

Review Article

Role of Cellular Magnesium in Human Diseases

Samantha Long and Andrea MP Romani*
Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University, USA

***Corresponding author:** Andrea MP Romani, Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-4970, USA, Tel: 1-216-3681625; Fax: 1-216-3685586; Email: amr5@po.cwru.edu

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Abstract

Magnesium is required for many of the major organs to function and plays a crucial role in human and mammalian physiology. Magnesium is essential for the structure of bones and teeth, acts as a cofactor for more than 300 enzymes in the body, including binding to ATP for kinase reactions, and affects permeability of excitable membranes and neuromuscular transmission. Despite these essential roles, much is still unknown about magnesium physiology and homeostasis. Currently, nutritionists believe that the general population intakes insufficient magnesium daily through the diet. The effects of magnesium deficiency are, for the most part undetected, and simple, widespread assessments of magnesium intake remain unavailable for humans. Many of the patients admitted to hospitals or medical care facilities are unaware of their low magnesium levels. Moreover, because magnesium is predominantly an intracellular cation (>99%), serum magnesium levels remain a poor predictor of tissue magnesium content and availability. This review will discuss the effects of magnesium deficiency in various pathologies affecting the human population. The underlying causes for magnesium depletion in major physiological systems will be examined along with the involved signaling pathways and the main roles of magnesium homeostasis. Where possible (e.g. alcoholism), the implications of administering supplemental magnesium will be discussed. Ultimately, this review will advocate for the necessity of identifying easy and reproducible methods to assess serum and cellular magnesium levels and to identify magnesium deficiency in order to alleviate related pathological conditions.

Keywords: Magnesium homeostasis; Cardiovascular system; Renal system; Endocrine system; Bones; Reproductive biology; Alcoholism; Diabetes

Introduction

Magnesium (Mg^{2+}) is integral to cellular and systemic human physiology and its ability to function. Yet, this mineral is often overlooked in deference to other cations such as calcium or iron. As the fourth most abundant element in the human body, magnesium accounts for ~25 grams, most of which is stored within bones (50%) and soft tissues (47%). Magnesium enters the eukaryotic cell through a number of channels, including MagT1, MMgT, SLC1-A1, TRPM6, and TRPM7 [1]. TRPM6 and TRPM7 are two cation channels with unique C-terminal sequences and protein kinase domains, although TRPM7 seems central to magnesium homeostasis by regulating its uptake through coupling of the channel with the kinase domain activity [2]. TRPM6 assembles with TRPM7 via heterooligomerization in order to form channel complexes at the cell surface [3]. When disrupted by S141L mutation, autosomal recessive hypomagnesaemia with secondary hypocalcemia results (HSH, OMIM #602014) [3].

Whole body magnesium homeostasis

The absorption of magnesium in the gastrointestinal tract is not fully understood, although saturable magnesium accumulation mechanisms have been observed in the brush border cells of the ileum [4,5]. While an active transport process occurs in the ileum, the rest of the small intestine uses passive absorption for magnesium [5]. Magnesium from the diet is absorbed at the apical side of intestinal epithelial cells and released into the blood exits at the basolateral side through a Na^+/Mg^{2+} exchanger [5]. The apical membrane possesses two magnesium channels from the melastatin subfamily of transient

receptor potential channels. TRPM6 resides in the colon and also in the distal convoluted tubule of the nephron, while TRPM7, ubiquitous in most in most mammalian cells, is usually present in various parts of the small intestine [4]. One of the biggest differences between the two channels is that diet and estrogens modulate only TRPM6 expression and activity [4]. Normally, 17β -estradiol upregulates TRPM6 mRNA in the colon and kidney, while in its absence, the repressor of estrogen receptor activity - REA - binds to the 6th, 7th, and 8th β -sheets of TRPM6 kinase domain and inhibits TRPM6 activity. Re-administration of estrogen, however, increases channel activity again [4]. Similarly, dietary magnesium restriction increases TRPM6 mRNA expression in the kidney and colon, while high magnesium intake upregulates TRPM6 mRNA expression only in the colon [4]. RACK1, the receptor for activated protein kinase C, also regulates TRPM6 and possibly TRPM7 channel activity by binding to their -kinase domain, as well as the β -sheets that REA regulates [4]. This binding will inhibit channel activity of both TRPM6 and TRPM7. Magnesium exits the intestinal cell and enters the bloodstream through a Na^+/Mg^{2+} exchanger, which is activated by cAMP phosphorylation [4]. Once in the bloodstream, one-third of the circulating magnesium is bound to proteins, mainly albumin, or is complexed to anions, whereas two-thirds are in the free form [4]. In the kidney, 15% to 30% of the magnesium filtered by the glomeruli is reabsorbed in the proximal tubule [4,5], and 65-70% is reabsorbed in the thick ascending limb (TAL) of the Henle's loop [4,5]. The TAL is the main site of control of magnesium reabsorption in the kidneys [5]. In this section of the nephron, hormones such as

AVP and PTH, and various drugs including diuretics can increase or decrease magnesium reabsorption [4]. The increase occurs through passive paracellular transport mediated by claudins, and active transcellular magnesium transport mechanisms that regulate magnesium absorption to a finer extent [4].

Daily magnesium intake

The current recommended daily dose of Mg^{2+} is 300mg for men and 250mg for women [6] but, in the last century, the amount has fallen from 500mg per day to around 175-225mg daily [7]. The US population, in particular, ingests less than this amount, to such an extent that reports in 2009 from the World Health Organization suggest that three quarters of Americans do not meet the Recommended Daily Intake (RDI) [7]. Dr. Sherry A. Rogers, author of *Wellness Against All Odds* complains that "...the average American diet, government studies show us, provides only 40% - less than half the magnesium that we all need in a day" [8]. Normally, magnesium is obtained from foods that include whole grains, nuts, and green leafy vegetables [9]. Large contributors to the sizeable losses in magnesium intake incurred by the general population are the refining and processing of food, whereby magnesium can be reduced by 82-97% in the processing of wheat to flour, rice to polished rice, or corn to starch [10].

Magnesium plays a role in the structure of bones and teeth, acts as a cofactor for more than 300 enzymes in the body, including binding to ATP for kinase reactions, and affects permeability of excitable membranes and neuromuscular transmission [11], as well as nervous tissue electrical potential [4]. Magnesium is crucial for controlling ECF volume, Na^+/K^+ -ATPase and cellular uptake of solutes, as a driving force for secondary active transport, and neuromuscular transmission [11s]. Certain groups are at higher risk for magnesium deficiencies, due to underlying medical conditions or insufficient consumption. Such populations include patients with gastrointestinal diseases, type II diabetes, older adults, and alcoholics. People with Crohn's disease and celiac disease encounter longitudinal magnesium depletion in their gastrointestinal tract, while small intestinal bypass can lead to malabsorption, which further aggravates magnesium loss [12]. Similar to gastrointestinal diseases, older adults intake less magnesium from the diet in addition to experiencing less magnesium absorption in the gut [12]. Furthermore, many studies have shown decreased plasma and intracellular free magnesium levels in diabetic (both type I and type II) patients versus control non-diabetic patients. The reasons for decreased serum and tissue magnesium content are several, including exogenous or endogenous factors, such as diets low in magnesium, high renal excretion of magnesium, insulin insensitivity, which affects magnesium transport and glucose metabolism (which, in turn, is negatively affected by low magnesium levels), and utilization of loop and thiazide diuretics, which enhance magnesium wasting [12]. Alcoholism works like diuretics, whereby renal magnesium excretion is increased. Ketoacidosis, hypophosphatemia, and hyper-aldoosteronism from alcohol-induced liver disease may also account for increased magnesium excretion [4].

In the following pages, we will highlight studies demonstrating specific effects of magnesium deficiency on different systems of the body, as well as the need for further research into the mechanisms relating to systemic effects of magnesium deficiency.

Magnesium and the cardiovascular system

Within the cardiac conduction system, magnesium plays several roles. The main role is as a natural calcium channel blocker or as an antagonist for sodium, thereby setting physiological parameters for cation content traffic through different channels [4]. Magnesium is also a cofactor for the Na-K-ATP pump, which maintains the membrane potential in all mammalian cells, including cardiac myocytes [6]. Hence, disruption of magnesium homeostasis or hypomagnesaemia can alter myocardial excitability, or change the functionality of the inward rectifying potassium channel, which will affect the effective and relative refractory period [13]. Thus, insufficient serum and tissue magnesium concentrations may lead to the onset of various cardiac arrhythmias, including congestive heart failure (CHF), ventricular tachycardia (VT), ventricular fibrillation (VF), long QT and torsades de pointes, atrial and ventricular extrasystoles or premature beats, all conditions that can result in sudden cardiac death [4]. The infusion of magnesium, on the other hand, prolongs atrioventricular node conduction time, in contrast to low serum magnesium concentration, which increases sinus node automaticity [13]. Increased loss of serum and cellular magnesium affects the way in which the body responds to digitalis therapy in CHF patients in terms of control of cardiac performance and rhythm [4]. Several studies have demonstrated that low serum magnesium concentrations, irrespective of other factors, correlated with long-term gain of left ventricular mass, which is a significant predictive factor for adverse cardiovascular events [14]. Recent studies have also demonstrated a statistically significant inverse relationship between higher dietary magnesium intake and the risk of a cardiovascular event, whereby the risk is lowered by 15% in individuals with the highest intake of magnesium [15].

Magnesium deficiency is believed to result from elevated circulating levels of catecholamine, aldosterone, and vasopressin, as well as from increased urinary magnesium excretion (as observed, for example, following diuretic and digoxin therapy) [4]. Reduction in dietary magnesium intake and low magnesium in drinking water have been identified as risk factors for the development of hypertension, atherosclerosis, vasospasm, inflammatory conditions, and sudden cardiac death. This is supported by work from Kobayashi, who studied water hardness and its correlation with cardiovascular effects [16]. Intake of "hard water" (i.e. water containing relatively high levels of calcium and magnesium) has an inverse association with cardiovascular mortality as supported by a follow-up study by the World Health Organization (WHO) from 1957-1979 [17]. Such effects can be circumvented by increasing magnesium levels to the point of hypermagnesaemia, which curbs hypertension, cardiac arrhythmias, and atherogenesis. Although Western cultures is often cited as having chronically deficient levels of magnesium in diets, research has indicated that non-Western cultures also easily develop pathologies related to low magnesium intake following changes in diet or drinking habits. Cultures that have low incidences of heart disease, hypertension, and stroke, while maintaining diets with high levels of magnesium and drinking, have been shown to develop high blood pressure, hypertension, and heart disease as often as native Westerners upon moving to and/or adapting to the water and diets of more industrialized areas [18].

Low magnesium intake has been linked to high blood pressure, arterial plaque build-up, calcification of soft tissues, cholesterol,

and hardening of arteries [18]. Additionally, inflammation from magnesium deficiency can also lead to increased production of reactive oxygen species, which can contribute to elevating blood pressure [19]. In humans, specific magnesium-selective electrodes hooked up to patients with hypertension, ischemic heart disease, stroke, and atherosclerosis revealed a significant decrease of serum/plasma ionized, but not total, magnesium, while in rat and rabbit models, dietary magnesium deficiency caused vascular remodeling associated with hypertension and atherosclerosis [20]. Carotid Intima Media Thickness (IMT), an index of atherosclerosis and associated with an increased risk for CVD, is improved in heart disease patients that were given magnesium supplementation [21]. Additionally, the serum magnesium levels were found to inversely correlate with carotid IMT in HD patients [21]. Hypomagnesaemia has also been linked to the development of atrial fibrillation following cardiac surgery. Intravenous magnesium supplementation can improve rate control in atrial fibrillation and help maintain sinus rhythm, while hypomagnesaemia increases the dose of digoxin required for rate control and lowers the threshold for digoxin-related arrhythmias [13].

Over the twenty years of observation in a Framingham follow-up, a positive association was found between low serum magnesium and the development of atrial fibrillation in patients without signs of cardiovascular disease [13]. Studies in both animals and humans have demonstrated a statistical relationship between decreased intake of magnesium and cardiovascular disease. In particular, diminished dietary intake of magnesium enhances the risk of developing hypertension, atherosclerosis, vasospasm, sudden cardiac death, stroke, and inflammatory conditions in the human population. The mechanisms, however, remain vague [22]. Dr. Andrea Rosanoff argues that "...by 1957, low magnesium was shown to be, strongly, convincingly, a cause of atherogenesis and the calcification of soft tissues... this research was widely and immediately ignored as cholesterol and the high saturated-fat diet became the culprits to fight" [23].

Rosanoff's statement ties in well with the Altura's group study, which reported that short term deficiency of magnesium (MgD) resulted in upregulation of PKC isoforms in left ventricular and aortic smooth muscle and serum, as well as the release of specific cytokines and an upregulation of NF- κ B in the left ventricle, aortic smooth muscle, and primary cultured aortic smooth muscle cells [22]. These results would link MgD to inflammation in various parts of the heart, which will ultimately result in poor cardiac health. This was the first study to demonstrate the activation of all classes of PKC isoforms in cardiac and VSM tissues and cells due to low levels of extracellular magnesium [22]. Furthermore, this study was also the first to display an upregulation of all classes of PKC isoforms by MgD in any cell type in any species. During the study, feedings rats a diet that was deficient in magnesium for 21 days led to a 300-400% increase in the LV muscle, as well as a 300-500% rise in all PKC isoform levels, except for PKC-epsilon, in aortic smooth muscle [22]. The elevation of many PKC isoforms in left ventricular and aortic smooth muscles was slightly decreased by feeding the magnesium deficient animals just 15mg/L/day of magnesium in the drinking water while feeding 100mg/L/day of magnesium restored the protein kinase C isoforms to normal [22]. Ultimately, cardiovascular tissue samples obtained

from the MgD rats revealed a three- to ten-fold increase in cytokine and chemokine levels, while such an increase was not present in MgD animals fed 15-40mg/L Mg²⁺ in their drinking water [22].

Altura and collaborators also demonstrated that short-term dietary deficiency of magnesium upregulated two important proto-oncogenes, c-Fos and c-Jun [24]. Specific inhibitors of neutral- and acid-sphingomyelinases led to reductions in the expression of c-Fos, c-Jun, and NF- κ B components. Hence, the authors hypothesize that activating sphingomyelinases under MgD condition upregulates these proto-oncogenes and activates NF- κ B, two pathways that are essential in atherogenesis and hypertensive disease states [24]. Short-term MgD resulted in greater than 150% increases in sphingomyelinase activity and proto-oncogene expression in both the left and right ventricular muscle, atrial muscle, and abdominal aortic smooth muscle [24].

Pro-inflammatory cytokines trigger the secretion of acid-sphingomyelinases in endothelial cells and patients with CHF demonstrate elevated levels of plasma acid-sphingomyelinase [25]. For these reasons, it is hypothesized that upregulating acid-sphingomyelinase in the chambers of the heart and in vascular smooth muscle cells of MgD animals may trigger, at least in part, upregulation of pro-inflammatory cytokines [25]. These changes in intracellular signal transcription molecules can also cause membrane oxidation, truncation of membrane fatty acids, and activation of apoptotic pathways connected with activation of sphingomyelinase and alterations in membrane sphingomyelin, which leads to the release of ceramides in the extracellular matrix of cultured vascular smooth muscle cells [20].

Magnesium in immunological responses

The article mentioned above and past research supports the idea of an association between an increase in systemic inflammation and magnesium deficiency. This association is supported by the observed increase in serum levels of TNF α and inflammatory cytokines with concomitant reduction in the production and release of anti-inflammatory cytokines [4]. Magnesium deficiency acts as a stressor effect that makes the body more susceptible to physiological stress, with consequent activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis and sympathetic nervous system [19]. This activation can increase oxidative stress or lead to elevation of NF κ B, which would promote translation of molecules involved in cell metabolism and apoptosis [19]. NF κ B signaling is one of the two mechanisms believed to trigger the increase in inflammatory cytokines in magnesium deficiency through the activation of a calcium-channel normally blocked by magnesium. Release of the inhibition under magnesium-deficient conditions increases calcium entry and, ultimately, promotes production of reaction oxygen species, with consequent membrane oxidation and NF κ B activation [4]. This inflammatory response in magnesium deficiency can extend to the liver and other tissues. Inducing magnesium deficiency in rats led to a clinical inflammatory syndrome with leukocyte and macrophage activation, synthesis of inflammatory cytokines, and acute phase proteins, extensive production of free radicals. Increasing extracellular magnesium concentration, on the other hand, decreases inflammatory effects [19]. The specific effects of magnesium deficiency on mast cells and their activation were examined [26]. A study by the Lenardo's group

further demonstrated that decreased intracellular free magnesium induces defective expression of natural killer activating receptor NKG2D in NK and CD8+ T cells [27].

Magnesium deficiency can stimulate hepatic fibrosis and steatosis, possibly through its effect on mast cells [26]. Experiments by Rayssiguier and Durlach indicate that lack of sufficient magnesium levels favors accumulation of collagen in the cardiovascular system following stress or inflammatory stimuli, such as the administration of a hepatotoxic agent [28]. Veilleux had previously demonstrated that magnesium deficiency led to increased proliferation of mast cells in the duodenum and the kidney [29], while Kraeuter and Schwartz indicated that mast cells developed during magnesium depletion were defective in storing and secreting histamine [30].

Takemoto and Matsui built upon this research to explore whether the immunological effects extended to the liver. They compared control Sprague-Dawley rats versus Sprague-Dawley rats exposed to a magnesium deficient diet for four weeks [26]. The magnesium deficient diet increased the mRNA levels of two immunoglobulin E-receptors found in mast cells and basophils of the liver, FC α R1a, FCER1B, as well as the mRNA of mast cell proteases Mcpt1 and Mcpt2. In contrast, control rats presented few mast cells in the portal triad area [26]. Additionally, enlarged vacuoles, possibly consisting of fat, were observed only in magnesium-deficient rats [26]. This observation supports previous research by Gueux and Alcindor [31], indicating that magnesium deficient rats presented inward levels of triglyceride and free cholesterol and decreased levels of esterified cholesterol as well as decreased activity of Lecithin-Cholesterol Acyltransferase (LCAT), the enzyme needed to prevent unesterified cholesterol from accumulating in the plasma [31].

Lenardo and his group took the research of magnesium deficiencies in a slightly different direction and studied the involvement of the magnesium transporter, MAGT1, in individuals with XMEN disease [27]. XMEN disease is an abbreviation of the various disorders observed under this condition. The disease is an X-linked immunodeficiency (X), with a magnesium defect (M), Epstein-Barr virus (E), and neoplasia disease (N), all due to null mutations in the MAGT1 gene [32]. Decreased intracellular free magnesium induces defective expression of natural killer activating receptor NKG2D in NK and CD8+ T cells in the affected individuals. It also impairs cytolytic responses against Epstein - Barr virus, which predisposes the patients to lymphoma onset [27]. The researchers noted that XMEN patients carrying MAGT1 mutations had lower basal free intracellular magnesium levels, and that magnesium supplementation to these patients would recover the cytotoxicity defect partially in cytotoxic T-lymphocytes (CTLs) and nearly completely in NK cells. Thus, intracellular free magnesium and NKG2D levels would elevate while EBV-infected cells would decrease [27]. Taken together, these results indicate that magnesium plays a relevant role in antiviral and anti-tumor immunity and that consistently low free magnesium levels account for impaired cytotoxicity.

Magnesium and the neurological system

As mentioned in the introduction, magnesium plays a key role in the activation of nervous system sympathetic activity. Magnesium deficiency has been shown to impair the affinity of serotonin and angiotensin II for their receptors in coronary vascular muscle, as

well as affect depolarization-induced contractions by interfering with potassium in a competitive manner [33]. In a recent study that spanned over six months in women with altered reasoning, changes were found in EEG readings [30]. When these women transitioned from daily consumption of about 115mg of magnesium (40% of the RDA) to 280mg of magnesium (slightly over the RDA), a great improvement in neurons' overexcitation and in reasoning coherence was observed, suggesting that deficient magnesium intake greatly impaired these functions [34]. Magnesium in the form of MgSO₄ has been shown to decrease inflammation-associated brain injury in fetal mice, suggesting a link between magnesium, inflammation, and neurologic injury, at least in rodents [4]. For these reasons, it cannot be excluded that magnesium may play a role in various neurological states, ranging from Parkinson's Disease to mood disorders (e.g. depression).

In 1921, Weston reported the beneficial effect of magnesium in the treatment of depression [35]. The study indicated that nearly all fifty patients injected with a magnesium sulfate solution underwent relaxation and successful sleeping [35]. In 2004, Singewald and Murck found that a magnesium-deficient diet exacerbates depression- and anxiety-related behavior in C57BL/6J mice [36]. The authors assessed their depression levels using the forced swim test, the standard for depression-like behavior in rodents. In this test, high depression levels are linked to mostly-immobile efforts to stay afloat. This behavior was more pronounced in mice with MgD [36]. Anxiety levels were also measured in these animals using a light/dark test, whereby anxiogenic behavior is associated with hesitation to explore more brightly-lit areas than familiar, darker territories, or the open-field test, whereby the amount of time spent in the center of a brightly-lit, new territory and away from the walls is considered anxiolytic behavior. Mice exposed to magnesium-depleted diets displayed more anxiogenic behavior [36].

This set of information led to a study on magnesium intake and its correlation with depression and anxiety in a community-dwelling of middle-aged and older adults that was published in the Australian and New Zealand Journal of Psychiatry. After letting subjects assess their own levels of anxiety, depression, and scoring the number of magnesium-rich or magnesium-poor foods that they ingested (using a checklist), Jacka and Mykletun observed that an inverse correlation existed between magnesium intake and depressive symptoms, which persisted after adjustments for age, gender, body habits, blood pressure, socioeconomic, and lifestyle factors [37]. A similar trend existed for magnesium intake and anxiety symptoms, but the associations were weaker and not significant following adjustments [37]. Interestingly, higher magnesium intake correlated with higher education, higher income, higher physical activity levels, lower alcohol consumption, lower BMI, and non-smoking, once energy-adjusted in men as well as in women [37].

In addition to mood disorders, brain magnesium levels are also reduced in other acute and chronic neural pathologies that include traumatic brain injury, migraine, cocaine exposure, ethanol intoxication, stroke, and subarachnoid hemorrhage [38]. A decrease in magnesium level has been hypothesized in neurodegenerative diseases as well [38]. Of particular interest is the effect of magnesium deficiency on Parkinson's disease, a neurodegenerative disorder characterized by tremors, rigidity, and a loss of neurons in the

substantia nigra. In a study on a population in Guam in 2005, Oyanagi and colleagues demonstrated that rats with induced Parkinson's Disease and exposed to a diet consisting of one-fifth the normal amount of magnesium showed marked atrophy in the substantia nigra, small tyrosine hydroxylase (TH)-immunopositive neurons, and a severely-reduced number of TH-immunopositive dendrites or axons of the substantia nigra, as well as reactive gliosis in the substantia nigra [39]. The authors concluded that a diet solely deficient in magnesium is more unsafe for nigral neurons than a diet deficient in both magnesium and calcium, and that nigral neurons only degenerate when a low magnesium intake persists from fetal and newborn periods through the prime of life [40]. Kolisek and collaborators took a different angle on this research by looking specifically at SLC41A1, a gene identified as a $\text{Na}^+/\text{Mg}^{2+}$ exchanger (NME) and the effects of a gain-of-function substitution mutation, pA350V [41]. Magnesium extrusion was blocked by >90% after administration of imipramine, which supported the notion that it occurred through SLC41A1-encoded NME activity. More importantly, this observation indicates that increased activity of NME might contribute to the development of intracellular magnesium deficient in the absence of compensatory cellular magnesium influx [41]. This further suggests that increased magnesium efflux leads to long-term systemic deterioration of cellular magnesium homeostasis in low extracellular magnesium, and that chronic magnesium deficiency in brain tissue may result in neurodegeneration.

Magnesium and the reproductive system

Magnesium is commonly used as a tocolytic in preterm labor in the form of i.v. MgSO_4 infusion. The mechanism of action is thought to be a limitation of calcium accumulation in uterine muscle cells, thereby reducing uterine contractility [4]. The use of MgSO_4 as a tocolytic dates back to early 1990. This intervention prevents preterm labor and also diminishes the incidence of cerebral palsy in newborns, as this condition represents the main complication of preterm labor. Indirectly, this therapeutic approach suggests that MgSO_4 may also act as a neuroprotective agent for newborns at risk [4]. Ex vivo research from Berstein's and Romani's laboratory provides compelling evidence that magnesium also acts as an anti-inflammatory agent for maternal and possibly neonatal monocytes, downregulating NF- κ B activation and therefore diminishing the synthesis and production of circulating proinflammatory cytokines (i.e. TNF α , IL-1) [42]. As a consequence, maternal and neonatal complications associated with preterm labor and inflammation, namely preeclampsia and cerebral palsy, are expected to decrease. This observation would also indicate that magnesium can be therapeutic as a broad-spectrum anti-inflammatory agent [42].

Multiple studies suggest that magnesium levels decrease during pregnancy, and that magnesium deficiency may lead to pre-eclampsia and pre-term birth. A study by Farzin and Sajadi in 2012 [43] show significantly lower serum calcium and magnesium levels in pre-eclamptic women, suggesting that the physiological effect of magnesium as a calcium channel blocker and as an antagonist of calcium-dependent arterial constriction is markedly attenuated, or lost altogether, in this pathological group. The authors also suggested that pre-eclampsia is associated with oxidative stress [43], in agreement with previous studies that proposed preeclampsia being associated with increased lipid peroxidation and decreased antioxidant enzyme

activities. Increased superoxide generation and decreased superoxide dismutase activity and mRNA expression have been observed in placental trophoblast cells [44]. Similarly, Enarunde and Okpere measured the clinical significance of low serum magnesium in pregnant women and found a significant inverse correlation with pre-eclampsia, leg cramps, and pre-term labor onset [45]. These authors also observed that women exhibiting hypomagnesaemia, a disorder characterized by extremely low serum magnesium levels and more profound than simply cellular magnesium deficiency, were 22-times more likely to progress toward pre-eclampsia than anticipated based on their age, and 47-times more likely to end in full-blown pre-eclampsia than predicted by their BMI [45].

Magnesium and the renal system

Numerous studies indicate that magnesium may play a protective role in vascular calcification via one of the following mechanisms: 1) magnesium inhibits formation of apatite crystals and forms smaller deposits that are more soluble; 2) magnesium functions as a calcium antagonist, which prevents calcium from entering cells; 3) magnesium restores balance between the expression of calcification promoters and inhibitors; 4) magnesium acts on CaSR and activates it, acting as calcimimetics that inhibit VSMC calcification [46].

Deficient magnesium levels, while prevalent, are often undetected. This increases the risk for other diseases, including diabetes mellitus type II, low bone mass, osteoporosis, and vascular calcification [46]. Previous research has demonstrated that magnesium deficiency in rats correlates to hypomagnesaemia, hypercalcemia, and the accumulation of additional calcium in several tissues, including the liver. This additional calcium in the liver has the potential to be associated with hepatotoxic effects, which enhance membrane permeability [47]. A study in 550 T2DM patients with no known kidney disease indicated that lower magnesium levels correlated with progressive deterioration of renal function [46]. Rodriguez explored the role of magnesium in different concentrations in Chronic Kidney Disease (CKD) and found that hypomagnesaemia translated into more negative outcomes, whereas moderate hypomagnesaemia seemed to have beneficial effects, and higher levels of hypomagnesaemia were associated with low parathyroid hormone (PTH) levels and hypoparathyroid complications [46]. It was also suggested that magnesium supplementation and mild hypermagnesaemia may have potential beneficial effects against calcification and mortality in CKD patients [46].

Magnesium in the skeletal/muscular systems

Tight magnesium control is essential for healthy bone growth. In the bones, over half of the whole body's magnesium store is found (60%), with an additional 30-40% contained in skeletal muscles and soft tissues, and just 1% in the extracellular fluid [4]. A depletion of magnesium can lead to endothelial dysfunction, which affects bone health, as well as inflammation, leading to the release of more inflammatory cytokines and subsequent bone remodeling and osteopenia [48]. Because magnesium also has mitogenic effects on osteoblasts, magnesium deficiency inhibits growth in cells and causes larger and more mineralized hydroxyapatite crystals to form [49]. For these reasons, osteoporosis, where bones become brittle and weak and bone formation is limited, may develop, and microfractures of the trabeculae become detectable, while tibial cortical thickness

markedly decreases [48]. A depletion of magnesium also promotes high levels of free radical production, which are shown to induce structural damage in skeletal muscle tissue of magnesium-deficient rats, mainly in sarcoplasmic reticulum and mitochondria [50]. Magnesium deficiency indirectly affects bone structure and functioning by altering PTH and 1,25 (OH)₂-Vitamin D levels, which ultimately leads to hypocalcemia. Lower levels of magnesium impair PTH secretion since magnesium is required as a cofactor for PTH signaling. The decreased secretion of PTH will eventually result in low serum concentrations of 1,25 (OH)₂-Vitamin D levels [48]. A study in 2007 on magnesium, zinc, and copper levels in postmenopausal women that were normal, osteopenic, or osteoporotic revealed that magnesium levels were significantly lower in osteoporotic women as compared to the levels measured in normal women [51].

Rude and Mills investigated the effects of magnesium deficiency on bone and mineral metabolism in a mouse model [49]. Mice were pair-fed, with the experimental group receiving a diet deficient in magnesium and water sources with lower levels of magnesium, before being sacrificed on the seventh day. The collected immunohistological samples indicated that magnesium deficiency resulted in significant osteoblast loss, impaired bone growth, and increased osteoclastic resorption in young animals by 135%, along with loss of trabecular bone [49]. Growth plate width decreased 33%, the number and length of chondrocyte columns were reduced, trabecular bone volume in the metaphysis of the tibia decreased, and there was an increased number of inflammatory cytokines in the bone, with a 140% increase in IL-1 by day three, and a 120% increase in TNF α and a 50% increase in megakaryocytes number by day five [49]. Taken together, these data point to magnesium deficiency as a risk factor for osteoporosis.

Interestingly, various studies have indicated that even short-term administration of oral magnesium supplementation has the capacity to partially reverse or mitigate damage. Aydin and coworkers measured the fasting plasma and urine of postmenopausal women who were given oral magnesium supplementation every five days for thirty days [52]. Among the substances the women were tested for was osteocalcin, which is secreted by osteoblasts, deoxypridinoline, a structural component in type I collagen in bones, and iPTH. The results indicate that even short-term dosage of magnesium raised serum levels of osteocalcin and significantly reduced urine deoxypyridinoline and serum iPTH, suppressing bone turnover [52].

Magnesium and hormones

Many endocrine-related diseases have associations with magnesium deficiency or reduced dietary magnesium intake although the specific underlying mechanisms remain undefined [4]. Among these endocrinopathies, diabetes mellitus type II and metabolic syndrome have been highly correlated to low magnesium levels [53]. Type I (T1DM) and type-II (T2DM) both involve hypomagnesaemia, hyper-magnesuria, and lower magnesium levels within tissues. More research has been conducted on T2DM, since 90-95% of diagnosed cases of diabetes are T2DM [4,54]. Numerous causes for low magnesium levels in diabetics can be listed including diets low in magnesium, osmotic diuresis that leads to high renal excretion of magnesium, insensitivity to insulin that affects intracellular magnesium transport and causes increased loss of extracellular magnesium, usage of loop and thiazide diuretics that

promote magnesium wasting, diabetic autonomic neuropathies, and reduced tubular reabsorption due to insulin resistance. Additionally, continuous magnesium deficiency correlates to higher levels of TNF α , which may also contribute to post-receptor insulin resistance [55]. Studies indicate that through not fully characterized mechanisms insulin increases cellular magnesium content, so that in pathologies like diabetes, where insulin functionality is impaired, cellular magnesium levels markedly decrease. Consistent with this observation, in magnesium-deficient animals, the levels of insulin receptor phosphorylation are decreased in skeletal muscles, thus impairing glucose accumulation in these tissues [4]. During severe hyperglycemia, the kidneys may lose the ability to reabsorb magnesium, excreting too much of the cation with the urine, which will further lower serum magnesium levels [56]. Significant negative correlations between magnesium and fasting plasma glucose, HbA1c, and HOMA-IR have also been observed (55).

In 2012, Dasgupta and collaborators detailed incurring pathological complications in one hundred and fifty non critically-ill T2DM patients and the effects of magnesium supplementation [55]. Patients with hypomagnesaemia had a nearly 20% higher incidence of retinopathy and 35% higher incidence of foot ulcers [55]. In contrast, patients with low intracellular magnesium levels and diabetic peripheral neuropathy experienced improvement in nerve conduction following magnesium supplementation [55]. The Nurses' Health Study (NHS) and Health Professionals' Follow-up Study (HFS) examined risk factors for developing T2DM in 127,932 research subjects with no prior history of diabetes, CVD, or cancer and found that the risk was much greater in those with lower magnesium intake levels [56]. Similarly, the Iowa Women's Health Study found that greater intake of whole grains, dietary fiber, and magnesium decreased the risk of women developing diabetes in the future [56].

One of the main complications of T2DM is diabetic retinopathy. This pathology is one of the leading causes of blindness, and hypomagnesaemia has been reported to occur at an increased rate among patients with T2DM [54]. A recent study has investigated serum magnesium levels in 120 patients, where 30 had T2DM without retinopathy, 30 had T2DM with retinopathy, and 60 had neither T2DM nor retinopathies. The results indicate that lower levels of serum magnesium were present in diabetic patients without retinopathy, and those with retinopathy had the lowest levels of serum magnesium [54]. As diabetic retinopathy has a strong inflammatory component, these results suggest that hypomagnesaemia may act as a risk factor for diabetic retinopathy, perhaps by negatively modulating the underlying inflammation.

Magnesium and alcoholism

Animal and human models reveal that alcohol consumption causes magnesium loss from tissues and, ultimately, through urine [4]. Specifically, animal studies demonstrate that magnesium deficiency will aggravate hepatic damage caused by alcohol [57]. Ethanol decreases cytoplasmic ATP, which removes an essential complexing agent for cellular magnesium. The consequent increase in cellular free magnesium will result in marked extrusion of magnesium from the hepatocyte through the NME. Inhibiting the NME or the alcohol dehydrogenase prevents magnesium loss [4]. Additionally, acute and chronic ethanol administrations both inhibit PKC signaling, a

pathway involved in magnesium accumulation, whereby magnesium cannot effectively accumulate until ethanol is removed from the system and PKC can properly translocate to the cell membrane, a process that can take more than an hour [4]. Alcohol consumption causes serum magnesium to decrease while urinary magnesium excretion increases two-three-fold, further exacerbating magnesium loss from the body [58].

Numerous lines of evidence indicate that ethanol consumption correlates to a decreased hepatic magnesium content after prolonged administration, while after acute administration, larger doses of ethanol result in larger depletions of cellular pools, which inhibits catecholamine-induced magnesium extrusion [58]. The effects of ethanol consumption on magnesium homeostasis are so profound that administering a dose of ethanol even as small as 0.01% is enough to inhibit the accumulation of magnesium for up to 60 minutes [58]. Physiological magnesium levels normally inhibit the release of proinflammatory cytokines, while promoting the release of anti-inflammatory cytokines. Alcohol consumption promotes production and release of pro-inflammatory cytokines from Kupffer cells, as well as circulating monocytes, and lymphocytes, which may have profound impacts for the well-being of hepatocytes [4]. In addition, persistent Kupffer cell activation promotes fibrosis and ultimately cirrhosis by upregulating hepatic stellate cells responsible for collagen deposition [56]. Earlier research demonstrates that loss of hepatic magnesium by ethanol or CCl_4 administration correlates to elevated collagen deposition, which may lead to the development of hepatic cirrhosis [58]. This potential for inflammation following alcohol consumption may play a role in the progression of Alcohol Liver Disease (ALD) from alcohol intake to steatohepatitis to cirrhosis [58].

Recent years yielded research that looked into the administration of supplemental magnesium to chronic consumers of alcohol. In a Norwegian study on chronic alcoholics, magnesium treatment decreased abnormally high activities of serum gamma-glutamyltransferase (S-GGT), aspartate-aminotransferase (S-AST), and alanine-aminotransferase (SALT), all of which are involved in liver function [51]. These results suggest that magnesium treatment may help restore part of liver function in alcoholics. In 2008, Poikolainen and Alho followed up on this study to determine whether oral magnesium treatment of alcoholics for eight weeks would decrease elevated S-GGT levels and neutralize S-AST or S-ALT activity. While these expectations were not confirmed, the authors ceded that alcohol intake level, its change, baseline enzyme levels, age, and body weight may influence after-treatment enzyme activity, suggesting that the randomized patient setup may not have been ideal for the proper undertaking of the study [51]. Additionally, the authors did find that S-AST levels were lower in the magnesium-treated group than in the placebo, which means that magnesium treatment may speed up the decrease in S-AST and reduce the risk of death from alcoholic liver disease [51].

Magnesium and the respiratory system

Asthma is a pathological condition characterized by inflammation and narrowing of the respiratory airways. Therapeutically, the narrowing of the respiratory airways is resolved by inducing rapid bronchodilation, usually through the usage of β_2 -adrenergic receptor agonists [59]. Co-administration of anticholinergic agents with β_2 -

adrenergic receptor agonists is also beneficial in that inhibition of cholinergic receptors potentiate the bronchodilation to resolve the asthma attack [59]. Whether other therapeutic agents can alleviate the acute attack or are more suitable to prevent future recurrences of the disease remain contentious [59]. In 1995, the potential role of magnesium in pulmonary functions was investigated by Fantidis and Cacho [60]. After excluding candidates displaying signs of pathologies with the potential to affect magnesium concentration (aside from asthma), they used a control group of 21 hospital employees and 50 patients with either mild, moderate, or severe bronchial asthma. In the study, they classified mild asthmatics as those with intermittent attacks and free of symptoms between attacks, moderate asthmatics as those whose pulmonary functions interfered with usual activities, and severe asthmatics as those with incapacitating dyspnea, cough, and obstructed airways despite treatment, as well as persistently abnormal pulmonary function [60]. Comparison of the polymorphonuclear magnesium content among control group and patient groups revealed a statistical difference. Although the study did not evidence a correlation between intracellular magnesium content and degree of asthmatic severity, it did demonstrate a difference between the control group and subgroup of asthmatics with low polymorphonucleate magnesium content [60].

The beneficial role of magnesium in pulmonary dysfunction was further investigated by Rowe and collaborators. These authors conducted a retrospective analysis of five adult and two pediatric trials with 668 patients to determine whether hospital admissions for asthma attacks were statistically reduced by the therapeutic usage of magnesium sulfate [59]. When pooled, the results indicated that magnesium sulfate treatment did not significantly improve the rates of maximum expiration speed or peak expiratory flow rate (PEFR), although this rate increased in those with severe acute asthma [4,59]. However, in patients with severe acute asthma treatment with magnesium sulfate increased absolute FEV_1 (the exhaled volume in the first second of a forced expiration) [4,59]. Patients with severe asthma also showed a reduced propensity towards hospital admission [59]. All together, the results of the study indicated that the benefits of intravenous magnesium sulfate administration in patients with acute asthma were rather questionable while they appeared to be beneficial in patients with severe acute asthma [59].

These results were mirrored by those obtained by Cheuk, Chau, and Lee [61]. These authors reviewed 5 randomized studies that used asthmatics under 18 years of age, and evaluated whether these patients were hospitalized or admitted to the ICU, as well as their clinical symptom scores and pulmonary function tests [61]. The results of the meta-analysis revealed that intravenous magnesium sulfate ultimately proved effective in avoiding hospitalization, with statistically significant results for those patients treated with magnesium sulfate as opposed to a placebo [61]. Consistent with the results by Rowe et al. [59], this new study indicated that magnesium sulfate was beneficial in conjunction with inhaled bronchodilators and systemic steroids for patients affected by moderate to severe acute asthma admitted to the Pediatric Emergency Department [61]. Blitz and Blitz took a slightly different approach, in that they reviewed whether aerosolized as opposed to intravenous magnesium sulfate had better efficacy in treating acute asthmatics brought to the Emergency Department [62]. For their study, the authors

used six trials from 1995 onward drawn from databases EMBASE, CENTRAL, MEDLINE, and CINAHL for a total of 296 patients treated with nebulized magnesium sulfate alone or with B₂-agonists. The results were compared to those obtained from patients treated with B₂-agonists alone or placebo [62]. Studies using intravenous magnesium sulfate administration were excluded. Interestingly, most studies revealed no change in pulmonary function, and the pooled results did not differentiate between baseline pulmonary function in treated vs. control groups [62]. However, patients who used nebulized magnesium sulfate alone showed a significant difference in pulmonary function and a trend toward decreased hospitalizations, as well as improved lung function, when nebulized magnesium sulfate was used with a B₂-agonist [62]. Ultimately, these studies revealed that magnesium sulfate seems to be most effective when used in conjunction with a B₂-agonist, and in patients with severe asthma attacks [59-62]. These aspects of magnesium sulfate treatment have been consistently confirmed by more recent studies in both adult and children [63], although some significant differences in effectiveness exist between adult and children most likely as a result of different dosages used and differences in susceptibility of smooth muscle cells based on age [63]. Consensus is there for the use of magnesium sulfate (nebulized or intravenous) especially for severe and life-threatening asthma exacerbation [64,65].

Despite these positive outcomes, the mechanism of action underlying the beneficial effects of magnesium needs further elucidation. Data in the field suggest that magnesium can induce bronchial smooth muscle cell relaxation by inhibiting cytosolic calcium increase in the cells [66], or by inhibiting the release of histamine from mast cells [67] or the release of acetylcholine from cholinergic nerve endings [68], or by increasing the bronchodilator effect of β 2-adrenergic agonist through changes in receptor affinity [69]. Additionally, administration of magnesium sulfate has a stabilizing effect on the atria, attenuating the tachycardia that is usually observed following β 2-adrenergic agonist intake [65].

Future directions

This review briefly highlighted that magnesium deficiency has wide-ranging systemic effects on the body in a multitude of different ways, stemming to the myriad roles magnesium plays in signaling pathways, or acting as a co-enzyme in scores of enzymatic interactions, and the varying roles it plays in different bodily responses. Although intake of too much magnesium and hypermagnesaemia can prove detrimental, mechanisms upregulating intestinal uptake and renal reabsorption of magnesium usually prevent this possibility. Hypomagnesaemia or magnesium deficiency, on the other hand, may prove far more deleterious, especially in the long run. Many different sources have revealed the beneficial effects of administering magnesium in depleted individuals for varying pathologies, thus hinting to a role for magnesium in the treatment of specific diseases and their complications.

A large limitation to the development of therapies providing appropriate magnesium administration in disease states is the identification of magnesium deficiency and its extent. There is no clear approach to positively identify magnesium deficiency in patients or even animals, other than giving the recipients a magnesium-load test or a serum ionized magnesium test, neither of which are in current

clinical usage [22]. This review has summarily listed the wide-ranging effects of magnesium deficiency in CVD, neuropathies, nephrology, osteoporosis, T2DM, chronic alcohol consumption, pre-eclampsia, and asthma. All of these pathologies affect a large percentage of the population, some with more fatal outcomes than others. In the review, the beneficial effects of magnesium supplementation in many of these pathologies have also been addressed. Ultimately, establishing a method to identify magnesium deficiency in patients could prove very useful for their identification and for the prevention (or attenuation) of often-fatal diseases. Ultimately, this field remains very promising and full of potential to develop therapeutic approaches aimed at alleviating various disease states and their complications.

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