

## Solutions to [Test Your Knowledge: Hypomagnesemia](#)

### 1B. Thick ascending limb of the loop of Henle

99% of total body magnesium ( $\text{Mg}^{2+}$ ) is located in the intracellular compartment and bone, leaving only 1% of total body  $\text{Mg}^{2+}$  in the extracellular compartment. Of all the extracellular  $\text{Mg}^{2+}$ , 30% is bound to proteins and therefore not filtered by the glomerulus. Hypomagnesemia is mainly due to kidney-related or non kidney-related loss. [The fractional excretion of  \$\text{Mg}^{2+}\$  \(FEMg<sup>2+</sup>\) can help in differentiating between these two situations.](#) The formula is  $\text{FEMg}^{2+} = (\text{UMg} \times \text{PCr} / 0.7 \times \text{PMg} \times \text{UCr})$  with 0.7 adjusting for the amount of magnesium that is available for glomerular filtration. The nephron reabsorbs about 95% of filtered  $\text{Mg}^{2+}$ . In contrast to the higher resorptive capacity for other ions such as  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Ca}^{2+}$ , the proximal tubule reabsorbs only 15% of the filtered  $\text{Mg}^{2+}$  load. The majority of filtered  $\text{Mg}^{2+}$  (70%) is reabsorbed in the thick ascending limb of the loop of Henle (TALLH). The distal convoluted tubule (DCT) reabsorbs 10% of the filtered load. Despite this, the DCT is considered the site for fine-tuning of  $\text{Mg}^{2+}$  reabsorption.

### 2D. TRPM6

$\text{Mg}^{2+}$  transport in the proximal tubule occurs through the paracellular route, although the [molecular mechanisms](#) and transport proteins have not been elucidated. The reabsorption of  $\text{Mg}^{2+}$  in the TALLH follows a similar process as calcium reabsorption. The basolateral  $\text{Na}^+$ - $\text{K}^+$ -ATPase generates the chemical gradient for  $\text{Na}^+$  reabsorption.  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  are reabsorbed via the apical  $\text{Na}^+/\text{K}^+/2\text{Cl}^-$  cotransporter (NKCC2).  $\text{K}^+$  is immediately recycled across the apical membrane via the renal outer medulla  $\text{K}^+$  (ROMK) channel. The  $\text{Cl}^-$  is extruded via the basolateral CLC-Kb channel. The disproportionate transport of two  $\text{Cl}^-$  to one  $\text{Na}^+$  and the secretion of  $\text{K}^+$  contribute to the generation of a lumen-positive transepithelial voltage gradient which drives calcium and  $\text{Mg}^{2+}$  reabsorption through the paracellular route via the claudin 16-19 complex. [Claudin 10](#) also seems to be involved in paracellular cation transport in TALLH, and deletion of the claudin 10 gene causes the opposite effect of deletion of the claudin 16 or 19 genes: increase paracellular transport of  $\text{Mg}^{2+}$  causing hypermagnesemia. The reabsorption of  $\text{Mg}^{2+}$  in the DCT occurs transcellularly via the apical [TRPM6 channel](#). There is no  $\text{Mg}^{2+}$  reabsorption beyond this segment. TRPM6 is also present in the apical membrane of enterocytes in the small intestine. Mutations in the TRPM6 gene lead to the autosomal recessive disorder known as hypomagnesemia with secondary hypocalcemia (HSH), mainly due to decreased intestinal absorption of  $\text{Mg}^{2+}$ , and to a lesser degree, decreased renal reabsorption of  $\text{Mg}^{2+}$ . Individuals suffering from HSH present in early infancy with symptoms of muscle spasms, tetany, and generalized convulsions. For years, studies have predicted the existence of an active extrusion mechanism in the transcellular movement of  $\text{Mg}^{2+}$  in the DCT, either via a  $\text{Mg}^{2+}$  pump or a  $\text{Na}^+/\text{Mg}^{2+}$  exchanger, however, the molecular identity of this mechanism have not been identified.

### 3A. Competitive inhibition of EGFR

Epidermal growth factor receptor (EGFR) activation is essential for the expression of TRPM6, the main apical  $Mg^{2+}$  transporter in the DCT. EGF starts as pro-EGF. Pro-EGF is cleaved by a series of extracellular proteases to generate the active EGF on the basolateral side of the DCT cells. A mutation in the pro-EGF gene leads to faulty basolateral sorting of EGF, resulting in impaired stimulation of the EGFR and isolated hypomagnesemia. [Certain chemotherapeutic agents, such as the monoclonal antibodies against EGFR](#) (cetuximab and panitumumab), and less commonly the EGFR tyrosine kinase inhibitors (gefitinib, erlotinib, and lapatinib), are also associated with significant hypomagnesemia.

### 4C. Hypomagnesemia causes low intracellular $Mg^{2+}$ levels which relieve inhibition of $K^+$ secretion via ROMK channels.

In the distal nephron,  $K^+$  is taken into cells across the basolateral membrane via the  $Na^+-K^+-ATPase$  pump and secreted into luminal fluid via apical  $K^+$ -channels. One of those channels is the renal outer medulla  $K^+$  channel (ROMK). ROMK is an inward-rectifying  $K^+$  channel. Inward rectification means that  $K^+$  flow into the cells through ROMK more readily than out. [Inward rectification of ROMK results when intracellular  \$Mg^{2+}\$  binds and blocks the pore of the channel from the inside, therefore limiting  \$K^+\$  efflux.](#) Inward  $K^+$  flux (influx) would displace intracellular  $Mg^{2+}$  from the pore and release the block. Normal intracellular magnesium levels will inhibit  $K^+$  efflux via the ROMK channel. Low intracellular magnesium levels are believed to relieve this inhibition, thereby causing ROMK-mediated potassium secretion.

### 5C. Amiloride

The DCT has a slight lumen-negative voltage of approximately -5 mV. Detailed experiments have shown that the concentration of  $Mg^{2+}$  in the luminal space is similar to the [concentration of  \$Mg^{2+}\$  inside the DCT cells](#). Thus, there is no chemical driving force for  $Mg^{2+}$  entry into these cells and the voltage difference plays the major role in  $Mg^{2+}$  transport within this segment. This is supported by the observation that mutations in channels and regulators implicated in the generation and maintenance of this negative membrane potential in DCT cells cause clinical hypomagnesemia. These include the  $\gamma$ -subunit  $Na^+/K^+-ATPase$  pump (FXVD2), the hepatocyte nuclear factor 1B (HNF1B), a transcription regulator of FXVD2, and the potassium channels Kir4.1 (apical membrane) and Kv1.1 (basolateral membrane). [Amiloride is used as an adjuvant treatment](#) of hypomagnesemia because by inhibiting sodium transport via ENaC, it would help to reestablish a negative membrane potential in these cells and therefore favor  $Mg^{2+}$  reabsorption.