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Hypomagnesemia in Type 2 Diabetes: A Vicious Circle?

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Over the past decades, hypomagnesemia (serum Mg^{2+} <0.7 mmol/L) has been strongly associated with type 2 diabetes mellitus (T2DM). Patients with hypomagnesemia show a more rapid disease progression and have an increased risk for diabetes complications. Clinical studies demonstrate that T2DM patients with hypomagnesemia have reduced pancreatic β-cell activity and are more insulin resistant. Moreover, dietary Mg^{2+} supplementation for patients with T2DM improves glucose metabolism and insulin sensitivity. Intracellular Mg^{2+} regulates glucokinase, K_{ATP} channels, and L-type Ca^{2+} channels in pancreatic β-cells, preceding insulin secretion. Moreover, insulin receptor autophosphorylation is dependent on intracellular Mg^{2+} concentrations, making Mg^{2+} a direct factor in the development of insulin resistance. Conversely, insulin is an important regulator of Mg^{2+} homeostasis. In the kidney, insulin activates the renal Mg^{2+} channel transient receptor potential melastatin type 6 that determines the final urinary Mg^{2+} excretion. Consequently, patients with T2DM and hypomagnesemia enter a vicious circle in which hypomagnesemia causes insulin resistance and insulin resistance reduces serum Mg^{2+} concentrations. This Perspective provides a systematic overview of the molecular mechanisms underlying the effects of Mg^{2+} on insulin secretion and insulin signaling. In addition to providing a review of current knowledge, we provide novel directions for future research and identify previously neglected contributors to hypomagnesemia in T2DM.

Globally, over 300 million people suffer from type 2 diabetes mellitus (T2DM), and the prevalence is predicted to rise to over 600 million over the next decades (1). T2DM is characterized by a combination of insulin deficiency and insulin resistance. The general pathophysiological concept is that hyperglycemia emerges when endogenous insulin secretion can no longer match the increased demand owing to insulin resistance (2).

Since the 1940s, it has been reported that T2DM is associated with hypomagnesemia (3,4). Low serum magnesium (Mg^{2+}) levels have been reported in large cohorts of patients with T2DM (5). In T2DM, the prevalence of hypomagnesemia ranges between 14 and 48% compared with between 2.5 and 15% in healthy control subjects (4). Hypomagnesemia is associated with a more rapid, and permanent, decline in renal function in patients with T2DM (6). In addition, epidemiological studies consistently show an inverse relationship between dietary Mg^{2+} intake and risk of developing T2DM (7). Several patient studies have shown beneficial effects of Mg^{2+} supplementation on glucose metabolism and insulin sensitivity (8–10). Recently, Rodríguez-Morán et al. (11) published an excellent overview of the clinical studies addressing the role of Mg^{2+} in T2DM. In our review, we will focus on the molecular mechanisms underlying these clinical observations.

Mg^{2+} is an essential ion for human health, as it is involved in virtually every mechanism in the cell, including energy homeostasis, protein synthesis, and DNA stability (12). Considering these divergent functions, it can be appreciated that serum Mg^{2+} levels are tightly regulated between 0.7 and 1.05 mmol/L in healthy individuals. However, impaired intestinal Mg^{2+} absorption or renal Mg^{2+} wasting can lead to hypomagnesemia. A wide range of genetic and environmental factors can affect the Mg^{2+}-deficient state, which have previously been extensively reviewed (12).

In this review, we address the following questions that are central to the role of hypomagnesemia in T2DM: *1)* Does Mg^{2+} regulate insulin secretion? *2)* How does Mg^{2+} affect insulin resistance? *3)* How does insulin regulate Mg^{2+} homeostasis? Taken together, these questions will aid the understanding of whether hypomagnesemia is a causative factor for or a consequence of T2DM.

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INSULIN RESISTANCE

Insulin Sensitivity in Normal Cell Physiology

Increased insulin resistance is the major pathophysiological cause for the development of T2DM. In healthy subjects, insulin increases glycogen production in the liver, lipid synthesis by adipose tissue, and glucose uptake in muscle (5,13–18). Insulin resistance is often the consequence of reduced sensitivity of the insulin receptor that is composed of two insulin-binding α-subunits and two β-subunits. Specifically, upon insulin binding, the tyrosine residues of the β-subunits become autophosphorylated, activating a wide signaling network in the cell (Fig. 1). Depending on the target tissue, direct substrates of the insulin receptor may be recruited to the receptor, of which insulin receptor substrates (IRSs)-1–4 are the most studied. These IRSs, in turn, phosphorylate downstream signaling pathways leading to glucose uptake, glycogenesis, lipid synthesis, and other insulin-dependent actions. Alternatively, the insulin receptor can activate IRS-independent pathways via Src homology 2 domain containing transforming protein causing the activation of mitogen-activated protein kinase signaling and regulation of cell proliferation (16,19). An overview of the main insulin signaling pathways is provided in Fig. 1.

Role of Mg^{2+} in Insulin Sensitivity

Many clinical studies have shown that hypomagnesemia is associated with increased insulin resistance in T2DM patients (4,20–22). In a cross-sectional study of patients with metabolic syndrome, it was shown that insulin resistance associates with reduced serum Mg^{2+} levels (21). Furthermore, a cohort study of adult black Americans showed that Mg^{2+} deficiency contributes to an insulin-resistant state (20). Similar results were found in healthy human subjects, where induced Mg^{2+} deficiency reduced insulin action and secretion (22). In this segment of the review, we will clarify the association between hypomagnesemia and insulin resistance by focusing on the effects of Mg^{2+} on the insulin receptor activity and downstream signaling events.

Insulin Receptor Phosphorylation

It is widely accepted that Mg^{2+} is essential for autophosphorylation of the β-subunits of the insulin receptor. The crystal structure of the insulin receptor tyrosine kinase shows that two Mg^{2+} ions can bind to the tyrosine kinase domain (23). The role of this Mg^{2+} binding has been shown by in vitro studies using isolated insulin receptors. Here, Mg^{2+} enhances tyrosine kinase activity by increasing the receptor's affinity for ATP (24,25). Indeed, rats with hypomagnesemia have reduced levels of insulin receptor phosphorylation, mimicking a state of insulin resistance (26,27). In contrast, increased insulin receptor phosphorylation was shown in liver tissue of rats fed Mg^{2+}-deficient diets for 11 weeks (28). However, the value of this study can be questioned because insulin

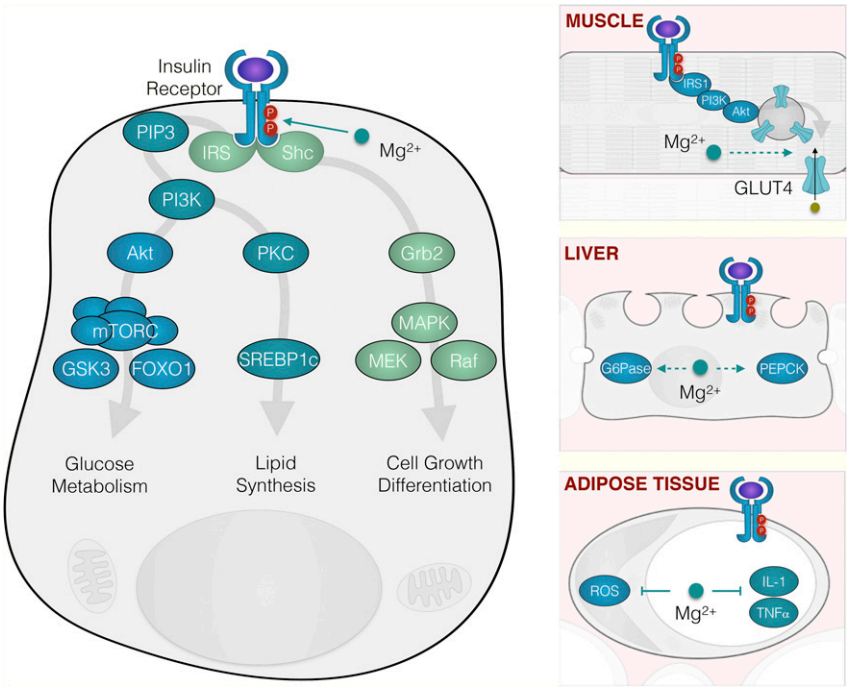

Figure 1—Mg^{2+} affects insulin sensitivity. Mg^{2+} regulates the insulin signaling pathway by increasing the affinity of the insulin receptor tyrosine kinase for ATP. Consequently, hypomagnesemia is associated with a reduced activity of all downstream pathways. In the muscle, Mg^{2+} therefore regulates the membrane trafficking of GLUT4. In the liver, Mg^{2+} is an important regulator of enzymes in gluconeogenesis, including G6Pase and PEPCK. In adipose tissue, Mg^{2+} acts as an anti-inflammatory factor reducing IL-1 and TNF-α secretion. FOXO1, forkhead box class O1; Grb2, growth factor receptor-bound protein 2; GSK3, glycogen synthase kinase 3; MEK/MAPK, mitogen-activated protein kinase kinase; P, phosphorylation; PIP3, phosphatidylinositol 3,4,5 trisphosphate; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; ROS, reactive oxygen species; Shc, Src homology 2 domain containing transforming protein.

phosphorylation was normal in rats with the same serum Mg^{2+} levels at 6 weeks or in muscle tissue. Altogether, Mg^{2+} seems to be an important factor in insulin receptor autophosphorylation. Defective insulin receptor phosphorylation is therefore regarded as the main mechanism by which hypomagnesemia contributes to insulin resistance in T2DM patients.

Glucose Metabolism in the Muscle

Glucose uptake in skeletal muscle accounts for the disposal of ~80% of the dietary glucose load via insulin-dependent glucose uptake using GLUT4 glucose transporters (29). In a recent study in rats with streptozotocin (STZ)-induced diabetes, oral Mg^{2+} supplementation increased GLUT4 expression in the rat muscle and thereby lowered serum glucose levels to the normal range (30). Similar results were obtained in STZ mice treated with Mg^{2+}-rich sea water, showing increased GLUT1 and GLUT4 expression in muscle (31). Although the molecular mechanism is still unknown, these findings suggest that Mg^{2+} regulates glucose uptake in muscle.

Glycogen Synthesis in the Liver

Although many enzymes in the liver require Mg^{2+} for their activity, the role of Mg^{2+} in gluconeogenesis and glycogenesis is poorly studied. The activity of several enzymes involved in gluconeogenesis including glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) were measured in Mg^{2+}-deficient rats, but only PEPCK activity was increased (32). In the same vein, mice with STZ-induced diabetes showed decreased PEPCK and G6Pase mRNA expression when supplemented with Mg^{2+}-rich deep sea water (31). However, it cannot be excluded that other molecules that are present in the sea water mediate these effects. In contrast, mRNA expression of PEPCK was decreased in Mg^{2+}-deficient rats (33).

Obesity and Inflammatory Aspects

Obesity is a major risk factor for the development of T2DM (16,34). In obese patients with T2DM, adipocytes produce proinflammatory mediators, such as interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α), and stimulate the production of reactive oxygen species. The inflammatory environment is regarded as an important contributor to insulin resistance and is one of the main reasons that obesity is associated with T2DM (15,16). Among others, chronic inflammation may induce insulin resistance by reducing GLUT4 activity and inhibiting IRS-1 action (reviewed in 35).

Mg^{2+} is an important anti-inflammatory molecule, and therefore hypomagnesemia increases the inflammatory environment in obesity. IL-1 and TNF-α are significantly increased in Mg^{2+}-deficient hamsters and rats (36). Moreover, low serum Mg^{2+} levels are associated with increased levels of TNF-α in obese people without diabetes (37). Additionally, Mg^{2+} deficiency contributes to neutrophil activation and oxidative stress (38). In a community-based cross-sectional study of 488 healthy children, an inverse correlation between serum Mg^{2+} levels and hs-CRP was demonstrated (39). A recent clinical randomized double-blind placebo-controlled trial of healthy subjects with prediabetes and hypomagnesemia showed reduced hs-CRP levels after Mg^{2+} supplementation for 3 months (40). Given that inflammation and oxidative stress are important factors in the development of insulin resistance (35,41), hypomagnesemia may cause increased insulin resistance.

Novel Perspectives

Although there is extensive evidence that hypomagnesemia reduces insulin receptor phosphorylation and increases chronic inflammation in T2DM patients, the consequences of these signaling events in affected tissues, such as muscle, liver, and adipocytes, have barely been studied. For instance, Ca^{2+} has been shown to increase glucose uptake via GLUT4 in the skeletal muscle (42). Given that Mg^{2+} is an antagonist of Ca^{2+}, GLUT4 membrane trafficking may be reduced in hypomagnesemia. Additionally, some early studies suggest that Mg^{2+} may affect adipokine levels (43). Therefore, future research should be aimed at identifying the effects of hypomagnesemia in target tissues, taking into account the intrinsic effects of Mg^{2+} on gene expression and Ca^{2+} antagonism.

INSULIN SECRETION

Insulin Secretion in Normal Cell Physiology

Acute insulin secretion from pancreatic β-cells is essential to the control of blood glucose homeostasis. Increased blood glucose levels stimulate the influx of glucose in pancreatic β-cells via GLUT2, where it is converted to glucose-6-phosphate (G6P) by glucokinase (44). This enzymatic reaction functions as a glucose sensor to determine the required amount of insulin secretion. G6P is further metabolized by glycolysis to generate ATP, which directly induces closure of K_{ATP} channel Kir6.2 (45). Closure of these channels induces depolarization of the plasma membrane and, consequently, opening of voltage-dependent Ca^{2+} channels (46). The influx of extracellular Ca^{2+} triggers the release of insulin via exocytosis (19) (Fig. 2).

In the early phases of T2DM, insulin release meets the increasing demands by expanding the pancreatic β-cell mass (47). For decades, insulin resistance was thought to be the major cause for T2DM. However, supported by genome-wide association studies studies, evidence has accumulated that impaired insulin secretion in the pancreatic β-cells is a major contributor to the development of T2DM (48,49).

Role of Mg^{2+} in Insulin Secretion

The clinical evidence for a role of Mg^{2+} in insulin secretion is limited and less well studied than the effects of Mg^{2+} on insulin sensitivity, but several recent clinical studies suggest that T2DM patients with hypomagnesemia display reduced insulin secretion. In individuals without diabetes, decreased serum Mg^{2+} concentrations are associated with a diminished insulin secretion (15). Conversely, HOMA of β-cell activity was negatively correlated with the serum

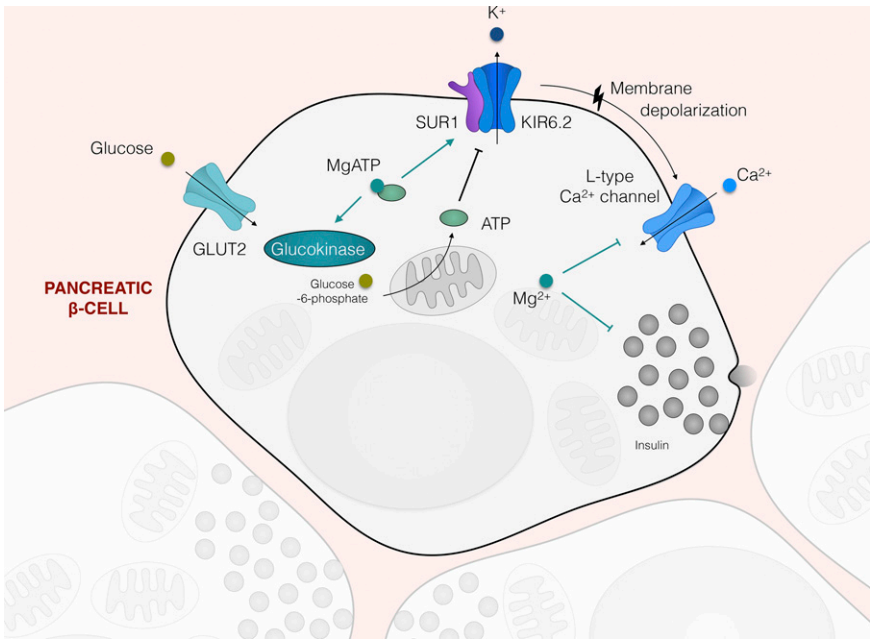

Figure 2—Mg^{2+} regulates insulin secretion in pancreatic β-cells. In pancreatic β-cells, Mg^{2+} directly influences the rate of glucokinase activity by acting as a cofactor for adenine nucleotides. The product of this enzymatic reaction, G6P, is further processed in glycolysis producing ATP. Closure of the K_{ATP} channel is dependent on ATP by its binding to the Kir6.2 subunits. Conversely, MgATP initiates channel opening by binding to the SUR1 subunits of the channel. Importantly, the physiological consequence of channel closure is the depolarization of the membrane, which triggers the influx of Ca^{2+} via the L-type Ca^{2+} channels. This final step initiates insulin vesicle release, which is negatively controlled by Mg^{2+} acting on both Ca^{2+} influx and the L-type Ca^{2+} channels.

Mg^{2+} concentration in a Canadian cohort of patients with T2DM (50). However, this study focused mainly on the lipid metabolism and did not fully explore the effects on insulin secretion. Supplementation of individuals without diabetes with $MgCl_2$ significantly increased β-cell function in a small randomized clinical trial (51). Here, we will examine the molecular mechanisms by which Mg^{2+} improves insulin secretion by showing how Mg^{2+} acts on the main actors involved in insulin secretion.

Glucokinase

After entering the pancreatic β-cells via GLUT2, glucose is converted to G6P by glucokinase. The action of glucokinase depends on $MgATP^{2-}$. Recent studies show that the activity of glucokinase is regulated by $MgATP^{2-}$ at subphysiological concentrations (reported K_M ~0.15 mmol/L), suggesting that there is always sufficient $MgATP^{2-}$ available in the cell for glucokinase activity (52). However, there may exist a small cooperative effect of $MgATP^{2-}$ on glucose binding (52,53). Mg^{2+} deficiency may, therefore, reduce glucose binding to glucokinase by slowing down metabolism and indirectly reducing insulin secretion.

Glycolysis

G6P is further metabolized via glycolysis and the Krebs cycle, resulting in increased ATP levels. Although many enzymes in this metabolic process use MgATP as a cofactor (54), studies in erythrocytes have shown that glycolysis depends on intracellular Mg^{2+} with a half-maximal glycolysis flux at 0.03 mmol/L Mg^{2+}. This is well below physiological intracellular Mg^{2+} values, even in Mg^{2+} deficient conditions (55).

K_{ATP} Channel

First described in 1984, K_{ATP} channels are the main regulators of the membrane potential in pancreatic β-cells (56). The K_{ATP} channel in β-cells consists of four Kir6.2 subunits and four regulatory sulfonylurea receptor (SUR)1 subunits, whose activity is controlled by the intracellular ATP-to-ADP ratio. In the presence of Mg^{2+}, it is the balance between MgATP, physiologically ranging between 1 and 5 mmol/L, and MgADP levels that determines channel activity. Binding of both MgATP and MgADP to the nucleotide-binding sites of SUR1 subunits results in opening of the channel. By contrast, in the absence of Mg^{2+}, ATP induces closure of the K_{ATP} channel by directly binding to Kir6.2 subunits (57). In high-glucose conditions, increased glycolysis shifts the balance toward ATP, concomitantly leading to reduced MgADP levels inducing channel closure and increased insulin secretion (58). This balance is the therapeutic target of sulfonylurea drugs, which induce channel closure by antagonizing the binding of Mg nucleotides on SUR1 (59). Thus, reduced intracellular Mg^{2+} concentrations, as a result of hypomagnesemia in T2DM, may reduce the MgATP and MgADP levels, favoring inhibition of the K_{ATP} channel and thereby indirectly stimulating insulin secretion. However, the exact effect of hypomagnesemia on intracellular MgATP levels remains to be examined.

L-Type Ca^{2+} Channel

Inhibition of K_{ATP} channels by increased ATP levels results in depolarization of the membrane, activating Ca^{2+} influx via the voltage-dependent L-type Ca^{2+} channel. In the pancreatic β-cell, the main L-type channels are of the $Ca_v1.2$ and $Ca_v1.3$ subtypes, of which $Ca_v1.2$ channels are the main contributors to insulin secretion (60,61). There is a wide range of literature showing that both intracellular and extracellular Mg^{2+} inhibits L-type Ca^{2+} channels (62–64). Although these findings suggest that hypomagnesemia would increase L-type channel activity in the short-term, in the long-term it has been indicated that hypomagnesemia reduces the expression of L-type Ca^{2+} channels and, thus, indirectly diminishes insulin secretion (65). However, it should be noted that these findings rely on measurements performed with cardiomyocytes and have not been confirmed in pancreatic β-cells.

Insulin Vesicle Release

Insulin vesicle release depends on Ca^{2+} binding that initiates exocytosis (45). Mg^{2+} fulfills an essential role in antagonizing Ca^{2+} and thus regulates insulin secretion by pancreatic β-cells. Atwater et al. (66) studied the importance of the Ca^{2+}-to-Mg^{2+} ratio in glucose-induced insulin release in perfused rat pancreas and mouse islets. The results indicated that only a reduction of the physiological Mg^{2+} concentrations stimulated insulin secretion. Conversely, insulin secretion was inhibited only by diminished Ca^{2+} levels. Interestingly, a simultaneous reduction in Ca^{2+} and Mg^{2+}, while maintaining their ratio constant, did not change the insulin release. Mg^{2+} deficiency inevitably changes the Ca^{2+}-to-Mg^{2+} ratio and may therefore affect insulin secretion.

Novel Perspectives

Altogether, the regulation of K_{ATP} channels, L-type Ca^{2+} channels, and vesicle release points toward an inhibitory effect of Mg^{2+} on insulin secretion. These studies suggest that Mg^{2+} deficiency stimulates insulin release by the β-cells. However, a recent clinical study showed a positive correlation between Mg^{2+} concentration and first-phase insulin secretion (15). Why do hypomagnesemic T2DM patients suffer from reduced insulin secretion then? There may be multiple factors contributing to this paradox: *1*) many of the studies of K_{ATP} channels and L-type Ca^{2+} channels are executed in cardiac cells and may therefore not be a good representation of the effects of Mg^{2+} in the pancreas; *2*) most studies investigate short-term effects of Mg^{2+} on channel activity and may not represent long-term Mg^{2+} deficiency; *3*) it has been shown that Mg^{2+} increases insulin synthesis at the transcriptional level (67); *4*) all studies have focused on the effects of intracellular Mg^{2+}, leaving the role of extracellular Mg^{2+} unknown; *5*) Mg^{2+} is an important regulator of protein synthesis and cell proliferation (68) and may therefore regulate β-cell viability; and *6*) Mg^{2+} deficiency may also regulate other factors, including serum K^+ and Ca^{2+}, and thereby indirectly affect insulin signaling. However, it should be emphasized that the role of Mg^{2+} in insulin secretion has only been examined by a small number of clinical studies. The current clinical evidence on the effect of Mg^{2+} on insulin secretion is too limited to draw firm conclusions on the physiological implications of Mg^{2+} in the pancreatic β-cell. Future clinical and experimental studies are necessary to resolve this question.

Mg^{2+} Homeostasis

Cellular Mg^{2+} Homeostasis

Since protein and DNA synthesis is highly dependent on intracellular Mg^{2+} availability, intracellular Mg^{2+} concentrations are tightly regulated (12). Intracellular Mg^{2+} concentrations are determined by the uptake via dedicated Mg^{2+} channels and transporters, including solute carrier family 41 member 1 (SLC41A1), magnesium transporter 1 (MagT1), and transient receptor potential melastatin type 6 and 7 (TRPM6 and TRPM7). The role of these Mg^{2+} transporters in the establishment of Mg^{2+} homeostasis has been reviewed in detail (12). Several groups have investigated the association between genetic variations in these Mg^{2+} transporters and risk for T2DM (69–71). Until now, a link between T2DM and TRPM7 or MagT1 has not been found. However, single nucleotide polymorphisms (SNPs) in TRPM6 or SLC41A1 have been associated with increased risk for T2DM (69,71). Interestingly, insulin may be an important regulator of cellular Mg^{2+} uptake. Studies in erythrocytes showed that insulin reduces serum Mg^{2+} levels and increases cytosolic Mg^{2+} concentrations (72). Indeed, insulin increases both glucose and Mg^{2+} uptake in pancreatic β-cells and cardiomyocytes, suggesting that glucose and Mg^{2+} homeostasis are linked (73,74). However, the molecular identity of the Mg^{2+} transporters involved in this mechanism has not been identified to date.

Body Mg^{2+} Homeostasis

Mg^{2+} homeostasis in the body is facilitated by the interplay of intestinal absorption, bone Mg^{2+} storage, and renal Mg^{2+} excretion (12). In the kidney, the bulk of filtered Mg^{2+} is reabsorbed passively in the proximal tubule (10–25%) and thick ascending limb of Henle loop (TAL) (60–80%). However, fine-tuning takes place in the distal convoluted tubule (DCT) (5–15%) where transcellular reabsorption determines the final urinary Mg^{2+} excretion, since no reabsorption takes place beyond the DCT (12).

In the DCT, Mg^{2+} is reabsorbed from the pro-urine by TRPM6 channels (12). Patients with TRPM6 mutations suffer from hypomagnesemia with secondary hypocalcemia (Mendelian Inheritance in Man: 602014) due to renal Mg^{2+} wasting (75,76). TRPM6 is highly regulated by dietary Mg^{2+} availability, epidermal growth factor, estrogen, pH, and ATP (77–81). Conversely, vitamin D and parathyroid hormone do not regulate Mg^{2+} reabsorption in the DCT (77). The transepithelial movement of Mg^{2+} is dependent on the electrochemical gradient that is set by Na^+-K^+-ATPase activity and depends on the local recycling

of K^+ transport via $K_v1.1$ at the apical membrane and Kir4.1 at the basolateral membrane (82–84). Although the basolateral Mg^{2+} extrusion mechanism in the DCT is still under debate, recent publications suggest that this might be facilitated by the SLC41A1 Na^+-Mg^{2+} exchanger and is regulated by cyclin M2 (85–87).

Insulin Regulates Mg^{2+} Reabsorption in the Kidney

Hypomagnesemia in T2DM is primarily due to renal Mg^{2+} wasting (88). Insulin extracts from animal pancreas were introduced in medicine in the early 1920s saving the lives of many T2DM patients (89). The first article reporting increased blood Mg^{2+} and Na^+ levels during treatment with impure insulin extracts was published in 1933 (90). However, it was not until the 1960s, when synthetic insulin was available and Mg^{2+} measurements were improved, that it became apparent that insulin regulates Mg^{2+} reabsorption in the kidney (91). Indeed, microperfusion experiments in mouse TAL segments show increased Mg^{2+} permeability after insulin stimulation (92). Moreover, in mouse DCT cells insulin stimulates Mg^{2+} uptake (93). Therefore, we will present an overview of the molecular targets of insulin in the regulation of renal Mg^{2+} transport.

TRPM6

TRPM6 was identified as the molecular target of insulin signaling in 2012 (94) (Fig. 3). Upon insulin binding to the receptor, an intracellular signaling cascade including phosphatidylinositol 3-kinase, Akt, and Rac1 is activated resulting in increased insertion of TRPM6 in the plasma membrane. Two SNPs in TRPM6 (p.Val1393Ile [rs3750425] and p.Lys1584Glu [rs2274924]) are associated with increased susceptibility for gestational diabetes mellitus (71). Patch clamp analysis showed that these mutations render the channel insensitive to insulin stimulation (94). Follow-up studies could not confirm associations between serum Mg^{2+} values and these two SNPs (69,95). However, these differences may be explained by the dietary Mg^{2+} intake of the subjects, since patients in the original study showed reduced Mg^{2+} intake (<250 mg/day) (71). The mRNA expression of TRPM6 is changed in diabetic rats, although results are contradictory and may depend on the experimental model used (96,97). Whereas some report increased TRPM6 expression (96), others show that TRPM6 is downregulated (97). Given that hypomagnesemia itself also stimulates TRPM6 expression (77,98), it is difficult to distinguish between the effect of hypomagnesemia and T2DM in these studies.

Na^+-Cl^- Cotransporter

In addition to its actions on TRPM6, insulin signaling in DCT increases Na^+ reabsorption via the thiazide-sensitive Na^+-Cl^- cotransporter (NCC) (99–103). Insulin activates an intracellular signaling cascade that includes mTOR complex 2 (mTORC2) and stress-activated protein kinase/oxidative stress responsive kinase (SAPK/OSR1) to increase NCC phosphorylation and activity (99,101,102) (Fig. 3). It has been hypothesized that

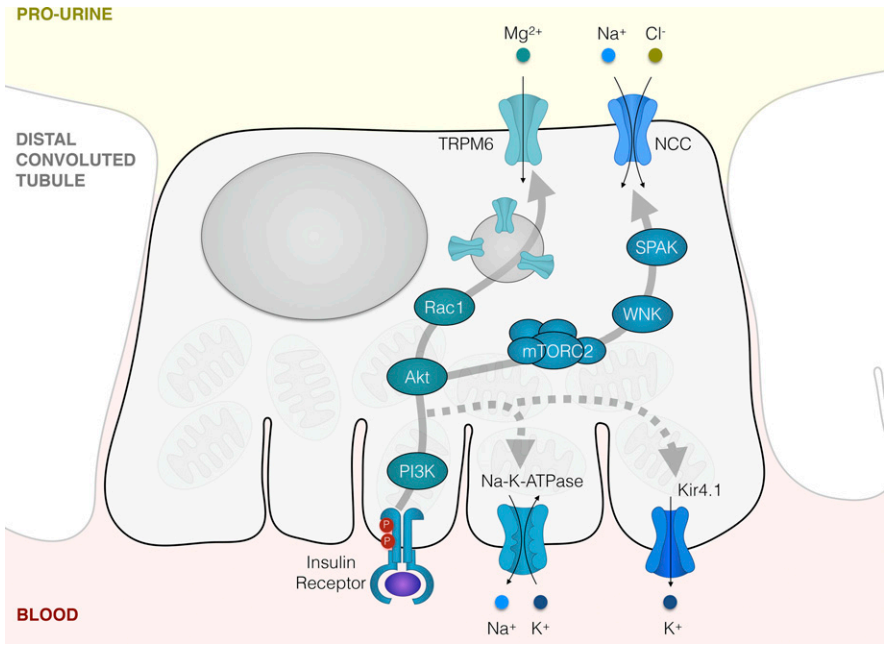

Figure 3—Insulin regulates electrolyte reabsorption in the distal convoluted tubule. Upon binding of insulin to its receptor, an intracellular signaling cascade including phosphatidylinositol 3-kinase (PI3K) and Akt increases the plasma membrane expression of the TRPM6 Mg^{2+} channel and the thiazide-sensitive NCC. Additionally, insulin may stimulate the activity of other channels, such as K^+ channel Kir4.1 and the Na^+-K^+-ATPase. SPAK, STE20/SPS1-related, proline-alanine-rich kinase; WNK, with-no-lysine [K] kinases.

hyperinsulinemia in T2DM causes increased NCC activation and thus renal Na^+ reabsorption, contributing to hypertension that is present in 75% of T2DM patients (104). This hypothesis is supported by studies in Zucker obese rats and db/db mice showing hyperinsulinemia, hypertension, and increased NCC activity (100,101).

Diabetes-DCT Paradox

Central in the theory of insulin-induced hypertension is that the hyperinsulinemic state causes overactivation of the insulin pathway resulting in increased NCC activity (99,101). In contrast, insulin resistance has been proposed to explain the reduced TRPM6 activity and hypomagnesemia in T2DM (94). Until now, it has been widely accepted that Na^+ and Mg^{2+} transport in the DCT go hand in hand, since reduced NCC activity causes hypomagnesemia as shown in patients with Gitelman syndrome and in users of thiazide diuretics (105,106). However, in T2DM, Na^+ and Mg^{2+} reabsorption in DCT may be uncoupled, resulting in increased Na^+ reabsorption and decreased Mg^{2+} reabsorption. Elucidation of the molecular mechanisms of Na^+ and Mg^{2+} cross talk and studies of the insulin resistance of DCT cells in T2DM may contribute to better understanding of the diabetes-DCT paradox.

Kir4.1 and Na^+-K^+-ATPase

Reports on diabetic retinopathy suggest that insulin regulates the potassium channel Kir4.1 in the retina (107,108). Given that Kir4.1 is also an important regulator of Na^+ and Mg^{2+} transport in DCT, and patients with mutations in Kir4.1 develop hypomagnesemia and hypokalemia, future studies should include regulation of Kir4.1 in T2DM patients (109,110).

Additionally, insulin has been shown to increase Na^+-K^+-ATPase activity in several tissues including heart and muscle (111,112). Na^+-K^+-ATPase activity is decreased in animal models of T2DM (reviewed in 113,114). Within the DCT, the membrane expression of the Na^+-K^+-ATPase is regulated by TBC1 domain family, member 4 (TBC1D4), which is a substrate of Akt in the insulin pathway (115). Given that the expression of TBC1D4 in the DCT is regulated by dietary Mg^{2+} intake (98), the regulation of the Na^+-K^+-ATPase may be changed in hypomagnesemia via TBC1D4.

Other Factors Regulating Mg^{2+} Transport in T2DM

In the complex context of T2DM, many factors may play a role in the development of hypomagnesemia. In contrast to the well-described effects of insulin on renal electrolyte handling, other contributing factors are poorly studied. Therefore, rather than providing a definitive overview of impaired renal regulation of Mg^{2+} transport in T2DM, this part of the article aims to highlight contributors to hypomagnesemia that are underappreciated in current literature and should be subject for further examinations.

Glucose

Initial experiments in 1970s and 1980s showed that glucose increases urinary Mg^{2+} excretion (116,117). However, the molecular mechanism by which glucose regulates renal Mg^{2+} handling is unknown, and these early experiments do not take into account the action of insulin or diuresis. Recently, the Na^+-GLUT2 sodium–glucose cotransporter 2 (sglt2) KO mouse, which is characterized by a marked glucosuria, was shown to have increased urinary Mg^{2+} excretion (118). These findings raise clinical concerns for the use of SGLT2 inhibitors. However, clinical trials with SGLT2 inhibitors have not provided any evidence that Mg^{2+} excretion is changed by SGLT2 inhibitor intake (119,120). Given the substantial glucosuria in T2DM, the physiological role of glucose in Mg^{2+} reabsorption can be questioned.

Hyperfiltration

Increased blood glucose values in T2DM result in hyperfiltration and increased renal urinary flow (121,122). Approximately 10–25% of the filtered Mg^{2+} is reabsorbed by the proximal tubules where water reabsorption precedes Mg^{2+} reabsorption, creating a favorable electrochemical gradient for Mg^{2+} reabsorption. Micropuncture studies have shown that a 1.9 ratio between the concentrations of Mg^{2+} in the tubular fluid and the interstitial fluid is necessary for passive Mg^{2+} reabsorption (123). Consequently, increased urinary volume in T2DM patients will dilute the Mg^{2+} concentration in the pro-urine reducing the transepithelial chemical Mg^{2+} gradient in the proximal tubule. Mg^{2+} reabsorption in TAL and DCT is inversely correlated to urinary flow. Given that increased glomerular filtration results in high urinary flow rates, hyperfiltration may reduce Mg^{2+} reabsorption in T2DM patients.

Oxidative Stress

One of the main contributors to diabetic nephropathy is the oxidative stress in the kidney (124). Interestingly, oxidative stress has been shown to reduce TRPM6 activity (125), and as a result, Mg^{2+} uptake may be diminished in people with diabetes. Previously, methionine sulfoxide reductase B1 (MSRB1) was shown to prevent the effects of oxidative stress on TRPM6 by reducing oxidation of the channel. However, studies of rats with STZ-induced diabetes showed reduced *Msrb1* expression (126). Thus, oxidative stress may contribute to hypomagnesemia in T2DM.

CONCLUSIONS AND PERSPECTIVES

Over the past two decades, there has been a staggering amount of clinical evidence showing a tight association between hypomagnesemia and T2DM. A recent cross-sectional study has shown that hypomagnesemia is associated with an increased risk for complications, including retinopathy, nephropathy, and foot ulcers (127). Importantly, Mg^{2+} supplementation improved insulin sensitivity and metabolic control in a double-blind randomized trial, suggesting that Mg^{2+} is an important factor in the etiology and management of T2DM (9,10). So far, the clinical trials that have been performed using Mg^{2+} supplementation to improve T2DM have mainly focused on general parameters such as blood glucose or HbA_{1c} levels. Therefore,

well-designed, double-blind randomized trials in T2DM patients with hypomagnesemia studying the long-term effects of Mg^{2+} supplementation on T2DM pathophysiology and disease progression are now warranted.

The prevalence of hypomagnesemia in T2DM has been reported to range between 14 and 48%, meaning that millions of people worldwide are affected (4). Nevertheless, serum Mg^{2+} levels are not routinely determined in T2DM patients. Provided that hypomagnesemia is associated with conditions that are often present in T2DM, including hypertension, hypokalemia, and muscle cramps, more clinical attention is necessary to address this problem (12). Patients using widely prescribed drugs such as thiazide diuretics, proton pump inhibitors, and calcineurin inhibitors are especially at risk for developing hypomagnesemia and should be closely monitored (128,129). Additionally, patients with diabetic neuropathy who have episodes of diarrhea may suffer from intestinal malabsorption of Mg^{2+}, which is another risk factor for hypomagnesemia.

Despite the widespread clinical evidence of the association of hypomagnesemia and T2DM, the molecular mechanisms of Mg^{2+} on insulin secretion and insulin resistance are still far from understood. Currently, the strongest line of evidence supports an effect of Mg^{2+} on insulin sensitivity (24–27). Small-scale fundamental studies have shown that Mg^{2+} is essential for insulin receptor phosphorylation, but the effect of Mg^{2+} on downstream targets in the muscle, liver, and adipocytes is largely unknown. The role of Mg^{2+} in the regulation of insulin secretion is more controversial and hampered by the limited number of clinical and experimental studies. In contrast, there have been significant advances demonstrating the important role of insulin in the regulation of Mg^{2+} reabsorption via TRPM6 in the kidney. Insulin resistance reduces renal Mg^{2+} reabsorption resulting in urinary Mg^{2+} wasting. As a consequence, people with diabetes may end up in a vicious circle in which hypomagnesemia enhances insulin resistance and insulin resistance causes hypomagnesemia. However, the picture is still far from complete, and more studies are required to fully understand the complex and dynamic role of Mg^{2+} in T2DM.

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