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## Relationship between low magnesium status and TRPM6 expression in the kidney and large intestine

Lusliany J. Rondón,<sup>1</sup> Wouter M. Tiel Groenestege,<sup>2</sup> Yves Rayssiguier,<sup>1</sup> and Andrzej Mazur<sup>1</sup>

<sup>1</sup>Institut National de la Recherche Agronomique, Unité de Nutrition Humaine, Centre de Recherche en Nutrition Humaine d'Auvergne, Theix, St Genès Champanelle, France; and <sup>2</sup>Department of Physiology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

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**Rondón LJ, Groenestege WM, Rayssiguier Y, Mazur A.** Relationship between low magnesium status and TRPM6 expression in the kidney and large intestine. *Am J Physiol Regul Integr Comp Physiol* 294: R2001–R2007, 2008. First published April 2, 2008; doi:10.1152/ajpregu.00153.2007.—The body maintains Mg<sup>2+</sup> homeostasis by renal and intestinal (re)absorption. However, the molecular mechanisms that mediate transepithelial Mg<sup>2+</sup> transport are largely unknown. Transient receptor potential melastatin 6 (TRPM6) was recently identified and shown to function in active epithelial Mg<sup>2+</sup> transport in intestine and kidney. To define the relationship between Mg<sup>2+</sup> status and TRPM6 expression, we used two models of hypomagnesemia: 1) C57BL/6J mice fed a mildly or severely Mg<sup>2+</sup>-deficient diet, and 2) mice selected for either low (MgL) or high (MgH) erythrocyte and plasma Mg<sup>2+</sup> status. In addition, the mice were subjected to a severely Mg<sup>2+</sup>-deficient diet. Our results show that C57BL/6J mice fed a severely Mg<sup>2+</sup>-deficient diet developed hypomagnesemia and hypomagnesuria and showed increased TRPM6 expression in kidney and intestine. When fed a Mg<sup>2+</sup>-adequate diet, MgL mice presented hypomagnesemia and hypermagnesuria, and lower kidney and intestinal TRPM6 expression, compared with MgH mice. A severely Mg<sup>2+</sup>-deficient diet led to hypomagnesemia and hypomagnesuria in both strains. Furthermore, this diet induced kidney TRPM6 expression in MgL mice, but not in MgH mice. In conclusion, as shown in C57BL/6J mice, dietary Mg<sup>2+</sup>-restriction results in increased Mg<sup>2+</sup> (re)absorption, which is correlated with increased TRPM6 expression. In MgL and MgH mice, the inherited Mg<sup>2+</sup> status is linked to different TRPM6 expression. The MgL and MgH mice respond differently to a low-Mg<sup>2+</sup> diet with regard to TRPM6 expression in the kidney, consistent with genetic factors contributing to the regulation of cellular Mg<sup>2+</sup> levels. Further studies of these mice strains could improve our understanding of the genetics of Mg<sup>2+</sup> homeostasis.

low Mg<sup>2+</sup> diet; low Mg<sup>2+</sup> inbred mice; active Mg<sup>2+</sup> (re)absorption

ONE OF THE MOST ABUNDANT CATIONS in the body is Mg<sup>2+</sup>. Mg<sup>2+</sup> is a cofactor for numerous enzymes and is required for many biological processes. It is essential for activation of many ATPases, including the Na<sup>+</sup>/K<sup>+</sup> and Ca<sup>2+</sup> ATPases, and more than 300 other enzymes (1, 31). Mg<sup>2+</sup> homeostasis is maintained by renal and intestinal absorption (22, 23).

The kidney is crucial for Mg<sup>2+</sup> homeostasis. About 70% of Mg<sup>2+</sup> is ultrafiltrable in the glomeruli (23), and 80–99% is reabsorbed in the nephron segments (29). Mg<sup>2+</sup> reabsorption in the kidney is mainly achieved by the paracellular pathway in the cortical thick ascending limb (cTAL ± 60%) (8). The distal convoluted tubule (DCT) is the critical segment of the nephron (28) where final Mg<sup>2+</sup> reabsorption occurs (10–15%) and final

excretion is determined. In the mammalian intestine, Mg<sup>2+</sup> absorption is almost exclusively passive, by a nonsaturable paracellular pathway. Active intestinal Mg<sup>2+</sup> absorption is important when dietary Mg<sup>2+</sup> intake is extremely low (13, 22).

Mg<sup>2+</sup> deficiency can result from low Mg<sup>2+</sup> intake or depletion, typically related to gastrointestinal or renal loss (23). Nowadays, clinical symptoms of Mg<sup>2+</sup> deficiency are rarely recorded. However, a high prevalence of hypomagnesemia has been reported in western countries, and a link between low Mg<sup>2+</sup> intake and chronic disease has been established (34).

Mg<sup>2+</sup> status is determined by a combination of environmental (including nutrition) and genetic factors, which regulate Mg<sup>2+</sup> metabolism (10). Henrotte et al. (18) studied inherited hypomagnesemia in populations with genetically induced low Mg<sup>2+</sup> status and found a polygenic influence and probably polymorphisms in the genes responsible for Mg<sup>2+</sup> homeostasis. Recently, different genetic diseases that specially interfere with Mg<sup>2+</sup> (re)absorption and retention have been described, and a number of genes involved in Mg<sup>2+</sup> homeostasis discovered: *CLDN16 (PCLN1)*, *FXSD2*, *CASR*, *CLCNKB*, *SLC12A3*, and *TRPM6*. Mutations in these genes can cause Mg<sup>2+</sup> metabolism disorders (5, 22, 23, 35). In familial hypomagnesemia with secondary hypocalcemia (HSH), transient receptor potential melastatin 6 (TRPM6) was identified as the first component directly involved in active kidney and intestinal epithelial Mg<sup>2+</sup> (re)absorption (36, 46).

TRPM6 is a member of the TRP channel family. It has a high affinity for Mg<sup>2+</sup>, resides in the apical membrane of kidney and intestinal epithelial cells (mainly the large intestine), and functions in active transepithelial transport. TRPM6 is expressed along the entire gastrointestinal tract (GIT) (36), in lung and in kidney (predominantly in the DCT) (4, 8, 14, 27). TRPM6 expression is sensitive to intracellular Mg<sup>2+</sup> levels and plays an important role in Mg<sup>2+</sup> reabsorption and excretion at critical reabsorption sites (DCT). This suggests that TRPM6 is a key component in the maintenance of Mg<sup>2+</sup> homeostasis. Furthermore, another member of the TRP family, the ubiquitously expressed TRP melastatin 7 (TRPM7), is necessary for cellular Mg<sup>2+</sup> and Ca<sup>2+</sup> conservation (15, 39, 42).

The goal of this study was to determine the role of TRPM6 in active transepithelial transport in selected tissues (kidney, cecum, colon, and lung) and in Mg<sup>2+</sup> homeostasis. To address these questions, we studied two low-Mg<sup>2+</sup> status models: 1) graded dietary Mg<sup>2+</sup> restriction in C57BL/6J mice and 2) inbred mice strains selected for high and low erythrocyte Mg<sup>2+</sup>

Address for reprint requests and other correspondence: Correspondence: Mazur A. INRA, Unité de Nutrition Humaine, Centre de Recherche en Nutrition Humaine d'Auvergne, Theix, 63122 St. Genès Champanelle, France (e-mail: mazur@clermont.inra.fr).

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levels (MgH and MgL, respectively). The MgL mice had inherited hypomagnesemia, consistent with a genetic defect in Mg<sup>2+</sup> handling, and therefore their response to a Mg<sup>2+</sup>-deficient diet was evaluated.

## MATERIALS AND METHODS

### Animals

**Experiment 1.** Female C57BL/6J mice, between 4 and 6 mo old, were used. During the first week of the experiment, the mice were fed a control diet (0.1% Mg<sup>2+</sup> wt/wt). Then, the mice were randomly divided into three groups and over the following 2 wk, each group received one of three diets: a control diet (0.1% Mg<sup>2+</sup> wt/wt), a mildly Mg<sup>2+</sup>-deficient diet (0.01% Mg<sup>2+</sup> wt/wt) or a severely Mg<sup>2+</sup>-deficient diet (0.003% Mg<sup>2+</sup> wt/wt). The Ca<sup>2+</sup> content of the diets was 0.4% (wt/wt). All diets were prepared in our laboratory. Distilled water and food were available ad libitum. The mice were housed in metabolic cages during the last three days of the experiment, and urine samples were collected. At the end of the experiment, the animals were killed and blood (collected from the heart), kidney, cecum, colon, and lungs were collected for further analysis. Tissues were immediately frozen in liquid nitrogen.

**Experiment 2.** Female mice were selected for high (MgH) or low (MgL) erythrocyte Mg<sup>2+</sup> levels (10, 11, 17), using our breeding colony. The animals were between 4 and 6 mo of age. During the first week of the experiment, the mice were fed a control diet (Mg<sup>2+</sup> and Ca<sup>2+</sup> content as above). Then, the MgH and MgL mice were divided into two different groups, and during the following 2 wk, each group received one of two diets: a control diet (0.1% Mg<sup>2+</sup> wt/wt) or a severely Mg<sup>2+</sup>-deficient diet (0.003% Mg<sup>2+</sup> wt/wt). Distilled water and food were available ad libitum. During the last three days of the experiment, the animals were housed in metabolic cages, and samples were collected as described above.

All animals were housed in temperature-controlled rooms (22°C), with a 12:12-h light-dark cycle and handled according to the recommendations of the Institutional Ethics Committee (Institut National de la Recherche Agronomique, Theix, France) and decree no. 87-848.

### Plasma and Erythrocyte Collection

Blood from the heart was collected in heparin-containing tubes. Plasma was obtained by centrifugation (10 min, 3,500 rpm, 4°C) and frozen for later analysis. For erythrocyte Mg<sup>2+</sup> determination, erythrocytes were washed three times with saline solution and then hemolyzed in water-containing tubes.

### Creatinine Analysis

Urine was diluted 20 times, and the creatinine content was determined using an automated chemical analysis kit, following the manufacturer's instructions (Kone Progress Plus, Kone Instruments Oy, Espoo, Finland).

### Mineral Analysis

Plasma, erythrocyte (only Mg<sup>2+</sup>) and urine Mg<sup>2+</sup> and Ca<sup>2+</sup> concentrations were determined after dilution with 0.1% (w/v) LaCl<sub>3</sub> (9, 11). Plasma and urine K<sup>+</sup> and Na<sup>+</sup> concentrations were determined after dilution with 0.1% (w/v) CsCl.

The mineral contents were determined by atomic absorption spectrophotometry (using a Perkin-Elmer AA800, Quebec, Canada) at 285 nm, 423 nm, 589 nm, and 767 nm for Mg<sup>2+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup>, respectively.

### Quantitative Real-Time PCR Analysis

Total RNA was extracted from complete segments of kidney, cecum, and colon using the TRIzol Total RNA Isolation Reagent

(Life Technologies BRL, Breda, the Netherlands), following the manufacturer's protocol. Subsequently, DNase treatment (Promega, Madison, WI) was performed to eliminate genomic DNA contamination. Two micrograms of RNA was used for reverse transcriptase reactions with the Moloney murine leukemia virus reverse transcriptase (Life Technologies), as previously described (20). The expression levels of TRPM6 and TRPM7 were determined by quantitative real-time PCR in kidney, cecum, and colon, using an ABI Prism 7700 sequence detection system (PE Biosystems, Rotkreuz, Switzerland). The expression level of the housekeeping gene hypoxanthine-guanine phosphoribosyl transferase (HPRT) was used as a control. Primers and probes for the target genes were designed using the Primer Express software as previously described (14).

### Immunohistochemistry (IHC)

Cryosections (7- $\mu$ m-thick) of periodate-lysine-paraformaldehyde-fixed kidney were stained as previously described (20, 44, 45). The

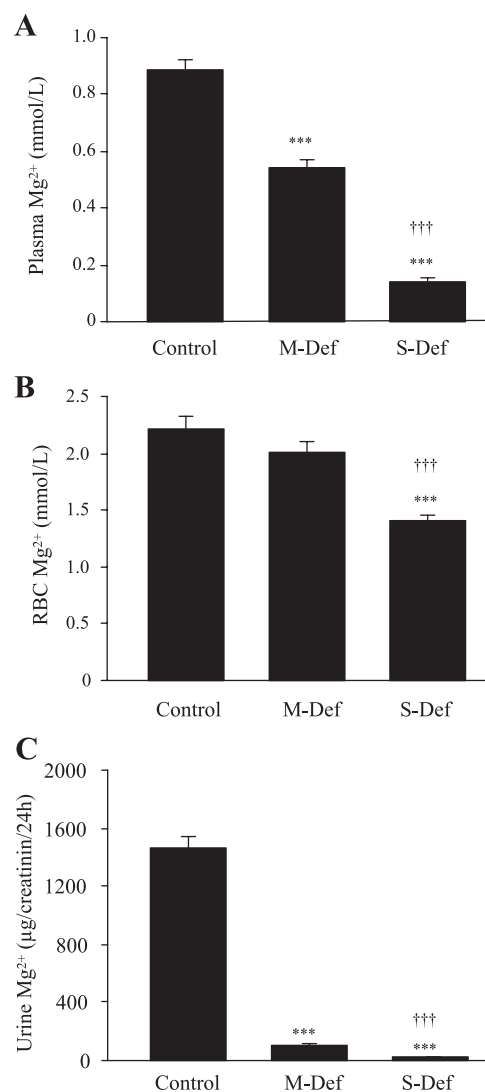


Fig. 1. Plasma, erythrocyte, and urinary Mg<sup>2+</sup> concentrations in mice fed a control, moderate (M-Def) or severely (S-Def) Mg<sup>2+</sup>-deficient diet. Plasma (A), erythrocyte (B), and urinary Mg<sup>2+</sup> (C) concentrations were determined by atomic absorption spectrophotometry. The results are expressed as means  $\pm$  SE of 10–16 values per group. ANOVA was performed followed by a Tukey test. \*\*\* $P$  < 0.001 vs. control and ††† $P$  < 0.001 vs. M-Def.

sections were incubated with affinity-purified guinea pig anti-TRPM6 antiserum [1:1,500 diluted with TNB buffer (TNB buffer containing blocking reagent)] (courtesy from R. J. Bindels laboratory) (45). Biotin-labeled, affinity-purified, goat anti-guinea pig IgG (1:2,000; Sigma Chemical, St. Louis, MO) was used as secondary antibody, followed by incubation with streptavidin-horseradish peroxidase (1:100; Perkin Elmer, Boston, MA). The sections were washed three times with TNB buffer (0.1 M Tris-HCL, 0.15 M NaCl 0.05% Tween 20 pH 7.5) and incubated with fluorescein tyramide in amplification diluent (1:50; Perkin Elmer). Sections were washed, dehydrated in 50 to 100% (vol/vol) methanol, and mounted in Mowiol (Hoechst, Frankfurt, Germany) containing 2.5% (wt/vol) NaN<sub>3</sub>. Micrographs of the cortex regions were acquired with a Zeiss fluorescence microscope (Slidrecht, The Netherlands) equipped with a Nikon DMX1200 digital camera.

#### Statistical Analysis

Values are expressed as means  $\pm$  SE. Differences between groups were tested by the Student's *t*-test or one-way ANOVA followed by a Tukey-Kramer test. Differences were considered

significant at  $P < 0.05$ . The SigmaStat 2.0 Statistical software (SPSS Inc, Chicago IL) was used.

## RESULTS

### Experiment 1: Dietary Mg<sup>2+</sup> Restriction

**Blood and urine mineral analysis.** The plasma Mg<sup>2+</sup> levels were significantly lower in animals fed the mildly (>39% reduction) and severely (>84% reduction) Mg<sup>2+</sup>-deficient diets compared with mice fed the control diet (Fig. 1A). Animals fed the severely Mg<sup>2+</sup>-deficient diet had significantly decreased erythrocyte Mg<sup>2+</sup> levels (about 36% reduction) compared with the control group (Fig. 1B). The urinary Mg<sup>2+</sup> concentrations (corrected for creatinine) are presented in Fig. 1C. As expected, Mg<sup>2+</sup> restriction led to hypomagnesuria in a dose-dependent manner. In addition, mice fed the severely Mg<sup>2+</sup>-deficient diet (0.003% Mg<sup>2+</sup> wt/wt) had altered plasma and urinary mineral concentrations, including a nonsignificant reduction of plasma Ca<sup>2+</sup> (about 12%) and K<sup>+</sup> (about 20%)

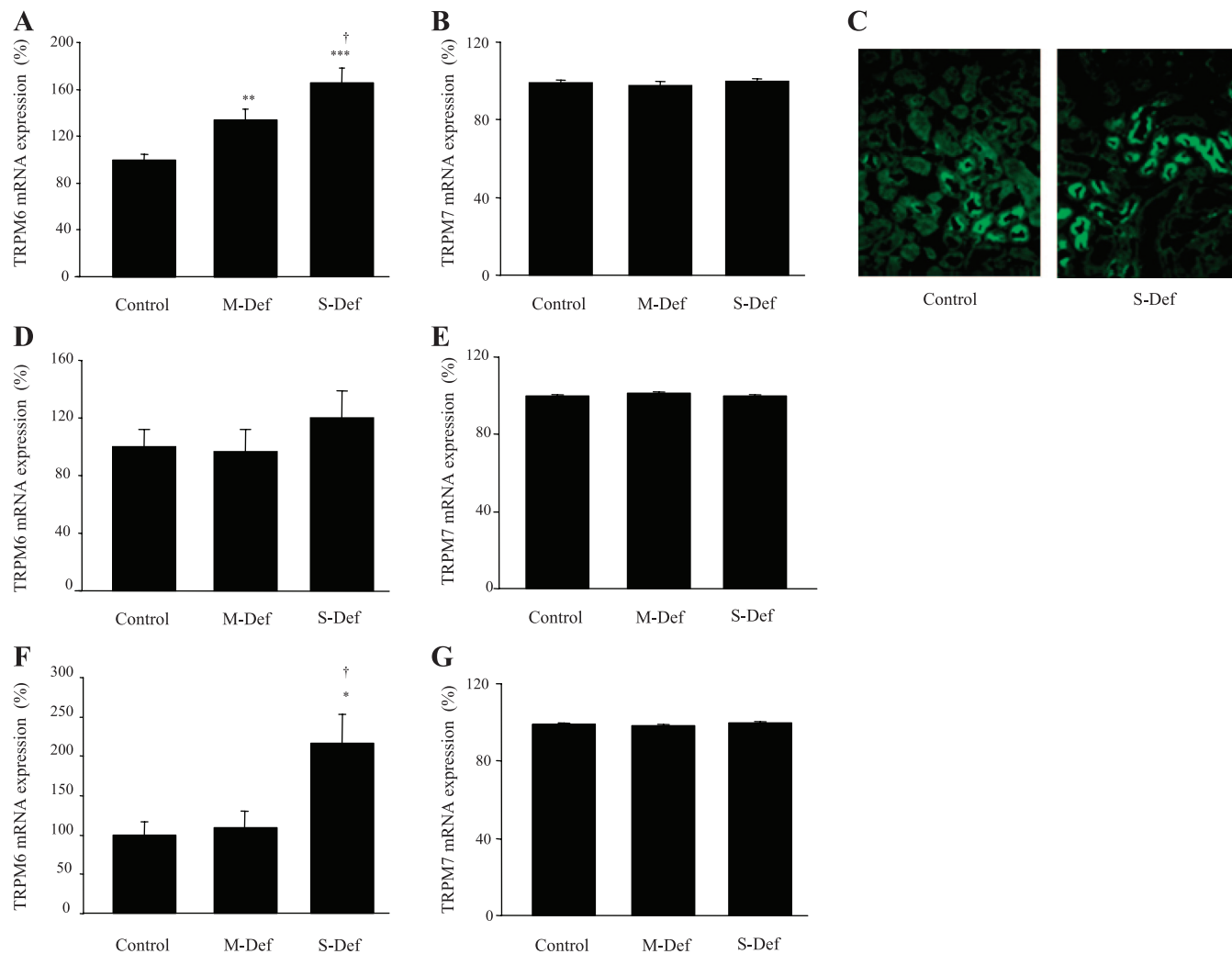


Fig. 2. TRPM6 and TRPM7 expression in kidney and large bowel of mice fed a control, moderate (M-Def), or severely (S-Def) Mg<sup>2+</sup>-deficient diet. TRPM6 (A) and TRPM7 (B) mRNA level in kidney measured by RT-PCR. C: immunohistochemical analysis of TRPM6 protein levels in kidney. TRPM6 (D) and TRPM7 (E) mRNA levels in cecum measured by RT-PCR. TRPM6 (F) and TRPM7 (G) mRNA levels in colon measured by RT-PCR. For the RT-PCR, the mean value of the control group was considered 100%, and the results are expressed as percentage means  $\pm$  SE of 10–16 values per group. ANOVA was performed followed by a Tukey test. \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , and \* $P < 0.05$  vs. control, and † $P < 0.05$  vs. M-Def.



content, and increased urinary Ca<sup>2+</sup> (about 8-fold) and K<sup>+</sup> (about 40%) excretion.

**Effect of dietary Mg<sup>2+</sup> on TRPM6 and TRPM7 expression.** The mildly and severely Mg<sup>2+</sup>-deficient diets induced kidney TRPM6 expression in a dose-dependent manner (Fig. 2A). IHC analysis of the kidneys confirmed that the severely Mg<sup>2+</sup>-deficient mice had increased levels of TRPM6 protein (Fig. 2C). The Mg<sup>2+</sup>-deficient diets induced no significant changes in cecum TRPM6 expression (Fig. 2D). Only the severely Mg<sup>2+</sup>-deficient diet induced TRPM6 expression in the colon (Fig. 2F). Mg<sup>2+</sup> status had no effect on TRPM6 expression in lung (100 ± 7.46% compared with controls, *n* = 8/group) and TRPM7 expression in any of the studied tissues (Fig. 2, B, E, G).

#### Experiment 2: Inbred Mice Selected for High or Low Erythrocyte Mg<sup>2+</sup> Levels

**Blood and urine mineral analysis.** MgL mice fed the Mg<sup>2+</sup> adequate diet (0.1% Mg<sup>2+</sup> wt/wt) had significantly lower Mg<sup>2+</sup> levels in plasma and red blood cells (RBCs) (−25% and −22%, respectively) compared with the MgH mice (Fig. 3, A and B). Interestingly, MgL mice fed the control diet had higher urinary Mg<sup>2+</sup> excretion compared with MgH mice (+86%). When fed a Mg<sup>2+</sup>-adequate diet, the plasma Ca<sup>2+</sup> levels did not differ between the MgL and MgH mice (not shown). K<sup>+</sup> plasma levels were significantly reduced in MgL mice (−10%). The MgL mice had lower Ca<sup>2+</sup> (about twofold), normal K<sup>+</sup>, and higher Na<sup>+</sup> (about 50%) urinary excretion compared with MgH littermates.

When fed the severely Mg<sup>2+</sup>-deficient diet (0.003% Mg<sup>2+</sup> wt/wt), both the MgH and MgL mice developed hypomagnesemia (−51% and −75%, respectively), hypomagnesuria (−89% and −94%, respectively), and had lower RBC Mg<sup>2+</sup> concentrations (−11% and −54%, respectively), compared with animals fed the (control) Mg<sup>2+</sup>-adequate diet. In the two strains, significantly different Mg<sup>2+</sup> concentrations in plasma and RBCs were observed even on the severely Mg<sup>2+</sup>-deficient diet. Furthermore, MgH and MgL mice fed the severely Mg<sup>2+</sup>-deficient diet (0.003% Mg<sup>2+</sup> wt/wt) had altered plasma and urinary mineral content (data not shown), including a nonsignificant reduction in plasma Ca<sup>2+</sup> and K<sup>+</sup> concentration, and a significant reduction in urinary Ca<sup>2+</sup> (−65% and −35%, respectively) and Na<sup>+</sup> excretion (+6% and −14%, respectively), compared with mice on the control diet. These trends were exacerbated in the MgL mice.

**TRPM6 and TRPM7 expression in MgL and MgH mice.** On the control diet, the MgL mice had significantly lower kidney TRPM6 expression (−38%) compared with the MgH mice (Fig. 4A). This was confirmed by IHC analysis of the TRPM6 protein level (Fig. 4C). Similarly, cecum TRPM6 expression was reduced in the MgL mice (−58%; Fig. 4D). The two strains showed no significant differences in colon (Fig. 4F) and lung TRPM6 expression (96.5 ± 7.9% in MgL compared with MgH, *n* = 6/group).

In the MgL mice, the severely Mg<sup>2+</sup>-deficient diet (0.003% Mg<sup>2+</sup> wt/wt) significantly induced kidney TRPM6 expression (+190%, Fig. 4A), compared with MgL mice fed the Mg<sup>2+</sup>-adequate diet. In contrast, the MgH mice showed no change in kidney TRPM6 expression in response to these two diets. Similarly, no significant differences in TRPM6 expression

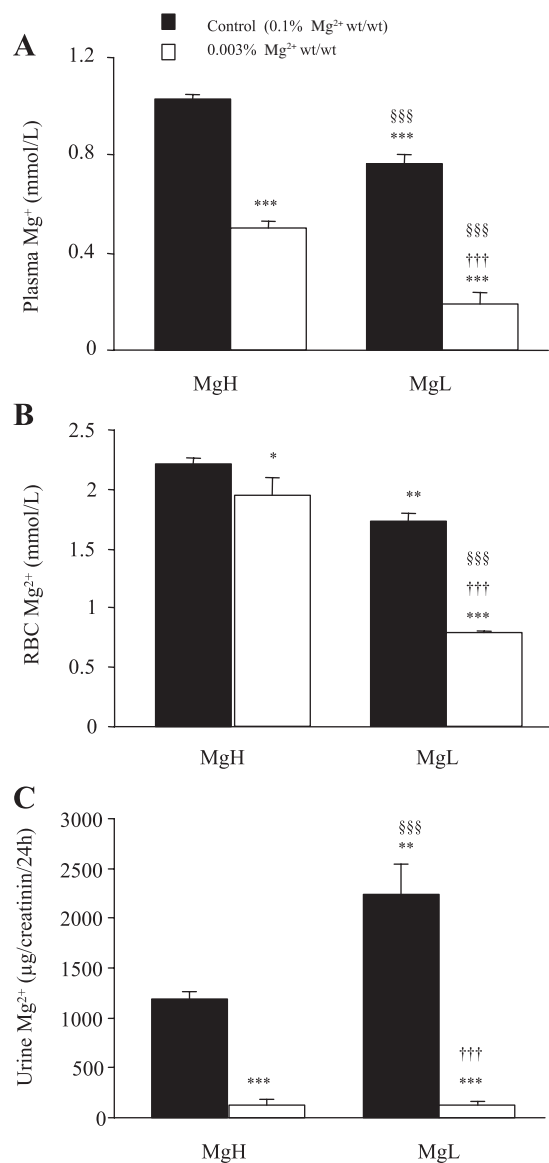


Fig. 3. Plasma, erythrocyte and urinary Mg<sup>2+</sup> concentrations in mice selected for high (MgH) or low (MgL) erythrocyte Mg<sup>2+</sup> levels, fed a control or severely (S-Def) Mg<sup>2+</sup>-deficient diet. Plasma (A), erythrocytes (B), and urinary Mg<sup>2+</sup> (C) concentrations determined by atomic absorption spectrophotometry. The results are expressed as means ± SE of 6–12 values per group. ANOVA was performed followed by a Tukey test. \*\*\**P* < 0.001, \*\**P* < 0.01 and \**P* < 0.05 vs. MgH fed a control Mg<sup>2+</sup> diet; ††††*P* < 0.001 vs. MgL fed a control Mg<sup>2+</sup> diet. ††††*P* < 0.001 vs. MgH fed S-Def Mg<sup>2+</sup> diet.

were observed in cecum, colon (Fig. 4, D and F) and lung (not shown) when the two strains were fed the severely Mg<sup>2+</sup>-deficient diet. TRPM7 expression was unchanged in all studied tissues (Fig. 4, B, E, G).

#### DISCUSSION

Here, we demonstrate a strong link between TRPM6 expression and Mg<sup>2+</sup> status in two mouse models of hypomagnesemia. TRPM6 and TRPM7 potentially contribute to the maintenance of Mg<sup>2+</sup> homeostasis. This is supported by the fact that TRPM6 has high affinity for Mg<sup>2+</sup>, which it transports across the apical membrane of epithelial cells, thereby acting as a gatekeeper in transepithelial Mg<sup>2+</sup> transport and uptake (3,

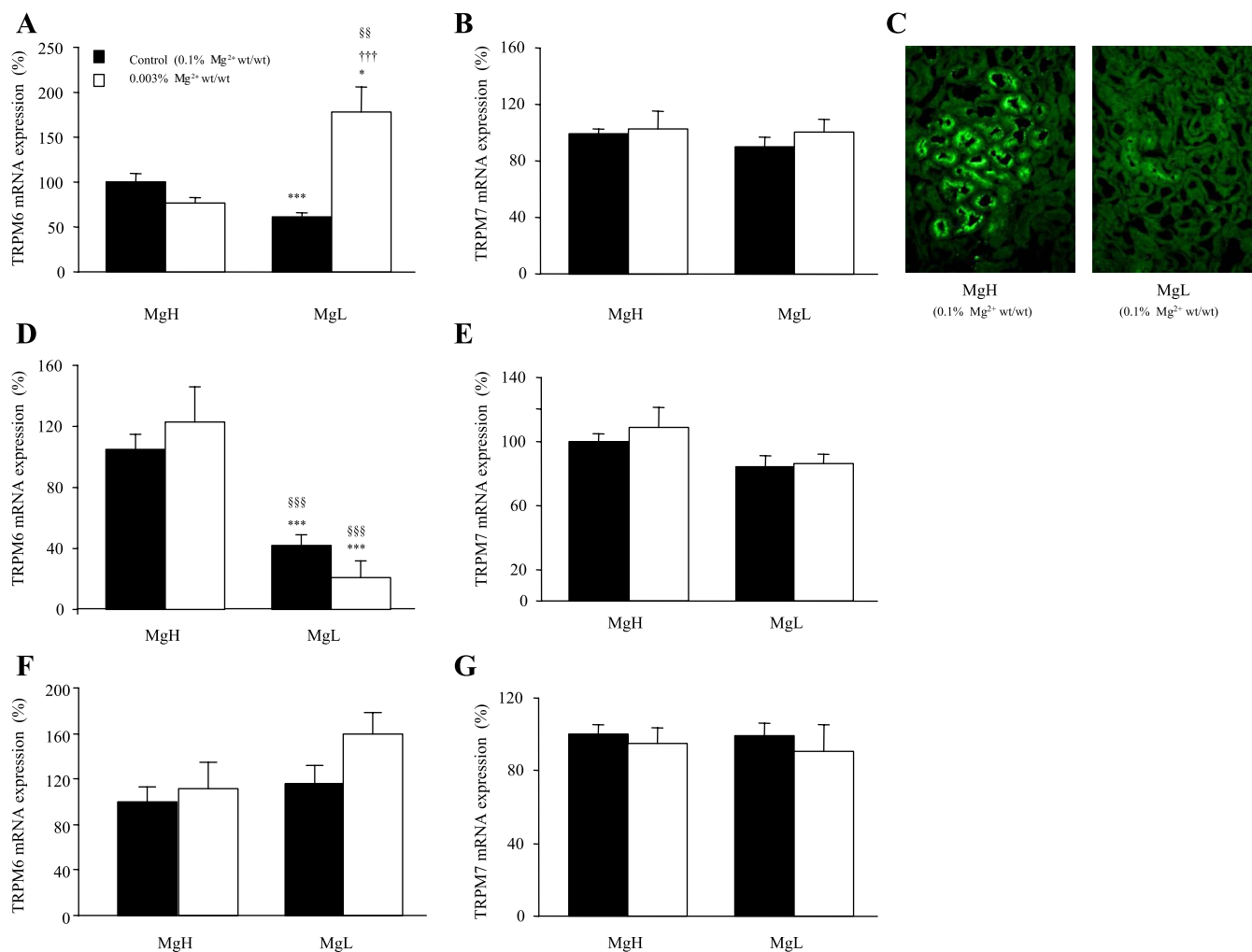


Fig. 4. TRPM6 and TRPM7 expression in kidney and large bowel of mice selected for high (MgH) and low (MgL) Mg<sup>2+</sup> status fed a control or severely (S-Def) Mg<sup>2+</sup>-deficient diet. TRPM6 (A) and TRPM7 (B) mRNA level in kidney measured by RT-PCR. C: immunohistochemical analysis of the TRPM6 protein level in kidney. TRPM6 (D) and TRPM7 (E) mRNA level in cecum measured by RT-PCR. TRPM6 (F) and TRPM7 (G) mRNA level in colon measured by RT-PCR. For the RT-PCR the mean value of the control group was considered 100%, and the results are expressed as percentage means  $\pm$  SEM of 6–12 values per group. ANOVA was performed followed by a Tukey test. \*\*\* $P < 0.001$  and \*\* $P < 0.01$  vs. MgH fed a control Mg<sup>2+</sup> diet; ††† $P < 0.001$  vs. MgL fed a control Mg<sup>2+</sup> diet; §§§ $P < 0.001$  and §§ $P < 0.01$  vs. MgH fed S-Def Mg<sup>2+</sup> diet.

36, 37). Similarly, TRPM7 regulates cellular Mg<sup>2+</sup> uptake (37). RT-PCR and in situ hybridization analyses have demonstrated that TRPM6 is expressed along the entire gastrointestinal tract, in kidney (predominantly in the DCT) (36), intestine (cecum, colon) (37), and lung (23, 27). It has been demonstrated that TRPM6 and TRPM7 are sensitive to intracellular Mg<sup>2+</sup> levels (37). On the basis of these data, we designed a study to determine TRPM6 and TRPM7 expression in the kidney and intestine of two mouse hypomagnesemia models.

One part of the study was focused on a mouse model of nutritionally induced hypomagnesemia. As predicted, two low Mg<sup>2+</sup> diets resulted in low Mg<sup>2+</sup> levels in plasma and RBCs. This is consistent with previous results from Rude et al. (32) and our laboratory (11). The nutritionally induced Mg<sup>2+</sup> deficiency was associated with hypomagnesuria consistent with previously published observations from animal and human studies (2, 14, 29, 31, 40, 41). Furthermore, graded levels of dietary Mg<sup>2+</sup> restriction resulted in reduced Mg<sup>2+</sup> excretion in a dose-dependent manner due to conservation of Mg<sup>2+</sup> by the kidney.

Mice fed a severely Mg<sup>2+</sup>-deficient diet presented normokalemia, normocalcemia, hyperpotassuria, and hypercalciuria. This is consistent with previous studies of Mg<sup>2+</sup> deficiency and associated electrolyte disturbances (1, 23, 25). Active and passive Mg<sup>2+</sup> transport in the kidney and intestine is regulated by hormonal and metabolic factors (23). Hormones such as aldosterone and arginine vasopressin can stimulate Mg<sup>2+</sup> uptake in mouse distal convoluted cells (6, 7). Hypomagnesemia promotes aldosterone secretion, reduces Na<sup>+</sup>-K<sup>+</sup>-ATPase activity, impairs parathyroid hormone release, decreases the activity of 1- $\alpha$ -hydroxylase, enhances ANG II action, and consequently affects the K<sup>+</sup>, Ca<sup>2+</sup>, and Na<sup>+</sup> balances (2, 16, 24, 30, 33).

Here, we show that gradually increased hypomagnesemia is correlated with increased kidney TRPM6 expression. The increased kidney TRPM6 expression in the Mg<sup>2+</sup>-deficient mice is consistent with a recent study of mice fed a Mg<sup>2+</sup>-depleted diet (0.005% wt/wt) by Groenestege et al. (14). This observation can be explained by increased transepithelial Mg<sup>2+</sup> transport induced by low Mg<sup>2+</sup> status. In fact, it has been shown

that the response to Mg<sup>2+</sup> status change involves transcriptional/translational control of membrane transporters (19, 29).

The severely Mg<sup>2+</sup>-deficient diet used in this study resulted in increased colon TRPM6 expression, whereas the mildly Mg<sup>2+</sup>-deficient diet had no effect. It has been reported that active Mg<sup>2+</sup> transport is only important during extremely low dietary Mg<sup>2+</sup> intake (13), and this could explain the increased expression of TRPM6 in response to the severely Mg<sup>2+</sup>-deficient diet. This is in contrast to data from Groenestege et al. (14), who showed that a Mg<sup>2+</sup>-enriched diet (0.48% Mg<sup>2+</sup> wt/wt), but not a Mg<sup>2+</sup>-deficient diet (0.005% Mg<sup>2+</sup> wt/wt), resulted in increased colon TRPM6 expression. These authors proposed that the absence of a specific hormonal control of Mg<sup>2+</sup> in the intestine can explain the upregulation of TRPM6 expression in colon during high Mg<sup>2+</sup> intake, similar to what has been described for TRPV6 in Ca<sup>2+</sup> transepithelial absorption (19, 21). Moreover, in this particular study, the unaltered colon TRPM6 expression was explained by adequate intestinal Mg<sup>2+</sup> absorptive capacity. This apparent discrepancy is likely due to differences in experimental parameters such as diet composition and regimen duration.

The severely Mg<sup>2+</sup>-deficient diet had no effect on lung TRPM6 expression. This supports the idea that Mg<sup>2+</sup> deficiency induces selective compensatory mechanisms, increasing active Mg<sup>2+</sup> (re)absorption at the specific sites responsible for Mg<sup>2+</sup> homeostasis. There are no previous data showing tissue-specific modulation of TRPM6 expression. Also, given that TRPM7 expression was unperturbed, our data support a role for TRPM6 in Mg<sup>2+</sup> homeostasis.

In the second part of this study, we used a genetic Mg<sup>2+</sup> status model to analyze TRPM6 expression in mice with high (MgH) or low (MgL) erythrocyte Mg<sup>2+</sup> levels. Consistent with previous results from our laboratory (10), the MgL mice had low Mg<sup>2+</sup> levels in plasma and RBCs when fed a Mg<sup>2+</sup>-adequate diet (0.1% wt/wt). However, high levels of urinary Mg<sup>2+</sup> were observed in the MgL mice when compared the MgH animals. Furthermore, hypokalemia, hypenatriuria, and hypocalciuria were observed in the MgL mice.

In the MgL mice, the observed hypermagnesuria could be due to impaired passive (paracellular) or active (transcellular) Mg<sup>2+</sup> transport in the kidney and/or intestine. Interestingly, kidney and cecum TRPM6 expression was significantly lower in the MgL mice compared with the MgH animals. The underlying mechanism of hypomagnesemia in the MgL mice is undoubtedly polygenic, but reduced kidney and intestinal TRPM6 expression may contribute to the phenotype.

Consistent with previous results from our laboratory (10, 26), the severely Mg<sup>2+</sup>-deficient diet induced hypomagnesemia and low Mg<sup>2+</sup> levels in RBCs in both mice strains, but the differences between the MgH and MgL mice persisted. The severely Mg<sup>2+</sup>-deficient diet induced and exacerbated hypocalciuria in the MgH mice and hyponatriuria in the MgL mice. Hypomagnesuria was a common finding in both strains when fed the severely Mg<sup>2+</sup>-deficient diet. Similar to the nutritionally induced Mg<sup>2+</sup> deficiency in C57BL/6J mice, MgL mice fed the severely Mg<sup>2+</sup>-deficient diet had increased kidney TRPM6 expression, which was not observed in the MgH mice. In both strains, intestinal TRPM6 expression was unaffected by the Mg<sup>2+</sup>-deficient diets.

The MgL mice showed higher sensitivity to Mg<sup>2+</sup> restriction than the MgH mice and had a greater adaptive response,

increasing the kidney TRPM6 level more than twofold. This is consistent with the fact that the kidney is the major organ responsible for Mg<sup>2+</sup> homeostasis (29) and could explain the specific upregulation of kidney TRPM6 expression during Mg<sup>2+</sup> deficiency in MgL mice. It is possible that the two strains show different genetic responses to changes in cellular Mg<sup>2+</sup> content and that the MgL mice have a lower Mg<sup>2+</sup> threshold, resulting in a more pronounced response to Mg<sup>2+</sup> deficiency.

From a comparative point of view, the low Mg<sup>2+</sup> mouse phenotype is different from that observed in HSH patients that carry *TRPM6* mutations and have pronounced hypomagnesemia. A high dose of oral Mg<sup>2+</sup> supplementation can reestablish normal Mg<sup>2+</sup> levels in these patients, which shows that there is no defect in the passive transport protein paracellular-1 (19).

In conclusion, dietary Mg<sup>2+</sup> restriction and hypomagnesemia can positively alter renal and intestinal Mg<sup>2+</sup> absorption by improving active Mg<sup>2+</sup> transport and modulating TRPM6 expression. TRPM6 expression is sensitive to the extracellular Mg<sup>2+</sup> concentration in kidney and intestine. No differences were observed in TRPM6 expression in lung under any of the studied conditions. TRPM7 expression was not affected in the different models. Our results support the idea that TRPM7 function is independent of TRPM6. TRPM6 and TRPM7 appear functionally nonredundant (38), but both may be involved in the process of epithelial Mg<sup>2+</sup> transport and absorption. The inbred MgL and MgH mice represent an interesting model for studying genetically determined Mg<sup>2+</sup> homeostasis. It clearly appears that their response to dietary Mg<sup>2+</sup> differs, but at present, the reasons for this are not understood.

#### *Perspectives and Significance*

Our observations strengthen the evidence that TRPM6 expression in epithelial cells is an important player of the genetic system involved in the maintenance of Mg<sup>2+</sup> homeostasis. Further studies are required to accurately determine the physiological roles of TRPM6 and TRPM7, and to further our understanding of the complex networks that control Mg<sup>2+</sup> homeostasis. This knowledge will also contribute to elucidate the origin and consequences of differences in Mg<sup>2+</sup> status between individuals.

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