# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**5,500**
Open access books available

**135,000**
International authors and editors

**165M**
Downloads

Our authors are among the

**154**
Countries delivered to

**TOP 1%**
most cited scientists

**12.2%**
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

## Interested in publishing with us?
## Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 4

# Role of Magnesium in the Regulation of Hepatic Glucose Homeostasis

Chesinta Voma and Andrea M.P. Romani

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/57564

## 1. Introduction

### 1.1. The liver and glucose metabolism

The liver comprises of hepatocytes, biliary epithelial cells, stellate cells (or Ito cells), Kupffer cells, sinusoid endothelial cells, and pit cells [1,2]. Most of the clinically quantifiable liver functions such as metabolic processes and protein synthesis take place within the hepatocytes, while non-hepatocyte cells are responsible for other functions including inflammatory response (Kupffer cells), collagen deposition (Ito cells), and cell orientation [2-5]. Regulation of blood glucose is one of the main functions exerted by the liver. The organ contains a dynamic storage of glycogen that is rapidly dismissed into the circulation as glucose to maintain glycemia and support brain functions. Hence, hepatocytes are enzymatically specialized to switch rapidly between glycogenolysis and glycogenosynthesis based upon hormonal stimuli and metabolic conditions.

Glucose enters the hepatocytes through the low-affinity transporter GLUT2 (Km=15-20 mM, Table 1). At variance of GLUT1 and GLUT4 glucose transporter that possess a Km=1-5mM and are therefore constitutively active near their maximal rate under euglycemic conditions (i.e. between 60 to 100 mg/dl), GLUT2 is maximally activated following a meal. The high Km of GLUT2 (~15-20mM) correlates well with the high Km glucokinase responsible for the conversion of glucose to glucose 6-phosphate [6].

Glucose 6-phosphate (G6P) can be routed towards glycogenosynthesis, glycolysis, or oxidation by the cytoplasmic glucose 6-phosphate dehydrogenase, *de facto* entering the pentose shunt pathway, an alternative path that generates ribose 5-phosphate utilized in nucleic acid formation (cell cycle) or return as glucose 6-phosphate to be used once again as most convenient for the cell (Fig.1). Glucose 6-phosphate is also transported into the endoplasmic reticulum

INTECH
open science | open minds

© 2014 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

| Transporter | Affinity for Glucose (Km) | Location |
|---|---|---|
| Glut1 | 1-2 mmol/L | ubiquitous |
| Glut2 | 15-20 mmol/L | hepatocytes, β-cells |
| Glut3 | 1-2 mmol/L | ubiquitous |
| Glut4 | 3.5-8 mmol/L | skeletal muscles, adipocytes |

**Table 1.** Glucose Transporters

to undergo hydrolysis via glucose 6-phophatase, or oxidation via the hexose 6-phosphate dehydrogenase, the reticular variant of the glucose 6-phosphate dehydrogenase [7].

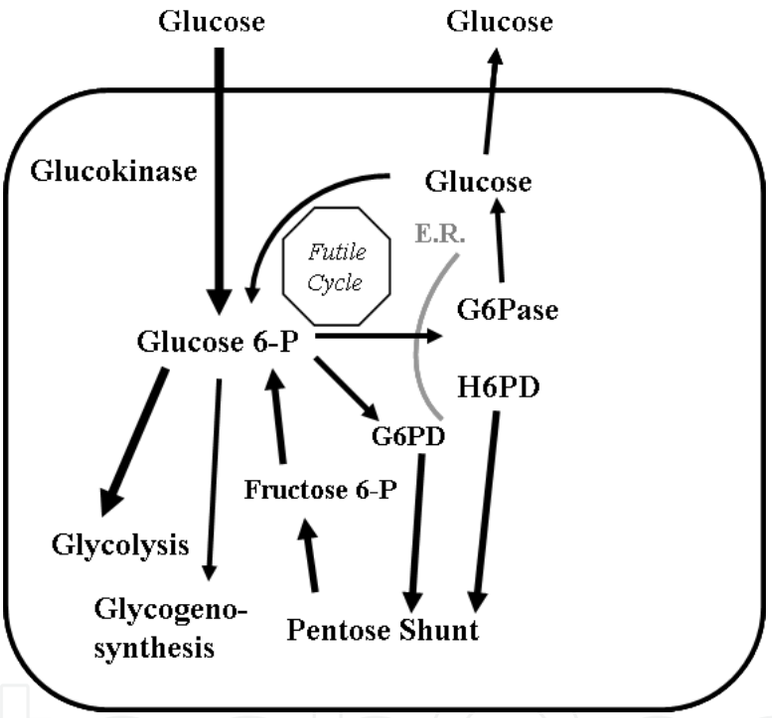


**Figure 1.** Cartoon depicting the different destinies of glucose 6–phosphate (G6P) within the hepatocyte.

The modality whereby glucose 6-phosphate enters the hepatic E.R. lumen is still debated. The *substrate-transport* theory postulates that G6P enters the ER lumen via a specific transporter (T1) distinct from the glucose 6 phosphatase. In this model, T1 represents the rate-limiting factor for the G6Pase system [8]. The *conformational flexibility substrate-transport* theory proposes that the G6Pase enzyme possesses a hydrophilic region that spans the E.R. membrane and project into the cytoplasm. This region is specific for substrate binding and is distinct from the hydrolytic site. Upon binding to glucose 6-phosphate this cytoplasmic site of the protein undergoes a conformational change and delivers the substrate to the intra-luminal catalytic site. According to this model, the substrate binding site and a hydrolytic site of the G6Pase are two parts of the same protein, and the enzyme is not specific for a particular substrate [9].

http://dx.doi.org/10.5772/57564

Irrespective of the modality of entry, the hydrolysis of glucose 6-phosphate by the hydrolytic site of the glucose 6-phosphatase generates two byproducts, glucose and inorganic phosphate (Pi), which are released into the cytoplasm via two additional, specific transport mechanisms [8-11]. The glucose exported out of the ER is either earmarked for glucose output from the hepatocyte into the bloodstream or is converted anew to glucose 6-phosphate by the glucokinase thereby contributing to the glucose-glucose 6-phosphate *futile* cycle [12]. The inorganic phosphate (Pi) is either exported out of the ER lumen through its specific transporter, or forms a complex with the $Ca^{2+}$ions that are actively transported into the ER lumen by the SERCA pumps [13]. Far from being static and irreversible, this Ca*Pi complex promotes an enlargement of the reticular $Ca^{2+}$pool within the hepatocyte, and it can be dynamically reversed to $Ca^{2+}$and Pi, with both moieties being mobilized out of the ER following $IP_3$–induced $Ca^{2+}$-release [13]. Thus, this enlargement of the reticular $Ca^{2+}$pool is an integral part of the hepatic response to hormones such as vasopressin or norepinephrine that tap into the $IP_3$-related $Ca^{2+}$-response for metabolic and functional purposes [14].

Further investigation is required to fully elucidate the functional implications of the reticular hexose 6-phosphate dehydrogenase. This enzyme also utilizes the glucose 6-phosphate transported into the E.R., oxidizing it to 6-phosphogluconolactone [7]. Essentially, this enzyme performs the first two steps of the pentose shunt pathway within the E.R. [7.] and is responsible for maintaining a reduced pyridine nucleotide pool ($NADPH/H^+$) within the E.R. to be utilized in various reticular functions including E.R. stress regulation [15]. Presently, it is unknown whether the expression and activity of the hexose 6-phosphate dehydrogenase (H6PD) change as a result of hormonal stimuli, metabolic conditions, or liver pathologies.

The liver plays a critical role in maintaining blood glucose levels within the normal range during the fed-fast cycle. During early fasting, hepatic glycogenolysis and glucose output from the organ maintains glycemia within a suitable range for brain function and metabolism. As the amount of glycogen stored within the liver (i.e. ~10% of the organ weight) is not sufficient to maintain glycemia over an extended period of time or prolonged fasting, gluconeogenesis becomes essential to synthesize glucose from amino acids, lactate and pyruvate dismissed into the circulation by skeletal muscles through glycogenolysis and glycolysis, and from glycerol dismissed by adipose tissue through lipolysis.

The complex metabolic scenario of fed to fast cycling is maintained through the antagonistic roles of insulin on one side, and glucagon, catecholamine and glucocorticoids on the other side. All these hormones modulate liver metabolism through the glucose to glucose 6-phosphate futile cycle [12], with insulin inhibiting the glucose 6-phosphatase activity and expression, and the pro-glycemic hormones increasing them.

During fed and postprandial states, elevation in blood glucose level promptly increases insulin secretion from pancreatic β-cells, which in turn, decreases glucagon release from pancreatic $\alpha$-cells. The combined effect of these hormonal changes decreases hepatic glucose output and production by suppressing gluconeogenesis and glycogenolysis while increasing glucose storage within skeletal muscles via glycogenosynthesis and adipocytes via lipogenesis. In addition, insulin promotes glucose utilization in peripheral tissues through activation of glycolysis [16].

## 1.2. Physiological magnesium homeostasis

### *1.2.1. Cellular magnesium distribution*

Our body absorbs minerals through food and drinks consumed daily. However, industrial food processing techniques limit to a varying extent the dietary content and intake of minerals and vitamins, making necessary the utilization of supplements. This is indeed the case of the macro mineral magnesium. Overall, $Mg^{2+}$is the fourth most abundant cation in vertebrates and the second most abundant cation within cells after potassium. In humans, total body magnesium ($Mg^{2+}$) is found mostly in the bones (60-65% of total content), soft tissues and cells in general [17]. Only 1% of total body magnesium is found in the extracellular fluid, thus making serum magnesium level a poor indicator of total magnesium content and availability in the body. Of the 1% total body $Mg^{2+}$present in the extracellular fluid, about sixty percent (60%) is free, the reminder (~33%) being bound to proteins, citrate, bicarbonate, ATP[1] and phosphate (≤7%) [18].

Whole body $Mg^{2+}$homeostasis changes overtime. At an early stage, most $Mg^{2+}$in the bones can readily exchange with serum, representing an optimal store to compensate for occasional dietary deficiency. As age progresses, however, the proportion of readily exchangeable $Mg^{2+}$in the bones decreases significantly due to a change in bone crystal size with age [19]. In individuals consuming $Mg^{2+}$enriched diet, a positive association between bone mineral density and $Mg^{2+}$content within the erythrocytes has been reported [20].

At the cellular level, $Mg^{2+}$is highly compartmentalized within nucleus, endoplasmic or sarcoplasmic reticulum, mitochondria, and cytoplasm [18], the only notable exception being the erythrocytes, in which $Mg^{2+}$is merely cytoplasmic [21]. In the majority of mammalian cells examined, including the hepatocytes, total cellular $Mg^{2+}$concentrations range from 15 to 20mM as measured by various techniques including electron X ray microprobe analysis (EXPMA), fluorescent dyes, and scanning fluorescence x-ray microscopy [21]. Total $Mg^{2+}$concentrations between 15 and 20mM have also been measured within the nucleus, the mitochondria, and the rough endoplasmic reticulum of various cell types by EPXMA [21]. In the cytoplasm, $Mg^{2+}$is present as a complex with ATP (~4-5mM=Mg*ATP) and other phosphonucleotides [22]. Consequently, the free $Mg^{2+}$concentration ($[Mg^{2+}]_i$) within the cytoplasm and the mitochondrial matrix ranges between 0.5 and 1.2 mM [21,23], i.e. slightly below or at the concentration present in the extracellular environment. These measurements suggest that the majority of mammalian cells are near *zero trans* conditions as far it concerns the cellular distribution of $Mg^{2+}$.

Despite the large amount of $Mg^{2+}$present within the majority of mammalian cells, limited information is available about the physiological role of $Mg^{2+}$for specific cell function. In liver cells, $Mg^{2+}$controls ATP production by the mitochondria and its utilization by various ATPases including the $Na^+/K^+$-ATPase [21] and the reticular $Ca^{2+}$-ATPase [21]. As a result, 90% of cytoplasmic ATP is in the form of a complex with $Mg^{2+}$[24]. Moreover, in hepatocytes $Mg^{2+}$is a cofactor for many enzymes involved in energy metabolism, including glycolysis and Krebs cycle [25]. The list of $Mg^{2+}$-regulated glycolytic enzymes includes hexokinase, phosphofructokinase, aldolase, phosphoglycerate kinase and pyruvate kinase [26]. The regulation of specific

http://dx.doi.org/10.5772/57564

enzymes or channels by $Mg^{2+}$is not restricted to the cytoplasm occurring also in the cellular organelles in which $Mg^{2+}$is compartmentalized. In liver mitochondria, changes in matrix $Mg^{2+}$content regulate the activity of succinate and glutamate dehydrogenases but not $\alpha$-ketoglutarate dehydrogenases [23]. In addition, $Mg^{2+}$regulates the opening of the inner mitochondrial anion channel (IMAC), the permeability transition pore (PTP), $K_{ATP}$-channels, and possibly the $H^+/K^+$exchanger, thus regulating the organelle volume [23]. It is still unresolved as to whether $Mg^{2+}$is required for the adenine nucleotide translocase to operate [23]. At the level of the hepatic rough endoplasmic reticulum (R.E.R.), $Mg^{2+}$regulates $Ca^{2+}$uptake via the Ca-ATPase, and its release through the $IP_3$ receptor [27], as well as the rate of protein synthesis and dismissal into the cytoplasm via the translocon [28]. Experimental evidence suggests that $Mg^{2+}$inversely regulates the rate of glucose 6-phosphate entry into the E.R. lumen, thus providing higher level of substrate to the glucose 6-phosphatase (G6Pase), and the hexose 6-phosphate dehydrogenase (H6PD) under conditions in which cellular $Mg^{2+}$levels are reduced [29]. In the nucleus, changes in $Mg^{2+}$content have been associated with inhibition of specific endonucleases and chromatin folding [21]. Less known is the function of $Mg^{2+}$within the Golgi lumen. The recent localization of a $Mg^{2+}$transporter in the Golgi cisternae, however, suggests a possible role of the cation in regulating protein glycosylation [23]. As for endosomal and lysosomal vesicles, nothing is known about the $Mg^{2+}$concentration within these vesicles and its role in modulating their physiological processes.

Despite its large total concentration within the cell, $Mg^{2+}$is not a static cation. Major $Mg^{2+}$fluxes have been detected across the cell membrane of the hepatocyte and other mammalian cells. Various hormones and pharmacological agents modulate total and free $Mg^{2+}$concentrations within the hepatocyte, supporting the hypothesis that many of the metabolic changes elicited by these agents are attained by changing the concentration of $Mg^{2+}$within the cells and/or within specific cellular compartments, which then results in the up-or down-regulation in the activity of Mg-sensitive enzymes.

### *1.2.2. Cellular magnesium transport mechanisms*

The current understanding of $Mg^{2+}$transport across the hepatocyte cell membrane indicates that $Mg^{2+}$exits the liver cell via a $Na^+/Mg^{2+}$exchanger [30,31], which functionally depends on the physiological concentration of extracellular $Na^+$[30,32] and the cellular level of cAMP [33], which activates the exchanger through phosphorylation [34]. Under conditions in which limited inward $Na^+$gradient is present across the cell membrane, or $Na^+$transport is inhibited by agents like amiloride or imipramine, cellular $Mg^{2+}$is extruded via a $Na^+$-independent mechanism that utilize different cations or anions in counter-transport for or co-transport with $Mg^{2+}$, respectively [32].

As for $Mg^{2+}$entry, hepatocytes appear to utilize predominantly the TRPM7 channel [35]. Protein kinase C (PKC) appears to regulate this channel directly via phosphorylation of its C terminus, or indirectly by removing RACK1-inhibition [36].

Several other $Mg^{2+}$entry mechanisms have been observed to be present in liver cells [32] but it is still unclear to which extent these mechanism cooperate with TRPM7 in mediating $Mg^{2+}$entry and in regulating hepatic $Mg^{2+}$homeostasis.

*1.2.3. Regulation of magnesium transport*

The specific modality of operation and regulation of the various $Mg^{2+}$transport mechanisms have been extensively addressed in recent review articles [32,37-39], and we refer to those reviews for further information. For the purpose of this chapter, we will only mention that in liver cells both $Mg^{2+}$entry and extrusion are under hormonal control. Hormones like catecholamine and glucagon, which increase cAMP level within the hepatocyte, all promote $Mg^{2+}$extrusion by phosphorylating the $Na^+/Mg^{2+}$exchanger mentioned above [40]. Activation of $\alpha_1$-adrenoceptors by catecholamine also induces $Mg^{2+}$extrusion. Stimulation of this class of adrenergic receptors activates PLC$\gamma$, which in turn hydrolyzes phosphatidyl-inositol bisphosphate (PIP2) to diacylglycerol and inositol 1,4,5-trisphosphate ($IP_3$). The $IP_3$-induced $Ca^{2+}$release from the endoplasmic reticulum and the subsequent capacitative $Ca^{2+}$entry through the hepatocyte plasma membrane promote $Mg^{2+}$extrusion from the hepatocyte, most likely by displacing $Mg^{2+}$for $Ca^{2+}$from organelle and cytosolic binding sites [41]. This is consistent with the observation that distinct $Na^+$and $Ca^{2+}$-dependent $Mg^{2+}$extrusion mechanisms operate in the basolateral and the apical portion of the hepatocyte cell membrane, respectively [34,42]. The differential activation of the $Na^+$-dependent ($\beta$-adrenergic receptors and glucagon receptor) and the $Ca^{2+}$-dependent $Mg^{2+}$extrusion mechanism ($\alpha_1$-adrenergic receptors) points to the ability of the hepatocytes to activate $Mg^{2+}$extrusion by different modalities and circumvent possible inhibitory mechanisms. It has to be noted, in fact, that insulin pre-treatment abolishes the $Mg^{2+}$extrusion mediated by cAMP but not that mediated via $\alpha_1$-adrenoceptor activation [43]. Conversely, hormones or agents that maximize $Ca^{2+}$release from the ER elicit a time-dependent inhibition of $\alpha_1$-adrenergic receptor mediated $Mg^{2+}$extrusion that leaves unaffected the extrusion occurring via $\beta$-adrenergic receptors stimulation and cellular cAMP elevation [44]. In this contest, it has to be noted that cytoplasmic free $[Mg^{2+}]i$ modulates adenylyl cyclase activation in a variety of cell types including hepatocytes [45]. Under resting conditions, cytoplasmic $[Mg^{2+}]i$ is insufficient to activate the adenylyl cyclase maximally. Following $\beta$-adrenoceptor or glucagon receptor stimulation the cytoplasmic $Mg^{2+}$pool increases markedly but transiently via the release of $Mg^{2+}$from other cellular pool (namely mitochondria and endoplasmic reticulum) promoting adenylate cyclase activity and cAMP synthesis [45]. Elevation of cytoplasmic $[Mg^{2+}]i$ also inhibits $IP_3$-induced $Ca^{2+}$-release [27] most likely via a direct modulatory effect of $Mg^{2+}$on the $IP_3$ receptor subunits.

Cellular $Mg^{2+}$accumulation is also under hormonal regulation. Among the hormones involved in the process there are insulin and vasopressin. These hormones either counteract cAMP production by acting at the level of the $\beta$-adrenergic receptor (inhibition) or the cytoplasmic phosphodiesterase that converts cAMP to AMP (stimulation), and/or activate PKC signaling, which acts as cAMP *alter ego*. Due to its ubiquitous presence and abundance, the TRPM7 channel is the mechanism most likely responsible for $Mg^{2+}$accumulation in the hepatocyte [46]. It is presently unclear whether PKC activates the channel by binding RACK1 and removing this protein from a specific site near the C terminus of the channel through which RACK1 inhibits TRPM7 conductance [36], or whether phosphorylation of the channel C-terminus is also required for full activation [46]. In the case of insulin, a direct modulatory effect on the putative $Na^+/Mg^{2+}$exchanger has also been observed [47].

Both $Mg^{2+}$extrusion and $Mg^{2+}$accumulation are quantitatively and timely limited processes [48,49], implying the movement of $Mg^{2+}$from and to specific cellular compartments. The cytoplasm is but one of the cellular compartments involved in $Mg^{2+}$transport out of the cell or into the cells [21,22], other compartments being the mitochondria and the endoplasmic reticulum [21]. This notion is supported by the observation that the co-stimulation of hepatic $\beta_2$-and $\alpha_1$-adrenergic receptors by the mix agonist epinephrine results in a $Mg^{2+}$extrusion that is quantitatively similar to the sum of the $Mg^{2+}$amounts mobilized by the stimulation of each adrenoceptor class by specific agonists [40]. However, the mechanisms involved in $Mg^{2+}$transport in-and-out of these compartments have not been fully elucidated. It is known that mitochondria accumulate $Mg^{2+}$through Mrs2, a $Mg^{2+}$-specific channel, the absence of which affects complex I expression and activity [50]. Less certain is whether $Mg^{2+}$extrusion from the mitochondria occurs via the adenine nucleotide translocase [21]. As for the cytoplasm, this compartment acts as a temporary step-in between the extracellular compartment and the cellular organelles both in the extrusion and in the accumulation of $Mg^{2+}$due to the high concentration of ATP that buffers $Mg^{2+}$with a very high Kd (~75μM) and the presence of other phosphonucleotides and binding proteins [22]. The role of ATP is further supported by the observation that pathological conditions that decrease cellular ATP content through dysmetabolic processes (namely diabetes and alcoholic liver disease) ultimately cause $Mg^{2+}$loss from the cell [51-53].

## 2. Insulin signaling in the liver

Insulin signaling is mediated by a complex and highly integrated signaling network that controls several processes including whole body glucose homeostasis. The liver is the first organ 'seen' by insulin following its release from the β-cells into the portal vein, and is responsible for the clearance of 50% of the released insulin at the first pass. Stimulation of the insulin receptor in liver cells is a key event to regulate hepatic glucose homeostasis. In addition, insulin acts indirectly on hepatic glucose homeostasis in that insulin released from β-cells inhibits glucagon release from pancreatic $\alpha$-cells thus limiting the drive on hepatic gluconeogenesis. The impairment of both these processes observed in insulin resistance is linked to major health problems including type 2-diabetes.

Insulin initiates its signaling cascade by interacting with the insulin receptor on the cell surface. Binding of insulin to the extracellular $\alpha$-subunits of the insulin receptor results in a conformation change that translates to the intracellular β-subunits of the receptor. The consequent activation of the kinase domain in the β-subunits of the receptor results in the autophosphorylation of specific tyrosine residues in the intracellular β-subunits. The phosphorylated insulin receptor now recruits the insulin receptor susbstrate (IRS), which upon phosphorylation on tyrosine residues acts as a docking unit for numerous cellular proteins including the phosphatidyl inositol 3-kinase (PI3K) [54]. Recruitment of these proteins to the IRS results in their activation. Activation of PI3K results in the phosphorylation of PIP2 to PIP3 and in the subsequent activation of protein kinase B (PKB or Akt), which then phosphorylates Forkhead box protein O1 (FoxO1), preventing its translocation to the nucleus. In its un-phosphorylated

state FoxO1 localizes in the nucleus, binds to the insulin response element sequence of gluconeogenesis-related genes, chiefly glucose 6 phosphatase and PEPCK, and increases their transcription rate, indirectly increasing the rate of hepatic glucose production. In its phosphorylated state, FoxO1 is unable to translocate to the nucleus and to activate the gluconeogenesis-related genes. Inhibition of FoxO1 could then improve hepatic metabolism in cases of insulin resistance and metabolic syndrome [55].

### 2.1. Role of $Mg^{2+}$on insulin receptor activation and signaling

The human insulin receptor homodimer is heavily glycosylated and contains a total of 19 predicted N-linked glycosylation sites in each monomer. The presence of sialic acid residues on molecules and cells is critical to their biological function and the presence of sialic acid residues on glycoproteins is partly responsible for the binding and transport of molecules, masking of the surface charge, aggregation and shape of cells [56]. Most recently, neuraminidase-1(Neu-1) an enzyme responsible for hydrolyzing sialic acid (neuraminic acid), has been associated with the positive regulation of insulin signaling [57]. Neu-1 is transported to the cell surface and gets involved in the regulation of cell signaling. Insulin binding to its receptor rapidly induces interaction of the glycan chains of the receptor with Neu-1 which hydrolyzes sialic acid residues in the glycan chains of the receptor consequently inducing activation of the insulin receptor. Impaired insulin-induced phosphorylation of Akt, thus identifies Neu1 as a novel component of the signaling pathways of energy metabolism and glucose uptake. Insulin binding to the insulin receptor has been shown to induce the interaction of the receptor with a pool of Neu-1 near the cell surface [57]. Also, insulin signaling is partially impaired in tissues of Neu-1-deficient mice [3], and desialylation of the insulin receptor by Neu1 promote the receptor activation [57]. While $CaCl_2$ has no significant effect on human liver neuraminidase activity, 10mM $MnCl_2$ or $MgCl_2$ shows a mild stimulatory effect (112% and 125% over control activity, respectively) [56].

Additional experimental evidence indicates that $Mg^{2+}$is required for the activated insulin receptor to phosphorylate IRS [54].

## 3. Magnesium and hepatic glucose metabolism

In liver cells, adrenergic stimulation of $\alpha_1$-and β-adrenergic receptors, and glucagon receptors elicit a $Mg^{2+}$extrusion that is associated with activation of glycolysis and glucose output on functional and temporal bases [40]. Although the nature of this association requires further clarification, it is fairly evident that conditions that limit the amplitude of $Mg^{2+}$extrusion decrease the amount of glucose outputted from liver cells, and vice versa [40]. This association is further supported by several pieces of observation. Overnight starvation, which depletes the liver of its glycogen content, decreases total hepatic $Mg^{2+}$content by 10-15% [58] rendering liver cells unresponsive to any subsequent adrenergic stimulation [58]. Both type-1 and type-2 diabetes present with a marked decrease in hepatic $Mg^{2+}$content [59], and treatment with the anti-diabetic drug metformin, which operates predominantly on liver metabolism, increases

intra-hepatic $Mg^{2+}$content [60]. The loss of hepatic $Mg^{2+}$observed under diabetic conditions depends on the enhanced phosphorylation of the $Na^{+}$.$Mg^{2+}$exchanger [61,62], and can be attenuated to a significant extent by the presence of glycogen, amylopectin, or glucose within liver plasma membrane vesicle [62].

The functional association between $Mg^{2+}$and glucose is also observed for $Mg^{2+}$accumulation. Insulin, one of the hormones involved in $Mg^{2+}$accumulation, is also responsible for glucose accumulation and conversion to glycogen [58]. Following insulin administration, $Mg^{2+}$accumulation is directly proportional to the amount of glucose present in the system [63]. Conversely, decreasing $Mg^{2+}$content in the extracellular system decreased the accumulation of glucose within the cells [40,63]. In part, the limited accumulation of glucose into insulin-stimulated cells in the presence of low extracellular $Mg^{2+}$concentration can be explained with the reduced activation of the insulin receptor occurring in these cells as $Mg^{2+}$is essential for the proper autophosphorylation of the insulin receptor and the subsequent recruitment of the insulin receptor substrate to the activated receptor [54]. All together, these pieces of evidence and observation support an essential role of $Mg^{2+}$in glucose regulation and pose for the cation as an important player in the onset and development of insulin resistance and diabetes in human patients.

### 3.1. Magnesium and enzyme activation in glucose metabolism

The physiological role of magnesium is principally related to enzyme activity. All enzymes utilizing ATP require Mg for substrate formation. Intracellular free magnesium also acts as an allosteric activator of enzyme action including critical enzyme systems such as adenylate cyclase, phosphofructokinase, phospholipase C, and $Na^{+}/K^{+}$-ATPase [64]. Magnesium is an enzyme substrate (ATPMg, GTPMg) to enzymes such as ATPase ($Na^{+}$, $K^{+}$ATPase, $Ca^{2+}$ATPase), cyclases (adenylate cyclase, guanylate cyclase), and the kinases (hexokinase, protein kinase) [64]. Recently, our laboratory has provided evidence that $Mg^{2+}$also modulates the amount of glucose 6-phosphate being routed into the endoplasmic reticulum (E.R) to be hydrolyzed to glucose plus Pi by the glucose 6-phosphatase, or to be converted to 6-phosphogluconolactone by the hexose 6-phosphate dehydrogenase, the reticular version of the G6PD. Moreover, our laboratory has provided significant evidence that both glucose and $Mg^{2+}$homeostasis are altered under pathological conditions such as diabetes [61] and alcoholic liver disease [65].

Many of the enzymes of glycolytic pathway that utilizes glucose have a requirement for $Mg^{2+}$ [26] and utilize $MgATP^{2-}$as a cofactor [66]. The Km values for $Mg^{2+}$in the glycolytic enzymes of the human erythrocyte are between 1 and 2.3 mM for hexokinase, 0.025 mM for phospho-fructokinase (PFK), 0.3 mM for phosphoglycerate kinase (PGK), and 1 mM for pyruvate kinase [26]. Magnesium ions ($Mg^{2+}$) and $MgATP^{2-}$regulate the most important glycolytic enzymes, namely hexokinase, phosphofructokinase, aldolase, phosphoglycerate kinase, and pyruvate kinase [66]. Glucokinase (Hexokinase IV or D), an enzyme expressed predominantly in liver and pancreatic β-cells of vertebrates, shows marked deviations from Michaelis-Menten kinetics when the glucose concentration is varied at a constant $MgATP^{2-}$concentration, but shows no deviations from Michaelis-Menten kinetics with respect to $MgATP^{2-}$[26,67]. Compared to the other hexokinase isoenzymes, this isoform has a low affinity for glucose (Table

1). Maximum binding of glucokinase and its regulatory protein to the hepatocyte matrix occurs at low [glucose] (<5mM) in a $Mg^{2+}$-dependent manner (Table 2, [68]). The regulatory protein binds to the hepatocyte matrix with ionic characteristics similar to those of glucokinase but, unlike glucokinase, it does not translocate from the binding site. Since the binding of glucokinase to its regulatory protein is associated with a decrease in the affinity of the enzyme for glucose, the bound enzyme in the presence of $Mg^{2+}$represents an inactive state and the translocated enzyme a more active state [69].

| Substrates | Dissociation Constants ($K_d$) |
|---|---|
| 0 mM Glucose | 0.14 ± 0.02 μM |
| 5 mM Glucose | 0.27 ± 0.03 μM |
| 10 mM Glucose | 0.54 ± 0.09 μM |
| 20 mM Glucose | 0.66 ± 0.07 μM |

**Table 2.** Effect of [Substrate] on $K_d$ of the high affinity binding sites of Glucokinase

## 4. Magnesium and gluconeogenesis

Gluconeogenesis is the process of glucose synthesis from non-carbohydrate precursors. Phosphoenolpyruvate Carboxy kinase (PEPCK), fructose1,6-bisphosphatase (F1,6BP), pyruvate carboxylase and glucose-6-phosphatase (G6Pase),catalyze irreversible reactions in the pathway and have lower activities compared to the other enzymes in the pathway and are thus considered rate limiting for glucose synthesis. Experiments by McNeill et al, [70] suggest that magnesium deficiency alters PEPCK by affecting secretion of pancreatic hormones. Of these four enzymes, $Mg^{2+}$is required by three, that is, pyruvate carboxylase, PEPCK, and F1, 6BP reactions. Though hormones such as insulin, glucagon, glucocorticoids and epinephrine influence the key enzyme activities of gluconeogenic enzymes, $Mg^{2+}$plays a role in the secretion of all these hormones [70]. Thus in $Mg^{2+}$deficiency, enzyme activities may change, as a result of altered circulating levels of one or more hormones. In this study like in earlier studies, an increase in PEPCK activity was observed in magnesium deficient rats making $Mg^{2+}$deficiency a possible contributing factor to the maintenance of low insulin levels an increased PEPCK in diabetes [70].

## 5. Conclusion

In the last two decades, our understanding of the importance of $Mg^{2+}$ions for numerous cell and body functions has increased significantly. The initial experimental evidence has been corroborated to a significant extent in clinical conditions such as diabetes, alcoholism, and dysendocrinopathies.

http://dx.doi.org/10.5772/57564

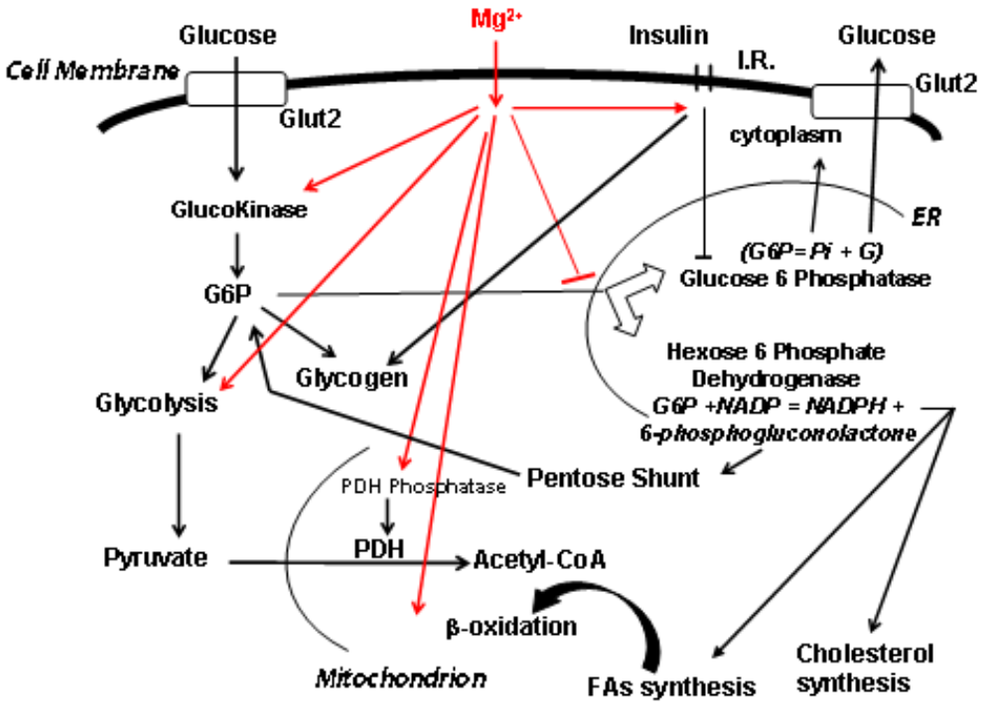


**Figure 2.** Graphic representations of the different glucose-related functions controlled by $Mg^{2+}$in the hepatocyte.`

In the case of liver cells, we have moved from the initial observation that $Mg^{2+}$is abundantly represented within the hepatocyte as a whole to the notion that the cation's homeostasis is controlled by hormones, which promotes the movement of $Mg^{2+}$in-and-out of the cell membrane to support and regulate specific liver metabolic functions. The observation that $Mg^{2+}$is highly compartmentalized within cellular compartments and organelles support the notion that the cation plays a key role in regulating enzymes, channel activities, and metabolic processes within each of these organelles. Figure 2 recapitulates the relevance of $Mg^{2+}$for the regulation of glucose homeostasis and bioenergetics within the hepatocyte. In the cytoplasm, $Mg^{2+}$regulates glucokinase and glycolytic enzymes but also ATP utilization. In the mtiochondria, $Mg^{2+}$regulates mitochondrial dehydrogenases and pyruvate dehydrogenase by promoting the activity of the pyruvate dehydrogenase phosphatase, responsible for dephosphorylating the enzyme to its active conformation. In the endoplasmic reticulum (ER), $Mg^{2+}$regulates protein synthesis and the entry of glucose 6 phosphate (G6P), the limiting step for the utilization of this substrate by the glucose 6-phosphatase (G6Pase) and the hexose 6-phosphate dehydrogenase (H6PD), the reticular variant of the cytosolic glucose 6 phosphate dehydrogenase (G6PD). The oxidation of G6P by the H6PD generates high levels of NADPH within the ER lumen to be used for other metabolic processes within the organelle and in the rest of the cell, including fatty acid synthesis and cholesterol synthesis. Far from being complete, the picture is a dynamic scenario in need to further clarification and study in the years to come.

## Author details

Chesinta Voma[1,2] and Andrea M.P. Romani[1*]

*Address all correspondence to: amr5@po.cwru.edu

1 Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University, Cleveland, OH, USA

2 Department of Clinical Chemistry, Cleveland State University, Cleveland, OH, USA

## References

[1] Hoganson DM, Prior HI, Vacanti JP. Tissue Engineering and Organ Structure: A Vascularized Approach to liver and lung. Pediatric Research. 2008;63(5): 520-526

[2] Crawford JM. The liver and Biliary Tract. In: Robbins Pathologic Basis of Disease (Cotran RS, Kumar V, Collins J eds.), W.B. Saunders Company, Philadelphia, PA, 1999 pp 845-901

[3] Jungermann K, Katz N. Functional hepatocellular heterogeneity. Hepatology 1982;2: 385-395

[4] Jungermann K, Katz N. Functional specialization of different hepatocyte populations. Physiol Rev 1989;69: 708-764

[5] LeCluyse EL, Audus KL, Hochman JH. Formation of extensive canalicular networks by rat hepatocytes cultured in collagen-sandwich configuration. Am J Physiol 1994; 266:C1764-C1774

[6] Robey, RB, Hay N. Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt. Oncogene 2006;25(34): 4683–4696

[7] Bánhegyi G, Benedetti A, Fulceri R, Senesi S. Cooperativity between 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum. J Biol Chem. 2004;279(26):27017-27021

[8] Berteloot A, Vidal H, van de Werve G. Rapid kinetics of liver microsomal glucose-6-phosphatase. Evidence for tight-coupling between glucose-6-phosphate transport and phosphohydrolase activity. J Biol Chem 1991;266: 5497–5507.

[9] Foster JD, Nordlie RC. The biochemistry and molecular biology of the glucose-6-phosphatase system. Exp Biol Med 2002;227:601–608

[10] Banhegy G, Marcolongo P, Fulceri R, Hinds C, Burchell A, Benedetti A. Demonstration of a metabolically active glucose-6-phosphate pool in the lumen of liver microsomal vesicles. J Biol Chem 1997;272:13584–13590.

[11] St-Denis J-F, Berteloot A, Vidal AH, Annabi B, van de Werve G. Glucose transport and glucose 6-phosphate hydrolysis in intact rat liver microsomes. J Biol Chem 1995; 270:21092–21097.

[12] Jungermann K, Heilbronn R, Katz N, Sasse D. The glucose/glucose-6-phosphate cycle in periportal and perivenous zone of rat liver. Eur J biochem 1982;123:479-436.

[13] Romani A, Fulceri R, Pompella A, Benedetti A. MgATP-dependent, glucose 6-phosphate-stimulated liver microsomal $Ca^{2+}$accumulation: difference between rough and smooth microsomes. Arch Biochem Biophys 1988;266(1):1-9.

[14] Fulceri R, Bellomo G, Gamberucci A, Romani A, Benedetti A. Physiological concentrations of inorganic phosphate affect MgATP-dependent $Ca^{2+}$storage and inositol trisphosphate-induced $Ca^{2+}$efflux in microsomal vesicles from non-hepatic cells. Biochem J 1993;289(Pt 1):299-306.

[15] Száraz P, Bánhegyi G, Benedetti A. Altered redox state of luminal pyridine nucleotides facilitates the sensitivity towards oxidative injury and leads to endoplasmic reticulum stress dependent autophagy in HepG2 cells. Int J Biochem Cell Biol 2010;42(1): 157-166

[16] He L, Cao J, Meng S, Ma A, Radovick S, Wondeisford FE. Activation of basal gluconeogenesis by coactivator p300 maintains hepatic glycogen storage. Mol Endocrino. 2013:27(8):1322-1332.

[17] http://www.whfoods.com

[18] http://bestpractices.bmj.com/best-pratice/monograph/1137.html

[19] Alfrey AC, Miller NL, Trow R. Effect of age and magnesium depletion on bone magnesium pools in rats. J Clin Invest 1974;54:1074-1081.

[20] Rude KK, Olerich M. Magnesium deficiency: possible role in osteoporosis associated with gluten-sensitive enteropathy. Osteoporos Int 1996;6:453-461.

[21] Romani A, Scarpa A. Regulation of cell magnesium. Arch Biochem Biophys 1992;298(1): 1-12

[22] Scarpa A, Brinley FJ. In situ measurements of cytosolic free magnesium ions. Fed Proc. 1981;40(12): 2646-2652

[23] Romani AM. Magnesium homeostasis in mammalian cells Met Ions Life Sci 2013;12:69-118

[24] Lüthi D, Günzel D, McGuigan JA. Mg-ATP binding: its modification by spermine, the relevance to cytosolic $Mg^{2+}$buffering, changes in the intracellular ionized

$Mg^{2+}$concentration and the estimation of $Mg^{2+}$by 31P-NMR. Exp Physiol 1999; 84(2): 231-52

[25] Cowan JA. Structural and catalytic chemistry of magnesium-dependent enzymes. Biometals 2002;15: 225-235.

[26] Garfinkle L, Garfinkle D. Magnesium regulation of the glycolytic pathway and the enzymes involved. Magnesium 1985;4(2-3):60-72.

[27] Volpe P, Alderson-Lang BH, Nickols GA. Regulation of inositol 1,4,5-trisphosphate-induced $Ca^{2+}$release. I. Effect of $Mg^{2+}$.Am. J. Physiol 1990; 258:C1077-C1085.

[28] Rubin H. Central roles of $Mg^{2+}$and $MgATP^{2-}$in the regulation of protein synthesis and cell proliferation: significance for neoplastic transformation. Adv Cancer Res 2005;93:1-58

[29] Barfell A, Crumbly A, Romani A. Enhanced glucose 6-phosphatase activity in liver of rats exposed to $Mg^{2+}$-deficient diet. Arch Biochem Biophys 2011;509(2):157-163.

[30] Gunther T. Functional compartmentation of intracellular magnesium. Magnesium 1986; 5:53-59.

[31] Kolisek M, Nestler A, Vormann J, Schweigel-Rontgen M. Human gene SLC41A1 encodes for the $Na^{+}/Mg^{2+}$exchanger. Am J Physiol. 2012, 302, C318-C326.

[32] Romani A. Cellular magnesium homeostasis. Arch. Biochem Biophys 2011;512:1-23.

[33] Romani A, Dowell E, Scarpa A. Cyclic-AMP-induced magnesium release from rat liver hepatocytes, permeabilized hepatocytes, and isolated mitochondria. J Biol Chem 1991;266(36): 24376-24384.

[34] Cefaratti C, Ruse C. Protein kinase A dependent phosphorylation activates $Mg^{2+}$efflux in the basolateral region of the liver. Mol Cell Biochem 2007;297(1-2): 209-214

[35] Mishra R, Rao V, Ta R, Shobeiri N, Hill CE. $Mg^{2+}$-and MgATP-inhibited and $Ca^{2+}$/calmodulin-sensitive TRPM7-like current in hepatoma and hepatocytes. Am J Physiol 2009; 297(4):G687-G694.

[36] Cao G, Thébault S, van der Wijst J, van der Kemp A, Lasonder E, Bindels RJ, Hoenderop JG. RACK1 inhibits TRPM6 activity via phosphorylation of the fused alpha-kinase domain. Curr Biol 2008;18(3):168-176.

[37] Romani A. In Physiology of Magnesium Homeostasis (Vink R, Nechifor M eds.) 2011, Univ. of Adelaide Publishing Co., Adelaide, Australia.

[38] Alexander RT, Hoenderop JG, Bindels RJ. Molecular determinants of magnesium homeostasis: insights from human disease.J. Am. Soc. Nephrol. 2008, 19, 1451-1458.

[39] Touyz RM, He Y, Montezano ACI, Yao G, Chubanov V, Gudemann T, Callera GE. Differential regulation of transient receptor potential melastatin 6 and 7 cation chan-

nels by ANG II in vascular smooth muscle cells from spontaneously hypertensive rats. Am J Physiol 2006;290:R73-R78.

[40] Fagan TE, Romani A. Activation of $Na^+$-and $Ca^{2+}$-dependent $Mg^{2+}$extrusion by $alpha_1$-and beta-adrenergic agonists in rat liver cells. Am J Physiol 2000;279:G943-G950

[41] Fagan TE, Romani A. $alpha_1$-Adrenoceptor-induced $Mg^{2+}$extrusion from rat hepatocytes occurs via $Na^+$-dependent transport mechanism. Am J Physiol 2001;280(6):G1145-G1156.

[42] Cefaratti C, Romani A, Scarpa A. Differential localization and operation of distinct $Mg^{2+}$transporters in apical and basolateral sides of rat liver plasma membranes. J Biol Chem 2000;275(6):3772-3780.

[43] Keenan D, Romani A, Scarpa A.Regulation of $Mg^{2+}$homeostasss by insulin in perfused rat livers and isolated hepatocytes. FEBS Lett 1996;395(2-3):241-244.

[44] Romani A, Marfella C, Scarpa A. Hormonal stimulation of $Mg^{2+}$uptake in hepatocytes. Regulation by plasma membrane and intracellular organelles. J Biol Chem 1993;268(21):15489-95.

[45] Maguire ME. Hormone-sensitive magnesium transport and magnesium regulation of adenylate cyclase. TIPS 1984:73-77.

[46] Romani AM. Magnesium homeostasis in mammalian cells. Front Biosci 2007;12:308-331.

[47] Ferreira A, Rivera A, Romero JR. $Na^+/Mg^{2+}$exchange is functionally coupled to the insulin receptor. J Cell Physiol. 2004;199(3):434-40.

[48] Romani A, Scarpa A. Hormonal control of $Mg^{2+}$transport in the heart. Nature 1990;346:841-844.

[49] Romani A, Scarpa A. Norepinephrine evokes a marked $Mg^{2+}$efflux from liver cells. FEBS Lett. 1990; 269(1);37-40.

[50] Piskacek M, Zotova L, Zsurka G, Schweyen RJ. Conditional knockdown of hMRS2 results in loss of mitochondrial $Mg^{2+}$uptake and cell death. J Cell Mol Med 2009;13(4): 693-700

[51] Gaussin V, Gailly P, Gillis J-M, Hue L. Fructose-induced increase in intracellular free $Mg^{2+}$ion concentration in rat hepatocytes: relation with the enzymes of glycogen metabolism. Biochem J 1997; 326:823-827.

[52] Dalal P, Romani A. Adenosine triphosphate depletion by cyanide results in a $Na^+$-dependent $Mg^{2+}$extrusion from liver cells. Metabolism 2010; 59:1663-1671.

[53] Fagan TE, Cefaratti C, Romani A. Streptozotocin-induced diabetes impairs $Mg^{2+}$homeostasis and uptake in rat liver cells. Am J Physiol 2004;286(2):E184-E193.

[54] Suarez A, Pulido N, Casla A, Casanova B, Arrieta FJ, Rovira A. Impaired Tyrosine-Kinase Activity of Muscle Insulin Receptors from Hypomagnesaemic Rats. Diabetologia 1995;38: 1262-1270.

[55] Cheng Z, Guo1 S, Copps K, Dong X, Kollipara R, Rodgers JT, Depinho RA, Puigserver P, White MF. Foxo1 integrates insulin signaling with mitochondrial function in the liver. Nature Medicine 2009;15:1307-1311.

[56] Alhadeff JA, Wolfe S. Characterization of Human Liver (4-Methylumbelliferyl-α-D-N-Acetylneuraminic Acid) Neuraminidase Activity. Int. J. Biochem 1981;13:975-980.

[57] Dribi L, Seyrantepe V, Fougerat A, Pan X, Bonneil E, Thibault P, Moreau A, Mitchell GA, Heveker N, Cairo CW, Issad T, Hinek A, Pshezheetsky AV. Positive Regulation of Insulin Signaling by Neuraminidase 1. Diabetes 2013; 62:2338-2346.

[58] Torres LM, Younger J, Romani A. Role of glucose in modulating $Mg^{2+}$homeostasis in liver cells from starved rats. Am. J. Physiol. 2005; 288(2) G195-G206.

[59] Barbagallo M, Dominguez LJ. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance.Arch Biochem. Biophys. 2007;458(1): 40-47

[60] Nadler J, Scott S. Evidence that pioglitazone increases intracellular free magnesium concentration in freshly isolated rat adipocytes. Biochem. Biophys, Res. Commun 1994; 202:416-421

[61] Guerrero-Romero F, Rodriguez-Moran M. Complementary therapies for diabetes: the case for chromium, magnesium, and antioxidants. Arch Med Res. 2005:36(3):250-257

[62] Cefaratti C, McKinnis A, Romani A. Altered $Mg^{2+}$transport across liver plasma membrane from streptozotocin-treated rats. Mol Cell Biochem 2004;262(1-2):145-54.

[63] Romani AM, Matthews VD, Scarpa A. Parallel stimulation of glucose and $Mg^{2+}$accumulation by insulin in rat hearts and cardiac ventricular myocytes. Circ Res 2000;86(3):326-33.

[64] Rude RK. Magnesium Defieciency: A Cause of Heterogenous Disease in Humans. J Bone Mineral Res 1998;13(4): 749-758.

[65] Romani AM. Magnesium Homeostasis and alcohol consumption. Magnesium Res 2008;21(4):197-204.

[66] Niemeyer H, Cardenas ML, Rabajille E, Ureta T, Clark-Turri, Penaranda J. Sigmoidal Kinetics of glucokinase. Enzyme 1975; 20:321-333.

[67] Storer AC, Cornish-Bowden A. Kinetics of rat-liver glucokinase: co-operative interactions with glucose at physiologically significant concentrations. Biochemical J 1976; 159:7-14.

[68] Agius L, Peak M, Newgard CB, Gomez-Foix AM, Guinovart JJ. Evidence for a Role of Glucose–induced Translocation of Glucokinase in the control of Hepatic Glycogen Synthesis. J Biol Chem 1996; 271(48):30479-30486.

[69] Agius L, Peak M, Schaftingen EV. The regulatory protein of glucokinase binds to the hepatocyte matrix, but unlike glucokinase does not translocate during substrate stimulation. Biochem J 1995; 309, 711-713.

[70] McNeill DA, Herbein JH, Ritchey SJ. Hepatic Gluconeogenic enzymes, plasma insulin and glucagon response to magnesium deficiency and fasting. J Nutr 1982; 112(4): 736-743.

IntechOpen

IntechOpen

IntechOpen