Magnesium Metabolism in Mice Selected for High and Low Erythrocyte Magnesium Levels

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A genetic control of blood magnesium (Mg) levels has been suggested. To investigate the mechanisms and the biologic significance of this genetic regulation, a mouse model, ie, mice selected for low magnesium level (MGL) and high magnesium level (MGH), was developed. The purpose of this study was to explore the Mg status and Mg metabolism in female MGL and MGH mice. We observed that MGL mice had reduced total and ionized plasma Mg, lower erythrocyte Mg, lower tibia, and kidney Mg levels. In contrast, total urinary Mg and ^{25}Mg levels were significantly higher in MGL mice. MGL mice had smaller total Mg exchangeable pool masses compared with MGH, and fractional transport rates of Mg (exchange constant) were different. In vitro ^{25}Mg enrichments in erythrocytes from MGL mice were significantly lower. Moreover, Mg efflux from erythrocytes was significantly higher in MGL. In conclusion, this work demonstrates that MGL mice present lower body stores of Mg than MGH mice and lower body Mg retention. This is confirmed at a cellular level by a lower enrichment of ^{25}Mg in erythrocytes. The lower retention of Mg by MGL erythrocyte in comparison to MGH appears to be partly due to a higher Mg efflux in MGL erythrocyte. It can be hypothesized that a genetic factor that modulates Na^+/Mg^{2+} exchanger activity may be important in the regulation of Mg metabolism. Further investigations on the mechanisms responsible for differences in Mg retention between MGL and MGH mice could contribute to a better understanding of the genetic regulation of cellular Mg.

MAGNESIUM (Mg), the second most abundant intracellular cation, is involved in many enzymatic reactions, playing a key role in at least 300 fundamental reactions. Mg is critical for phosphorylation reactions, protein synthesis, energy transfer, and lipid and carbohydrate metabolism.[1] However, the regulation of cellular Mg homeostasis remains unclear. In developed countries, marginal Mg intake may induce a high prevalence of Mg deficiency.[2,3] Mg depletion has been reported in many chronic illnesses, including neuromuscular disorders, alcoholism, diabetes mellitus, and cardiovascular diseases.[4] Whereas severe Mg deficiency is easy to detect, the diagnosis of mild or moderate deficiency is more difficult. Various biochemical markers of Mg status are available, but they all have some limitations. Moreover, interindividual variations of plasma and cell Mg are regulated, in part, by genetic factors. In fact, the existence of large intergroup variations (10% to 20%) in the levels of erythrocyte Mg has been shown in humans by comparing ethnic groups. This suggested a genetic control of blood Mg levels.[5] This hypothesis was subsequently confirmed by twin and family studies[6,7] and by the comparison of inbred mouse strains.[7,8] The genetics involved in this control are probably polygenic with large polymorphisms.[9] To investigate the mechanisms and biologic significance of this genetic regulation, a mouse model, ie, mice selected for low magnesium levels (MGL) and high magnesium levels (MGH), specifically designed to study effects of genetically controlled blood Mg levels was developed.[10] This animal model was used to study the overall response to pathologic conditions, particularly related to stress. However, little information is available on Mg metabolism in these animals.

The purpose of this study was therefore to explore Mg status and Mg metabolism in MGL and MGH mice, in particular by determination of Mg exchangeable pool and exploration of erythrocyte Mg flux. Findings from this study should contribute to a better understanding of the regulation of magnesium in animals characterized by low and high plasma cation level to explain the cause-effect relationship.

MATERIALS AND METHODS

Animals

Eighty-five female mice, aged 4 months selected for MGH and for MGL erythrocyte Mg levels, from the 28th or 32nd generation were used in this study. MGL and MGH mice, developed by Henrotte et al,[10] were bred in our laboratory animal colony (National Institute of Agronomic Research, Clermont-Ferrand-Theix, France). Also 20 OF1 mice, female, aged 4 months, were studied as controls (IFFA CREDO, L'Arbresle, France).

Mice were housed under conditions of constant temperature (20°C to 22°C), humidity (45% to 50%) and a standard dark cycle (8 PM to 8 AM). Our institutional guidelines for the care and use of laboratory animals were observed. MGL and MGH received a control diet, and OF1 mice were fed a control diet or a Mg-deficient diet for 15 days before the beginning of the experiments and during all experiments. The semipurified diet 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, AIN-76A vitamin mix 10 (ICN Biomedicals, Orsay, France). The Mg concentrations of diets were 60 mg/kg and 1,000 mg/kg for Mg-deficient and control diets, respectively.

Mg Status Evaluation in MGH and MGL Mice

Total and free plasma Mg, total erythrocyte Mg, urinary Mg, kidney, and tibia Mg were evaluated according to the following procedures. Forty-eight-hour urine was collected in 10 MGH and in 10 MGL mice and acidified with HCl (final pH <2). Blood samples were collected

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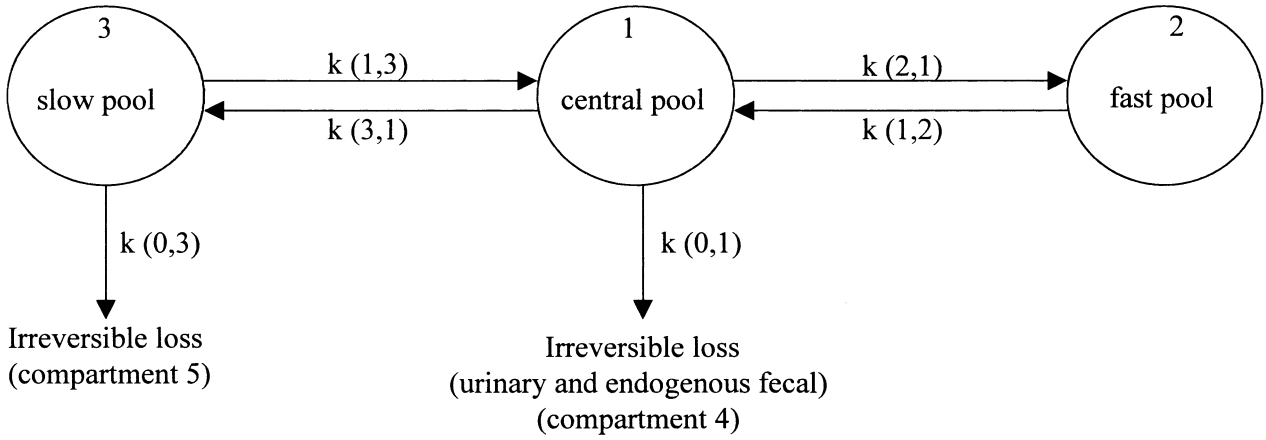

Fig 1. Three-compartmental model of Mg kinetics from Avioli and Berman.[12] Arrows represent intercompartmental movements of the cation as determined by appropriate rate constants and irreversible loss.

from the retroorbital sinus after anesthesia of 10 MGH and 10 MGL mice. Mg concentrations in plasma, erythrocytes, urine, and tissues were determined by atomic absorption spectrophotometry (Perkin Elmer 400, St Quentin en Yvelines, France) at 285 nm. Within- and between-run percentage residual standard deviations were 2.5% and 3.71% for Mg standard solution. Ionized plasma Mg was determined with AVL 988/4 analyzer (AVL Medical Instruments, Eragny, France).

Mg Exchangeable Pools in MGH and MGL Mice

A total of 55 MGL and 55 MGH mice received an intraperitoneal injection of 250 μg ^{25}Mg. Blood samples were obtained at 15, 30, 60, and 90 minutes and 2, 4, and 6 hours and 1, 2, 3, and 4 days after ^{25}Mg injection. Five mice were anesthetized at each time point and blood (0.5 mL) was collected from the retroorbital sinus. Urine was collected for 48 hours after ^{25}Mg injection. The ^{25}Mg concentration of plasma samples was determined by inductively coupled plasma mass spectrometry (ICP/MS) (PlasmaQuad II Systems; Fisons Instruments, Manchester, UK).[11] Mg kinetics were determined using a multicompartmental model as described by Avioli and Berman.[12] A schematic of the model is shown in Fig 1. Plasma data were expressed as tracer/tracee, with tracer = (^{25}Mg from the injection) and tracee = (Mg total – ^{25}Mg from the injection). The mean values (n = 5) at each sampling time were used in the model development. The mass of the different pools (M1, M2, M3), the fractional transport rate (exchange constant between pools (k1,2; k2,1; k1,3; k3,1) and the irreversible loss of Mg from pool 3 (k 0,3) were determined from the model using the SAAM II (Stimulation, Analysis, and Modelling) program (SAAM Institute, Seattle, WA). Irreversible loss from pool 1 (k 0,1) was approximated using the urinary excretion values obtained. Endogenous fecal losses were not taken into account in this calculation because they have been shown to contribute very slightly to the total Mg pool turnover.[13]

In Vitro ^{25}Mg Loading Test in MGH and MGL Erythrocytes

Blood was withdrawn from 10 MGH and from 10 MGL mice after anesthesia at the retroorbital sinus into heparinized tubes. Blood was then incubated with ^{25}Mg isotope (10 μg ^{25}Mg/mL blood) at 37°C for 2 hours.[14] Blood without added ^{25}Mg was also assayed to determine the ^{25}Mg/^{26}Mg ratio in cells from nonenriched blood. Blood was centrifuged at 1,500 × *g* (10 minutes, 20°C). Erythrocytes were then washed with saline and hemolized in distilled water (1/10). The ^{25}Mg and ^{26}Mg contents of erythrocytes were determined by ICP/MS (PlasmaQuad II Systems, Fisons Instruments).[11] Hemolized erythrocytes were diluted in 1% HNO_3, and natural Mg and beryllium were used as external and internal standards, respectively.

The ^{25}Mg enrichment of erythrocytes (relative and net enrichments) were then calculated according to the following equations: Relative ^{25}Mg enrichment (%) = (^{25}Mg/^{26}Mg in ^{25}Mg enriched cells − ^{25}Mg/^{26}Mg in cells from nonenriched blood)/(^{25}Mg/^{26}Mg in cells from nonenriched blood) × 100.

Fractional ^{25}Mg enrichment = (^{25}Mg/^{26}Mg in ^{25}Mg enriched cells − ^{25}Mg/^{26}Mg in cells from nonenriched blood)/(^{25}Mg/^{26}Mg in cells from nonenriched blood).

Net ^{25}Mg enrichment (mg/L) = (total Mg concentration, mg/L × fractional ^{25}Mg enrichment × natural abundance of ^{25}Mg)/(1 + [fractional ^{25}Mg enrichment × natural abundance of ^{25}Mg]).

Net ^{25}Mg enrichment was then expressed in milligrams per liter cells for erythrocytes.

Mg Efflux and Intracellular Adenosine Triphosphate in MGH and MGL Erythrocytes

Blood was withdrawn from 10 anesthetised MGH and 10 anesthetised MGL mice at the retroorbital sinus into heparinized tubes and centrifuged at 1,000 × *g* for 10 minutes. Plasma and buffy coat were recovered, and erythrocytes were washed twice with 150 mmol/L KCl. Erythrocytes were loaded with Mg by incubating a 10% cell suspension for 30 minutes at 37°C in KCl medium with the addition of 12 mmol/L $MgCl_2$ and 6 μmol/L A23187, according to Gunther and Vormann.[15] Mg efflux was measured by reincubating a 10% suspension of Mg-loaded cells at 37°C in Mg-free NaCl medium. Mg efflux was determined by the decrease of cellular Mg content. Erythrocyte intracellular adenosine triphosphate (ATP) was determined by an enzymatic method using a commercial kit (Sigma-Aldrich, L'Isle d'Abeau Chesnes, France).

Mg Status and Metabolism in Mg-Sufficient and Mg-Deficient OF1 Mice

Plasma and erythrocyte total Mg concentration, urinary Mg excretion, tibia, and kidney Mg levels in Mg-sufficient and Mg-deficient OF1 mice were determined as described for MGH and MGL mice. In vitro blood load test was performed, and Mg efflux was assayed in OF1 erythrocyte as described for MGH and MGL erythrocytes.

Table 1. Mg Status in Mice Selected for MGL and MGH Levels

	MGH	MGL
Total plasma Mg (mg/L)	25.1 ± 2.2	18.0 ± 1.7†
Ionized plasma Mg (mg/L)	11.8 ± 2.6	9.1 ± 0.7†
Erythrocyte Mg (mg/L)	54.5 ± 2.2	40.7 ± 1.3†
Tibia Mg (mg/g)	4.45 ± 0.28	3.56 ± 0.23†
Kidney Mg (mg/kg)	956 ± 39	869 ± 20†
Urinary Mg (μg/24 h)	258 ± 38	428 ± 45*
Urinary ^{25}Mg (μg/24 h)	106 ± 34	169 ± 34*

NOTE. Results are mean ± SD, n = 10. Statistical significance (Mann Whitney test).

*$P < .05$, †$P < .001$.

Statistical Analysis

Results are expressed as means ± SD, expect for kinetic characteristics. Statistical analysis was based on a Mann Whitney test. The limit of statistical significance was set at $P < .05$. Statistical analyses were performed using the GraphPad program (V3.00; GraphPad Software, San Diego, CA).

RESULTS

Blood and Tissue Mg

MGL mice presented lower total and ionized plasma Mg and lower erythrocyte Mg when compared with MGH (Table 1). Tibia and kidney Mg levels were significantly lower in MGL mice than in MGH mice. In contrast, total urinary Mg and ^{25}Mg levels were significantly higher in MGL mice. Most of these parameters were also explored in Mg-deficiency OF1 mice (Table 2). Tissue and blood Mg levels were decreased in Mg-deficient mice when compared with Mg-sufficient mice (Table 2). It must be noted, however, that urinary Mg level was significantly lower in Mg-deficient mice when compared with Mg-sufficient mice (Table 2), while it was increased in MGL compared with MGH mice (Table 1).

Exchangeable Mg Pool Masses in MGL and MGH Mice

MGL mice had smaller total Mg exchangeable pool masses compared with MGH (Table 3). Pools M1 and M3 were smaller in MGL than in MGH mice. M3 accounted for 83% of total exchangeable Mg in MGL and for 88% in MGH.

Fractional transport rates of Mg (exchange constant) were different between MGL and MGH mice for k1,2, k2,1, and k3,1, but coefficients of variation were high, except for k2,1. Irreversible loss from pool 3 was similar between MGL and MGH mice (Table 3).

Table 2. Mg Status and Metabolism in Mg-Deficient and Mg-Sufficient OF1 Mice

	Mg-Sufficient	Mg-Deficient
Total plasma Mg (mg/L)	22.6 ± 1.4	8.4 ± 2.2‡
Erythrocyte Mg (mg/L)	48.4 ± 2.2	36.6 ± 6.0‡
Tibia Mg (mg/g)	4.53 ± 0.08	3.15 ± 0.08‡
Kidney Mg (mg/kg)	891 ± 10	833 ± 8‡
Urinary Mg (μg/24 h)	228 ± 9	1.2 ± 0.3‡
Erythrocyte enrichment (%)	39 ± 5	62 ± 15‡
Erythrocyte ^{25}Mg enrichment (mg/L)	2.07 ± 0.33	2.56 ± 0.35†
Mg efflux mg/L cells/30 min	226 ± 19	195 ± 14*

NOTE. Results are mean ± SD, n = 10. Statistical significance (Mann Whitney test).

*$P < .05$, †$P < .01$, ‡$P < .001$.

Table 3. Mg Pools in Mice Selected for MGL and MGH Levels

	MGH	MGL
Exchangeable pool (mg)*		
M1	0.24 (18)‖	0.15 (20)
M2	0.41 (55)	0.64 (83)
M3	4.64 (14)	3.81 (29)
M total	5.29	4.60
Exchange constant†		
k1,2 (h^{-1})	0.481 (49)	0.312 (51)
k2,1 (h^{-1})	0.814 (28)	1.378 (38)
k1,3 (h^{-1})	0.050 (26)	0.050 (51)
k31 (h^{-1})	0.975 (28)	1.297 (55)
Irreversible loss		
k0,1 (h^{-1})‡	0.045	0.119
k0,3 (h^{-1})§	0.011 (19)	0.012 (22)

*M1, M2, M3 are the mass of the different exchangeable pools and were determined from the model of Avioli and Berman[12] using the SAAM II program. The mean values (n = 5 mice) at each sampling time were used in the model development.

†k1,2; k2,1; k1,3; k3,1 are the fractional transport rate and were determined from the model of Avioli and Berman[12] using the SAAM II program.

‡k0,1 is the irreversible loss from pool 1 and was approximated using the urinary excretion values obtained.

§k0,3 is the irreversible loss of Mg from pool and was determined from the model of Avioli and Berman[12] using the SAAM II program.

‖The numbers in parentheses are the estimated coefficient of variation provided by the SAAM II program.

In Vitro ^{25}Mg Erythrocyte Loading Test

^{25}Mg enrichments in erythrocytes from MGL mice were significantly lower compared with MGH erythrocytes (Table 4). On the contrary, ^{25}Mg enrichments in erythrocytes from Mg-deficient mice were significantly higher by comparison to those from Mg-sufficient mice (Table 2).

Mg Efflux

Mg efflux from erythrocytes was significantly higher in MGL than in MGH mice. Moreover, intracellular ATP, the principal ligand of intracellular free Mg, was significantly lower in MGL by comparison to MGH mice (Table 4). In

Table 4. In Vitro Blood Load Test and Mg Efflux in Erythrocytes From Mice Selected for MGL and MGH Levels

	MGH	MGL
Erythrocyte enrichment (%)	45 ± 5	39 ± 4†
Erythrocyte ^{25}Mg enrichment (mg/L)	2.57 ± 0.31	1.56 ± 0.17‡
Mg efflux (mg/L cells/30 min)	168 ± 20	209 ± 32†
Intracellular ATP (μmol/dL)	652 ± 52	534 ± 44*

NOTE. Results are mean ± SD, n = 10. Statistical significance (Mann Whitney test).

*$P < .05$, †$P < .01$, ‡$P < .001$.

Mg-deficient mice, Mg efflux was significantly lower compared with Mg-sufficient mice (Table 2).

DISCUSSION

As in all biologic processes, genetic factors are involved in the regulation of metal ion metabolism.[16] Concerning Mg, the existence of a genetic regulation of intracellular and extracellular Mg levels was first demonstrated by twin and family studies in man and by interstrain comparison in mice.[5,6,17,18] A segregation analysis performed in humans demonstrated that the genetic system implicated was polygenic and presented a large polymorphism.[9] To better characterize this genetic system, the possible association of erythrocyte Mg with genetic markers was investigated. Significant variation of erythrocyte Mg was found in association with human lymphocyte antigen (HLA) phenotypes in unrelated subjects.[7,19]

To further investigate the mechanisms and biologic significance of these genetic factors controlling Mg levels, Henrotte et al[20] undertook a bidirectional selective breeding for MGL and MGH erythrocyte Mg values. The initial population used to start the selection consisted of 160 segregant hybrids of the second generation between 4 inbred strains: C57BL/6J, DBA/2J, C3HeB/J, and AKR/J. Twelve pairs with the highest and 12 pairs with the lowest erythrocyte Mg concentrations were selected for reproduction. Similar assortive matings were repeated in 18 consecutive generations. According to the method recommended by Biozzi et al,[21] brother and sister matings were avoided to reduce inbreeding progression in the lines. Erythrocyte Mg values diverged rapidly and regularly in the 2 strains during the first 10 generations. Between the 14th and the 18th generations, the difference between 2 strains remained constant. The 2 strains were thus phenotypically stable for erythrocyte Mg, suggesting that the mice were homozygotes for all the relevant alleles. Various investigations were then performed in MGL and MGH mice. MGL and MGH mice exhibited significant differences in erythrocyte, plasma, kidney, and bone Mg contents.[10] Urine excretion of Mg was higher in MGL than in MGH mice. MGL mice had higher brain weight and noradrenaline content[22,23] and in stressful conditions, MGL mice exhibited higher urinary catecholamine levels than MGH mice and displayed a more aggressive behavior.[10,24] MGL mice also displayed a greater sensitivity and/or reactivity to stress,[10,25] higher blood pressure,[26] a greater number of gastric ulcers induced by immobilization,[27] and a greater reproductive longevity.[28] All these studies demonstrated distinct characteristics between MGL and MGH mice. But little is known about Mg metabolism in these animals. Thus, we conducted several studies to better characterize Mg metabolism in MGL and MGH mice.

Classical Mg status biomarkers were measured and compared with those of OF1 mice receiving a Mg-deficient or a Mg-sufficient diet. As described by Henrotte et al,[10] MGL mice presented lower plasma and erythrocyte Mg compared with MGH mice. Moreover tibia and kidney Mg level were significantly lower in MGL than in MGH mice. In Mg-deficient mice, tissue and blood Mg were decreased by comparison to Mg-sufficient mice. On the other hand, while urinary Mg levels were significantly lower in Mg-deficient mice compared with Mg-sufficient mice, they were significantly higher in MGL than in MGH mice. This reflects a lower retention of Mg by the body in MGL by comparison to MGH mice. The higher 24-hour urinary excretion of ^{25}Mg in MGL mice, after intraperitoneal injection of 250 μg ^{25}Mg, confirmed this interpretation. According to Henrotte et al,[10] the higher urinary Mg excretion in MGL compared with MGH mice cannot be attributed either to differences in diuresis or in body weights.

Recently, exploration of exchangeable Mg pools using stable isotopes has been proposed as a new approach for evaluating Mg status.[29-31] The assessment of exchangeable Mg pools is also important for a better understanding of Mg metabolism. For these reasons, we explored Mg exchangeable pools in MGL and MGH mice. In 1966, Avioli and Berman[12] proposed a multicompartmental model of exchangeable Mg pools, using ^{28}Mg as a tracer. In this model, there are 3 exchangeable Mg pools with varied rates of turnover. Pools M1 and M2 represent pools with a relatively fast turnover. Together, these 2 pools approximate the extracellular distribution of the cation. M3 is an intracellular pool, with a slower turnover. There is also a fourth pool, which represents urinary excretion and endogenous fecal loss, and a fifth pool, which is a loss pathway representing deposition into tissues.[32] Because of the very short half-life of ^{28}Mg, the turnover rate of the slowly exchangeable Mg pool cannot be determined accurately. With improvements in analytical techniques, methods using the stable isotopes ^{25}Mg and ^{26}Mg were developed, and Sojka et al[32] and Abrams and Ellis[13] validated the multicompartmental model described by Avioli and Berman.[12] Recent studies demonstrated that determination of exchangeable Mg pools using stable isotopes is an interesting approach to evaluate Mg status. In fact, Mg exchangeable pool sizes varied with dietary Mg in rats, Mg pool sizes being higher in Mg sufficient rats than in Mg-deficient rats.[29-31] We observed that MGL mice had smaller pool masses compared with MGH (except M2) and different exchange constants and kinetic losses, despite similar food consumption for MGL and MGH (2.76 ± 0.69 *v* 2.51 ± 1.09 g dry weight, respectively). In particular, M3, which represents the significant tissue Mg level that conventional markers of Mg cannot measure, was 18% lower in MGL mice. It is therefore surprising that Mg urinary excretion was higher in MGL than in MGH. In fact, the kidney regulates body stores of Mg, increasing excretion in normal state and retaining Mg in deficiency state. These results suggested that body Mg exchanges are different between MGL and MGH mice.

To better understand the cellular mechanism of the lower retention of Mg in MGL mice, flux studies have been performed. A novel in vitro ^{25}Mg erythrocyte loading study was developed in our laboratory[14] and was performed on MGL and MGH mice erythrocytes. This test is based on the hypothesis of an increasing cellular demand for Mg during Mg deficiency, thus leading to an increased in vitro cellular uptake of isotopic Mg. We demonstrated previously that blood cells from Mg-deficient rats had significantly increased ^{25}Mg enrichment by comparison with control rats.[14] In MGL erythrocytes, we observed lower enrichments of ^{25}Mg by comparison to MGH, despite a lower Mg level, contrary to what was observed in

Mg-deficient mice compared with Mg-sufficient mice. This phenomenon reflects differences in cellular uptake and/or release of Mg by the erythrocytes of MGL and MGH mice. To understand this observation, we then explored erythrocyte Mg efflux. Gunther et al[15,33] studied mechanisms of Mg^{2+} efflux in a variety of vertebrate erythrocytes and characterized a Na^{+}/Mg^{2+}antiport, which transports Na^{+} into the cell in exchange for Mg^{2+}, together with a Na^{+}-independent Mg^{2+} efflux. We observed that Mg efflux was higher in MGL mice in comparison to MGH, whereas, Mg efflux was lower in Mg-deficient mice compared with Mg-sufficient mice. Moreover and in accordance with the higher Mg efflux, intracellular ATP was lower in MGL than in MGH mice. Intracellular ATP is the principal ligand for Mg^{2+} in the cytosol of the cell, thus when ATP content decreases, the consequent increase in free Mg^{2+} concentration results in an extrusion across the plasma membrane.[34] These results suggest that the lower retention of Mg by MGL erythrocyte in comparison to MGH is partly due to higher Mg efflux. According to these studies, a genetic control of Mg homeostasis seems to be involved at the cellular efflux level of erythrocyte Mg. This is in accordance with the findings of Feray and Garay,[35] who observed in humans that interindividual differences in red blood cells Mg^{2+} content were related to the activity of the Na^{+}/Mg^{2+} exchanger. Moreover, Feray and Garay[35] suggested a possible relationship with HLA-associated genetic factors. The parallel increased Mg efflux from erythrocytes and kidney excretion in MGL mice, despite the low erythrocyte Mg level and the low Mg body stores in MGL by comparison to MGH mice, could involve the same or different mechanisms. It can be hypothesized from these results that a genetic factor that modulates the activity of the Na^{+}/Mg^{2+} transporter plays a role in the regulation of Mg^{2+} metabolism in erythrocyte and in kidney. However, considering that there are different Mg transport mechanisms at the cellular, subcellular, and tissue levels, it is now necessary to study at the molecular level the expression of transport proteins to better understand the significance of our results.

In conclusion, this work demonstrates that (1) MGL mice have lower body stores of Mg than MGH mice, as evidenced by the plasma, erythrocyte, tibia, and kidney Mg levels and (2) that total Mg exchangeable pool mass is reduced in MGL mice. Body Mg retention is lower in MGL mice than in MGH mice, as indicated by the larger urinary loss of Mg in MGL mice, despite a similar Mg intake in the 2 strains. This is confirmed at the cellular level by a lower enrichment of ^{25}Mg in erythrocytes after incubation of erythrocyte with ^{25}Mg. The lower retention of Mg by MGL erythrocyte in comparison to MGH appears to be partly due to a higher Mg efflux in MGL erythrocyte. It can be hypothesized that a genetic factor that modulates the activity of the Na^{+}/Mg^{2+} antiport may play an important role in the regulation of Mg metabolism. Further investigations on the mechanisms responsible for differences in Mg retention between MGL and MGH mice could contribute to a better understanding of the genetic regulation of cellular Mg.

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REFERENCES

1. Rayssiguier Y, Mazur A, Durlach J: Advances in Magnesium Research, Nutrition and Health. Eastleigh, England, Libbey, 2001

2. Galan P, Preziosi P, Durlach V, et al: Dietary magnesium intake in a French adult population. MagnesRes 10:321-328, 1997

3. Pennington JA, Schoen SA: Total diet study: Estimated dietary intakes of nutritional elements, 1982-1991. Int J Vitam Nutr Res 66:350-362, 1996

4. Durlach J: Le magnésium en biologie et en médecine (ed 2). Cachan, France, EMInter, 2000

5. Henrotte JG: Variabilité de la magnésémie des populations humaines. J Physiol Paris 67:197A, 1973

6. Darlu P, Michotte Y, Defrise-Gussenhoven E, et al: The inheritance of plasma and red blood cell magnesium and zinc levels from twin and family data. Acta Genet Med Gemellol 30:67-75, 1981

7. Henrotte JG, Pla M, Dausset J: HLA and H-2 associated variations of intra- and extracellular magnesium content. Proc Natl Acad Sci USA 87:1894-1898, 1990

8. Henrotte JG, Colombani J, Pineau M, et al: Role of H-2 and non-H-2 genes in the control of blood magnesium levels. Immunogenetics 19:435-448, 1984

9. Lalouel JM, Darlu P, Henrotte JG, et al: Genetic regulation of plasma and red blood cell magnesium concentration in man. II. Segregation analysis. Am J Hum Genet 35:938-950, 1983

10. Henrotte JG, Franck G, Santarromana M, et al: Mice selected for low and high blood magnesium levels: A new model for stress studies. Physiol Behav 61:653-658, 1997

11. Coudray C, Pepin D, Tressol JC, et al: Study of magnesium bioavailability using stable isotopes and the inductively-coupled plasma mass spectrometry technique in the rat: Single and double labelling approaches. Br J Nutr 77:957-970, 1997

12. Avioli LV, Berman M: Mg28 kinetics in man. J Appl Physiol 21:1688-1694, 1966

13. Abrams SA, Ellis KJ: Multicompartmental analysis of magnesium and calcium kinetics during growth: Relationships with body composition. Magnes Res 4:307-313, 1998

14. Feillet-Coudray C, Coudray C, Gueux E, et al: A new in vitro blood load test using a magnesium stable isotope for assessment of Mg status. J Nutr 133:1220-1223, 2003

15. Gunther T, Vormann J: Characterization of Na(+)-independent Mg2+ efflux from erythrocytes. FEBS Lett 271:149-151, 1990

16. Henrotte JG: Role of genetic factors in the regulation of metal metabolism, in Berthon G (ed): Handbook of Metal-Ligand Interactions in Biological Fluids. New York, NY, Marcel Dekker, 1995, pp 475-483

17. Henrotte JG: Genetic regulation of cellular magnesium content, in Birch NJ (ed): Magnesium and the Cell. London, UK, Academic, 1993, pp 177-195

18. Henrotte JG: Genetic regulation of red blood cell magnesium content and major histocompatibility complex. Magnesium 1:69-80, 1982

19. Henrotte JG: The variability of human red blood cell magnesium level according to HLA groups. Tissue Antigens 15:419-430, 1980

20. Henrotte JG, Franck G, Santarromana M, et al: Selection of two lines of mice for for high and low blood cell magnesium concentrations, called MGH (high) and MGL (low). Mouse News Lett 81:84-85, 1988

21. Biozzi G, Mouton D, Sant'Anna OA, et al: Genetics of immu-

noresponsiveness to natural antigens in the mouse. Curr Top Microbiol Immunol 85:31-98, 1979

22. Henrotte JG, Aymard N, Leyris A, et al: Brain weight and noradrenaline content in mice selected for low (MGL) and high (MGH) blood magnesium. Magnes Res 6:21-24, 1993

23. Amyard N, Leyris A, Monier C, et al: Brain catecholamines, serotonin and their metabolites in mice selected for low (MGL) and high (MGH) blood magnesium levels. Magnes Res 8:5-9, 1995

24. Henrotte JG, Soni A, Monier C, et al: High catecholamine excretion and aggressive behaviour in mice selected for low red blood cell magnesium levels. Magnes Res 4:236, 1991 (abstr)

25. Frances H, Monier C, Henrotte JG: Behavioral differences between mice selected for low (MGL) and high (MGH) red blood cell magnesium levels. Magnes Res 6:305-306, 1993

26. Osborne-Pellegrin MJ, Henrotte JG: The variability of blood pressure in mice selected for low (MGL) and high (MGH) blood magnesium levels. Magnes Res 8:11-17, 1995

27. Henrotte JG, Aymard N, Allix M, et al: Effect of pyridoxine and magnesium on stress-induced gastric ulcers in mice selected for low or high blood magnesium levels. Ann Nutr Metab 39:285-290, 1995

28. Motta R, Louis JP, Frank G, et al: Unexpected association between reproductive longevity and blood magnesium levels in a new model of selected mouse strains. Growth Dev Aging 62:37-45, 1998

29. Feillet-Coudray C, Coudray C, Brulé F, et al: Exchangeable magnesium pool masses reflect the magnesium status of rats. J Nutr 130:2306-2311, 2000

30. Feillet-Coudray C, Coudray C, Gueux E, et al: Compartmental analysis of magnesium kinetics in Mg-sufficient and Mg-deficient rats. Metabolism 49:1326-1329, 2000

31. Feillet-Coudray C, Coudray C, Gueux E, et al: A new approach to evaluate magnesium status: Determination of exchangeable Mg pool masses using Mg stable isotope. Magnes Res 15:191-198, 2003

32. Sojka J, Wastney M, Abrams S, et al: Magnesium kinetics in adolescent girls determined using stable isotopes: Effects of high and low calcium intake. Am J Physiol 273:R710-R715, 1997

33. Gunther T, Vormann J, Hollriegl V: Characterization of Na(+)-dependent Mg2+ efflux from Mg2(+)-loaded rat erythrocytes. Biochim Biophys Acta 1023:455-461, 1990

34. Romani AMP, Scarpa A: Regulation of cellular magnesium. Front Biosci 5:d720-734, 1990

35. Feray JC, Garay R: An Na+ stimulated Mg2+ transport system in human red blood cells. Biochim Biophys Acta 856:76-84, 1986