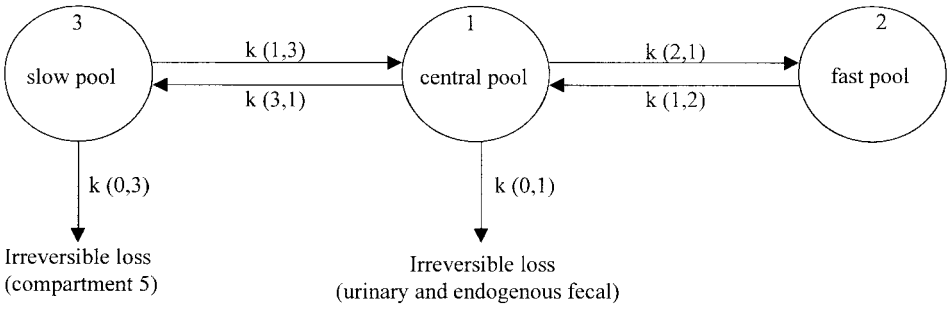

Fig 1. Three-compartment model of Mg kinetics from Avioli and Berman.[7] Arrows represent intercompartmental movements of the cation determined by appropriate rate constants and irreversible losses. Reprinted with permission of The American Physiological Society.

For total Mg determination, plasma and hemolyzed erythrocytes were diluted in 0.1% lanthanum chloride. For tibia analysis, the bone was first dried, mineralized, diluted with HNO_3 and H_2O_2, and heated at 110°C. The dry residue was then taken up with concentrated HNO_3 and diluted in 0.1% lanthanum chloride. The Mg concentration was determined by atomic absorption spectrophotometry (Perkin Elmer 400) at 285 nm.

Kinetic Analysis

Magnesium kinetics were determined using a multicompartmental model as described by Avioli and Berman[7] and Sojka et al.[8] A schematic of the model is shown in Fig 1. Compartmental modelling of the data was performed with the aid of the SAAM II (Simulation, Analysis, And Modelling) program.[9] Plasma data are expressed as the tracer to tracee ratio, with tracer = ^{26}Mg from the injection and tracee = (Mg total − ^{26}Mg from the injection). The mean values (n = 8) at each sampling time were used in the model development because of the use of different rats for the short-term and long-term kinetics experiments. The mass of the different pools (M1, M2, and M3), the fractional transport rate (exchange constant between pools (k1,2, k2,1, k1,3, and k3,1), and the irreversible loss of Mg from pool 3 (k0,3) were determined from the model using the SAAM II program. Irreversible loss from pool 1 (k0,1) was approximated using the urinary excretion values obtained from a previous experiment in our laboratory (data not shown).

Statistical Analysis

Results are expressed as the mean ± SD for the rat characteristics. The statistical significance of differences between mean values was assessed using Student's *t* test. The limit of statistical significance was set at *P* less than .05.

RESULTS

Characteristics of the Rats

Characteristics of the rats, including body weight and tissue magnesium content, are shown in Table 1. The body weight was significantly lower for rats fed the Mg-deficient diet versus the control diet. Moreover, conventional markers of magnesium status, including the Mg content of the plasma, erythrocytes, and tibia, were significantly lower in the Mg-deficient group compared with the control group.

Table 1. Characteristics of the Rats at the Beginning of the Isotopic Study

Characteristic	Control Rats	Mg-Deficient Rats
Weight (g)	259 ± 16	221 ± 11*
Mg content		
Plasma (mg/L)	16.5 ± 1.9	2.28 ± 0.56*
Erythrocyte (mg/L)	58.9 ± 4.3	30.1 ± 4.8*
Tibia (mg/g dried weight)†	4.29 ± 0.42	1.92 ± 0.16*

NOTE. Results are the mean ± SD (n = 16).

*$P < .001$.

†n = 8. Mg levels in tibia were determined only for rats in the short-term kinetics study.

Compartmental Model of Magnesium Metabolism

Semilogarithmic plots of the model fit to the plasma data for control and Mg-deficient rats are shown in Fig 2. The curves are similar for both experimental groups, showing a rapid disappearance of tracer during the first 10 hours, followed by a slower decline that extended through at least 170 hours. However, the tracer to tracee ratio was higher for Mg-deficient rats compared with the control rats.

Table 2 lists the kinetic parameters of the model and the mass of Mg pools determined from the model. Pools 1 and 2 primarily represent extracellular space, while pool 3 primarily represents intracellular Mg. Mg-deficient rats had smaller pool masses compared with control rats. Pool 3 accounted for 92% of the total exchangeable Mg in control rats and 95% in Mg-deficient rats. The mass of pools 1, 2, and 3 was 74%, 71%, and 58% lower in the Mg-deficient group versus the control group, which was associated with a higher fractional transport rate of Mg between pools. Moreover, irreversible loss from pool 1 was about 99% lower in Mg-deficient rats versus control rats. However, no differences were observed for irreversible loss from pool 3.

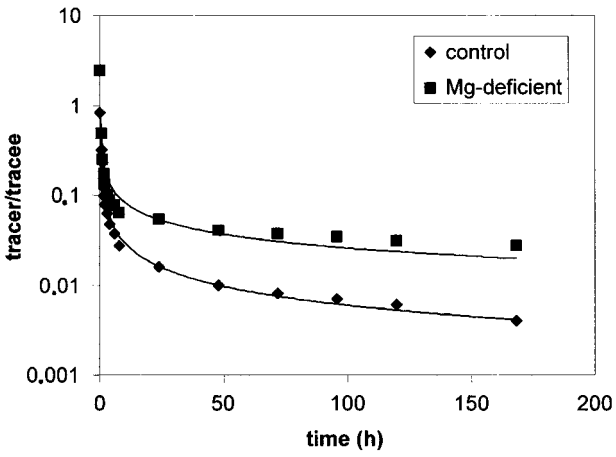

Fig 2. Semilogarithmic plot of the model fit to the plasma data for control rats and Mg-deficient rats.

Table 2. Calculated Tricompartmental Model Parameters of Rats Fed a Control Diet or Mg-Deficient Diet

Parameter	Control Rats	Mg-Deficient Rats
Mass of compartment (mg)		
M1	1.37	0.36
M2	2.46	0.72
M3	47.7	20.2
Exchange constant (h^{-1})		
k1, 2	0.46	0.86
k2, 1	0.82	1.70
k1, 3	0.03	0.05
k3, 1	0.97	2.87
Irreversible loss (h^{-1})		
k0, 1	0.18	0.002
k0, 3	0.007	0.006

DISCUSSION

In this study, we explored Mg kinetics in normal rats using the stable isotope ^{26}Mg to establish Mg kinetic data. We further determined the mass of the exchangeable pools of Mg in Mg-deficient rats to determine if the exchangeable pools of Mg can be used to estimate Mg status.

In the 1960s and 1970s, Mg kinetic studies were conducted using the radioactive isotope ^{28}Mg as a tracer. Rogers and Mahan[10] and Chutkow[11] evaluated ^{28}Mg metabolism in the normal rat. Rogers and Mahan determined 2 pools, a ''free'' pool of Mg with a turnover time of 1.2 hours and a ''bound'' pool with a turnover time of 25 hours. Chutkow hypothesized the existence of a large turnover pool consisting of bone and muscle Mg. Field and Smith[12] studied the effect of Mg deficiency in the rat on the uptake of ^{28}Mg by rat tissues. They did not detect any differences between Mg-deficient and control rats in the mass of the exchangeable pool of Mg in bone.

Mg kinetics were reported in humans by Aikawa et al,[13] Avioli and Berman,[7] and later by Watson et al.[14] Avioli and Berman first proposed a multicompartmental model for Mg. Recently, the use of stable isotopes of Mg, ^{25}Mg or ^{26}Mg, has been developed. The high natural abundance of these 2 isotopes (10% ^{25}Mg and 11% ^{26}Mg) made them very difficult to use, but improvements in analytical techniques have made Mg stable-isotope studies more feasible. Several studies of the compartmental analysis of Mg with stable isotopes were performed in infants,[15-17] in an adult male,[18] in adolescent girls with varying Ca intake levels,[8] and in children.[19] Sojka et al[8] used ^{26}Mg to validate the multicompartmental model described by Avioli and Berman.[7]

To analyze Mg kinetic data in rats, we therefore used a compartmental model based on the model of Avioli and Berman. In this model, there are 3 exchangeable magnesium pools with varied rates of turnover: pools 1 and 2, exemplifying pools with a relatively fast turnover, together approximating extracellular fluid in distribution, and pool 3, an intracellular pool containing over 70% of the exchangeable Mg, with a turnover rate half that of the most rapid pool. There is also a compartment 5, which is a loss pathway representing deposition into tissues.[8] We found that our dosing approach with ^{26}Mg allowed for the characterization of the 3 exchangeable Mg compartments, and in the control rats, the mass of pool 1 was half that of pool 2 and pool 3 accounted for more than 90% of the exchangeable Mg. The total exchangeable Mg was about 52 mg in the control rat. Assuming that a 250-g rat contains 70 mg total Mg,[20] the exchangeable Mg in the rat therefore represents 74% of total Mg. This result is much greater than the value observed in human adults, in whom exchangeable Mg content accounts for less than 16% of the estimated total body content of Mg.[7,13] This high percentage of exchangeable pool may be due to the fact that the rat was growing rapidly.

We also explored the exchangeable pools of Mg in Mg-deficient rats. Rats are often used as an experimental model to study Mg deficiency, as it is relatively easy to induce Mg depletion in the rat.[21] As expected, plasma, erythrocyte, and tibia Mg levels were considerably lower in Mg-deficient rats, versus the control rats. These conventional markers of Mg status thus confirmed a severe Mg deficiency in the Mg-deficient rats. Notably, the compartmental analysis of Mg pools in Mg-deficient rats also demonstrated a significant decrease in the size of the 3 exchangeable pools of Mg. The size of the exchangeable pools better reflects the state of Mg storage in the body. Indeed, the third pool of exchangeable Mg represents the tissue Mg level that the conventional biochemical markers of Mg cannot assess.

The fractional transport rate of Mg from pool 1 to pool 3 in Mg-deficient rats was 3 times the control level. Thus, in Mg deficiency, an increase in the fractional influx of Mg was demonstrated. This may prevent excessive depletion of intracellular Mg. The irreversible loss from pool 1 was lower in Mg-deficient rats. These irreversible losses largely account for the urinary excretion of Mg. This is in agreement with the compensatory mechanism occurring in the kidney in the case of Mg deficiency, where Mg is conserved when the Mg status is low, resulting in a low urinary excretion of Mg. Thus, in the growing rat with a Mg deficiency, urinary excretion is decreased but there is a greater avidity of various tissues for Mg.

We hypothesized that the exchangeable pool of Mg could be used to estimate Mg status. A sensitive marker to assess Mg status in the human[22] is still lacking. Thus, there is a pressing need to develop additional and potentially more suitable methods for the assessment of Mg status. The concept of the exchangeable pool of a given element has been developed to estimate trace element status, and has been validated for selenium[23] and zinc.[24,25] It is promising that this concept may be used to estimate Mg status.

In summary, this study allowed us to establish Mg kinetic data in Mg-sufficient and Mg-deficient rats. The present experiment supports the conclusion that the isotopic test effectively identifies animals with severe Mg deficiency. Further studies are required to determine whether this method is sufficiently sensitive to detect small changes in the kinetics of Mg exchange in the case of a marginal deficiency.

ACKNOWLEDGMENT

The authors thank Professor D. Pépin for her contribution to the study.

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