Magnesium deficiency regulates vitamin D metabolizing enzymes and type II sodium-phosphate cotransporter mRNA expression in rats

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Abstract. A magnesium (Mg) deficiency induces changes in calcium (Ca) and phosphorus (P) metabolism; however, the mechanisms responsible for these effects remain unclear. Since 1,25-dihydroxyvitamin D₃ and type II sodium-phosphate (Na/Pi) cotransporters are essential regulators of Ca and P metabolism, this study examined the effects of Mg deficiency on the mRNA expression of vitamin D metabolizing enzymes (25-hydroxyvitamin D-1α-hydroxylase (1α(OH)ase) and 25-hydroxyvitamin D-24-hydroxylase (24(OH)ase)), and Na/Pi cotransporters (type IIa and IIc) in the rat kidney. Rats were divided into two groups and fed a control diet (Mg concentration: 0.05%) or a Mg-deficient diet (Mg concentration: Mg-free) for 21 days. 1α(OH)ase mRNA levels were significantly decreased in rats fed the Mg-deficient diet, while 24(OH)ase mRNA levels were significantly increased, compared to rats fed the control diet. Type IIa and IIc Na/Pi cotransporter mRNA levels in rats fed the Mg-deficient diet were significantly decreased compared to rats fed the control diet. These results suggest that Mg deficiency induces downregulation of 1α(OH)ase and type IIa and IIc Na/Pi cotransporters, and upregulation of 24(OH)ase in the kidney.

Key words: magnesium deficiency, vitamin D metabolizing enzyme, Na/Pi cotransporter, rats

Fibroblast growth factor-23 (FGF-23) is a novel phosphaturic factor that influences regulators of calcium (Ca) and phosphorus (P) metabolism, such as 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) and type II sodium-phosphate (Na/Pi) cotransporters. Previous studies have reported that FGF-23 resulted in decreased levels of renal 25-hydroxyvitamin D-1α-hydroxylase (1α(OH)ase) mRNA and increased levels of renal 25-hydroxyvitamin D-24-hydroxylase (24(OH)ase) mRNA [1, 2]. Administration of recombinant FGF-23 also suppressed renal expression of type II Na/Pi cotransporters [1-3]. Previous reports found a decrease in serum 1,25(OH)₂D₃ levels in rats fed a magnesium (Mg)-deficient diet, indicating that Mg deficiency reduces 1,25(OH)₂D₃ production [4, 5]. Recently, we observed that rats fed an Mg-deficient diet exhibited decreased serum 1,25(OH)₂D₃ levels and increased serum FGF-23 levels [6]. This led...
to our suggesting that increased serum FGF-23 levels in Mg deficiency leads to changes in vitamin D metabolizing enzyme expression, which results in impaired 1,25(OH)₂D₃ production [6]. Moreover, our previous study suggested that decreases in renal P reabsorption by Mg deficiency may be due to a reduction in the expression of renal type II Na/Pi cotransporters, induced by an increase in serum FGF-23 levels [6]. However, the expression of vitamin D metabolizing enzymes and type II Na/Pi cotransporters under Mg deficiency requires clarification.

Accordingly, this study examined the effects of Mg deficiency on the expression of mRNA for vitamin D metabolizing enzymes (1α(OH)ase and 24(OH)ase) and Na/Pi cotransporters (type IIa and type IIc) in the rat kidney.

**Materials and methods**

**Animals and diets**

The mRNA expression data presented here are derived from a subset of data from the same animals reported recently [6]. The experimental protocol has been described in detail previously [6]. Therefore, except for the methods concerned with mRNA expression, the experimental protocol is described only briefly. Four-week-old male Wistar rats were housed in individual stainless steel wire-mesh cages. During the experimental period, cages were located in a room with controlled lighting under a 12-h light:dark cycle (light, 700-1,900 h), a temperature of 22 ± 1°C and relative humidity of 60% to 65%. The experimental diets were based on the AIN-93G diet [7] and contained Mg as follows: 0.05% Mg (control diet) and Mg-free (Mg-deficient diet). Prior to the initiation of the study period, rats were acclimatized over a four-day period, during which all rats were given free access to the control diet and deionized water. After the acclimation period, the rats were randomly divided into two groups and fed either the control or Mg-deficient diet for 21 days. Rats fed the control diet were fed the mean weight of food consumed by the rats fed the Mg-deficient diet on the previous day. All animals were given free access to deionized water. At the end of the experimental period, all rats were sacrificed, and right kidney was collected for analyses. The present study was approved by the Animal Use Committee of Ibaraki Christian University, and all animals were maintained in accordance with the university's guidelines for the care and use of laboratory animals.

**Quantitative analysis of vitamin D metabolizing enzymes and Na/Pi cotransporter mRNA**

Total RNA was isolated from the homogenized right kidney using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's specifications. The amount and purity of RNA were assessed by using NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA (cDNA) was synthesized using the High-Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA). For TaqMan real-time PCR, the reaction mixture was prepared using the TaqMan Gene Expression Master Mix (Applied Biosystems) with TaqMan gene expression assays (Applied Biosystems) for rat 1α(OH)ase (Assay ID: Rn00587137_m1), rat 24(OH)ase (Assay ID: Rn01423143_m1), rat type IIa Na/Pi cotransporter (Assay ID: Rn00564677_m1), rat type IIc Na/Pi cotransporter (Assay ID: Rn00595128_m1) and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Assay ID: Rn01775763_g1). Real-time PCR was performed using a StepOne Real-Time PCR System (Applied Biosystems). The mRNA expression of 1α(OH)ase, 24(OH)ase, type IIa Na/Pi cotransporter and type IIc Na/Pi cotransporter were normalized to GAPDH mRNA expression as a housekeeping gene.

**Statistical analysis**

Data are presented as the mean ± SEM. After conducting an F-test to determine the homogeneity of variances, Student’s t-test was used to determine significant differences between the two groups. If the homogeneity of variances was not equal, Welch’s t-test was used instead of Student’s t-test. Differences were considered significant at p < 0.05.

**Results and discussion**

Previous reports observed that serum 1,25(OH)₂D₃ levels decreased in rats fed a
Mg-deficient diet, and suggested that Mg deficiency adversely influences 1,25(OH)₂D₃ production [4-6]. It is well known that vitamin D metabolizing enzymes, such as 1α(OH)ase and 24(OH)ase, play a pivotal role in 1,25(OH)₂D₃ production. Therefore, the present study assessed the effects of Mg deficiency on the mRNA expression of vitamin D metabolizing enzymes. Results showed that 1α(OH)ase mRNA levels in rats fed the Mg-deficient diet were significantly decreased compared to rats fed the control diet (figure 1A). In contrast, 24(OH)ase mRNA levels were significantly increased in rats fed the Mg-deficient diet compared to control (figure 1A). These results suggest that Mg deficiency influences renal expression of vitamin D metabolizing enzymes. Moreover, this study suggests that the reduction in serum 1,25(OH)₂D₃ levels induced by Mg deficiency is due to the decrease and increase in the expression levels of 1α(OH)ase and 24(OH)ase, respectively.

It has been demonstrated that type IIa and IIc Na/Pi cotransporters are expressed in the proximal tubules of the kidney, and play important roles in renal P reabsorption [8, 9]. In this study, type IIa and IIc Na/Pi cotransporter mRNA levels were significantly decreased in rats fed a Mg-deficient diet compared to the control (figure 1B), indicating that Mg deficiency reduces renal expression of type IIa and IIc Na/Pi cotransporters. Consistent with this result, it has been reported that Mg deficiency decreases renal tubular P reabsorption and increases urinary P excretion [6, 10, 11], which is likely due to the observed reduction in renal type II Na/Pi cotransporter expression. Moreover, Thumfart et al. examined the effects of high-Mg intake on renal expression of Na/Pi cotransporters, and determined that the renal expression of type IIa and IIc Na/Pi cotransporters was increased in rats fed a high-Mg diet [12]. Based on these results, we suggest that Mg is probably involved in regulating renal Na/Pi cotransporter expression.

At present, a detailed mechanism responsible for the above-described changes remains unclear. However, we propose that these findings could be explained by the action of FGF-23. Previous studies reported that injection of recombinant FGF-23 resulted in decreased 1α(OH)ase expression, increased 24(OH)ase expression and decreased renal Na/Pi cotransporter expression [1-3]. Our previous study showed that Mg deficiency increased serum FGF-23 levels, and suggested that elevated FGF-23 levels contribute to altered 1α(OH)ase, 24(OH)ase and renal Na/Pi cotransporter expression [6]. Therefore, we conclude that Mg deficiency-induced serum FGF-23 levels alter the expression of vitamin D metabolizing enzymes and type II Na/Pi cotransporters.
the other hand, it is known that serum Ca levels influence 1,25(OH)2D3 and 24,25(OH)2D3 synthesis. Another possibility is that Mg deficiency-induced changes in 1α(OH)ase and 24(OH)ase expression may be caused by the increase in serum Ca levels, since serum Ca levels was increased in rats fed the Mg-deficient diet [6]. This study also suggests that Mg deficiency may influence Ca and P metabolism as a consequence of the alterations in vitamin D metabolizing enzymes and type II Na/Pi cotransporters.

In conclusion, this study examined the effects of Mg deficiency on the mRNA expression of vitamin D metabolizing enzymes and type II Na/Pi cotransporters in the rat kidney. The 1α(OH)ase and type Ila and IIc Na/Pi cotransporter mRNA levels were significantly decreased, while 24(OH)ase mRNA levels were significantly increased, in rats fed the Mg-deficient diet. These results indicate that Mg deficiency induces downregulation of 1α(OH)ase and type Ila and IIc Na/Pi cotransporters and upregulation of 24(OH)ase in the kidney. Furthermore, we suggest that Mg deficiency regulates expression of vitamin D metabolizing enzymes and type II Na/Pi cotransporters, leading to the changes in Ca and P metabolism.

Disclosure


References


