International Journal of Frontiers in
Chemistry and Pharmacy Research
Journal homepage: https://frontiersrj.com/journals/ijfcpr/
ISSN: 2783-0462 (Online)

(RESEARCH ARTICLE)

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Magnesium metabolism and its assessment

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International Journal of Frontiers in Chemistry and Pharmacy Research, 2021, 01(01), 029–043

Publication history: Received on 14 March 2021; revised on 19 April 2021; accepted on 22 April 2021

Article DOI: https://doi.org/10.53294/ijfcpr.2021.1.1.0055

Abstract

Magnesium is the fourth most abundant cation in the body. It has several functions in the human body including its role as a cofactor for more than 300 enzymatic reactions. Magnesium balance in the body is controlled by a dynamic interplay among intestinal absorption, exchange with bone, and renal excretion. Intestinal magnesium absorption proceeds in both a passive paracellular and an active transcellular manner. Regulation of serum magnesium concentrations is achieved mainly by control of renal magnesium reabsorption. In the kidney passive paracellular transport via claudins facilitates bulk magnesium absorption, whereas active transcellular pathways mediate the fine-tuning of magnesium absorption. There are no readily available and easy methods to assess magnesium status. Serum magnesium and the magnesium tolerance test are the most widely used. Measurement of ionized magnesium may become more widely available with the development of ion selective electrode analyzers.

Keywords: Magnesium metabolism, Magnesium transport; Serum magnesium; Magnesium loading test

1 Introduction

Magnesium is the second most abundant intracellular cation and the fourth most abundant cation in the body. Magnesium has several functions in the human body. It acts as a cofactor for more than 300 enzymes, regulating a number of fundamental functions such as muscle contraction, neuromuscular conduction, glycemic control, myocardial contraction, and blood pressure [1]. Moreover, magnesium also plays a vital role in energy production, active transmembrane transport for other ions, synthesis of nuclear materials, and bone development [1]. The assessment of magnesium status is important for the study of diseases associated with its chronic deficiency. In spite of intense research activities there is yet no simple, rapid, and accurate laboratory test to determine total body magnesium status in humans. However, serum magnesium level of 0·75 mmol/l is a useful cut off value for severe deficiency. In patients with values between 0·75 and 0·85 mmol/l a magnesium loading test can identify deficient individuals. The loading test seems to be the gold standard for magnesium status, but again unsuitable in patients with disturbed kidney and intestinal functions when administered orally. However, there is need for a consensus to be reached on a standardized protocol to enable comparison of results obtained in different clinical units. Other cellular magnesium measurements, such as total or ionized magnesium, frequently disagree and more research and systematic evaluations are needed. Muscle magnesium appears to be a good marker, but biopsies limit its usefulness, as in the case with bone magnesium, the most important but heterogeneous magnesium compartment. Non invasive techniques such as nuclear magnetic resonance (NMR) may provide valuable tools for routinely analyzing ionized magnesium in tissues [2].

2 Magnesium Functions

Magnesium is primarily found in the intracellular compartment, where it acts as a counter ion for the energy-rich ATP and nucleic acids. Magnesium is a cofactor in more than 300 enzymatic reactions [3]. Magnesium stabilizes enzymes,

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including many ATP-generating reactions. ATP is required universally for glucose utilization, synthesis of fat, proteins, nucleic acids and coenzymes, muscle contraction, methylation and many other processes. Thus, interference with magnesium metabolism impacts these functions [4]. Overall ATP metabolism, muscle contraction and relaxation, normal neurological function and release of neurotransmitters, are all magnesium dependent. It is also important to note that magnesium contributes to the regulation of vascular tone, heart rhythm, platelet-activated thrombosis and bone formation [5]. Some of magnesium's several functions are listed in Table 1.

Table 1 Physiological functions of magnesium

Enzyme function	Enzyme substrate (ATP-Mg^{+}, GTP-Mg^{+})
	Kinases B, Hexokinase, Creatine kinase Protein kinase, ATPases or GTPases Na^{+}/K^{+}-ATPase, Ca^{2+}-ATPase, Adenylate & Guanylate cyclise.
	Direct enzyme activation
	Phosphofructokinase, Creatine kinase 5-Phosphoribosyl-pyrophosphate synthetase Adenylate cyclise, Na^{+}/K^{+}-ATPase
Membrane function	Cell adhesion Transmembrane electrolyte flux
Calcium antagonist	Muscle contraction/relaxation Neurotransmitter release Action potential conduction in nodal tissue
Structural function	Proteins, Polyribosomes, Nucleic acids Multiple enzyme complexes, Mitochondria

In muscle contraction, magnesium stimulates calcium re-uptake by the calcium-activated ATPase of the sarcoplasmic reticulum [4]. Magnesium further modulates insulin signal transduction, cell proliferation, cell adhesion, transmembrane transport including transport of potassium and calcium ions. It also maintains the conformation of nucleic acids and is essential for the structural function of proteins and mitochondria. It has been proposed that magnesium may play a role in insulin secretion based on the findings that insulin secretion and sensitivity are altered in magnesium-deficient animals [6].

Magnesium is important in health and disease. Epidemiological studies have shown a high prevalence of hypomagnesemia with low intracellular magnesium concentrations in diabetics. Benefits of magnesium supplementation on the metabolic profile of diabetics have been observed in some clinical trials. However, larger prospective studies are needed to determine if dietary magnesium supplementation is beneficial for diabetics [7]. Studies have shown that a relatively young gestational age is associated with magnesium deficiency during pregnancy, which not only induces maternal and fetal nutritional disorders but also leads to other adverse effects throughout the life of the offspring's [8].

Evidence has been demonstrated in regards to competition between magnesium and calcium for the same binding sites on plasma protein molecules [9]. Thus, magnesium is considered a natural 'calcium antagonist'. It is shown that magnesium antagonizes calcium-dependent release of acetylcholine at motor endplates [10]. Based on the antagonist relationship between the two electrolytes, calcium may be considered a powerful 'death trigger', while magnesium salvages the negative impact of calcium [11]. Magnesium inhibits calcium-induced cell death [12]. It is anti-apoptotic in mitochondrial permeability transition and antagonizes calcium-overload-triggered apoptosis.

3 Magnesium Balance

There is considerable variation in the plasma and tissue exchange of magnesium between various organs of the body [13]. Myocardium, kidney parenchyma, fat tissue, skeletal muscle, brain tissue and lymphocytes exchange intracellular and extracellular magnesium at different rates. Humans need to consume magnesium regularly to prevent magnesium deficiency. The recommended daily allowance (RDA) for magnesium is 4.5 mg/kg/day for adults [14]. The RDA of magnesium proposed by the Institute of Medicine includes 310–360 mg and 400–420 mg for adult women and men, respectively [15]. The daily requirement is higher during pregnancy, lactation, following debilitating illness, those on high intakes of calcium, phosphate, and high fat diet, and those under environmental stresses [15]. Drinking water, especially 'hard water,' which contains up to 30 mg/l of magnesium, is an important source. While drinking water accounts for ~10% of daily magnesium intake, chlorophyll (and thus green vegetables) is the major source of magnesium [16]. Nuts, seeds and unprocessed cereals are also rich in magnesium. Legumes, fruits, meat and fish have an intermediate magnesium concentration. Low magnesium concentrations are found in dairy products [17]. Processed foods have much lower magnesium content than unrefined grain products and as a result, low intake of dietary magnesium occur in individuals that consume western diets which are heavily processed [18]. With the dominance of processed foods, boiling and consumption of de-mineralized soft water, most industrialized countries are deprived of their natural magnesium supply. Alternatively, magnesium supplements are very popular food supplements, especially in the physically active form. Serum magnesium concentration is regulated by a dynamic balance of interplay between intestinal, renal transport and bone exchange. Magnesium is absorbed in the gut, stored in bone mineral with excess magnesium excreted via the kidneys and faeces [13] (Figure 1).

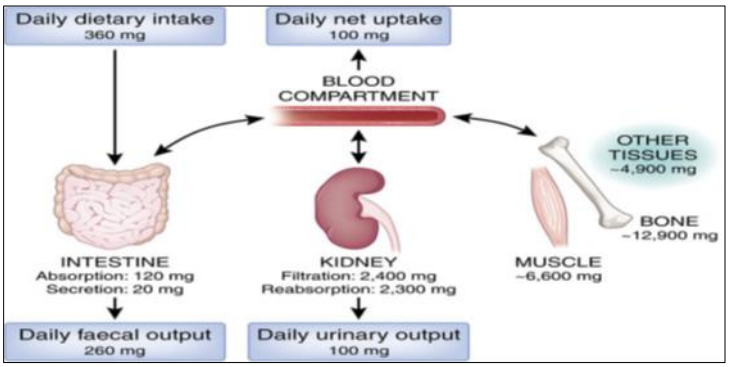


Figure 1 Magnesium balance; (Source: Jahnen-Dechent and Ketteler, 2012)

Typical magnesium ingestion is approximately 300 mg/d [19]. Intestinal absorption can range from 25% when eating magnesium-rich diets to 75% when eating magnesium depleted diets. Approximately 120 mg of magnesium is absorbed and 20 mg is lost in gastrointestinal secretions, amounting to a net daily intake of 100 mg/d. Assuming a normal GFR, the kidney filters approximately 2000–2400 mg of magnesium per day. This takes into account the fact that only 70% of total serum magnesium (30% is protein-bound) is available for glomerular filtration. Under normal conditions, 96% of filtered magnesium is reabsorbed in the renal tubules by several coordinated transport processes. In times of Mg^{2+} shortage, other tissues such as bone and muscle provide Mg^{2+} to restore blood Mg^{2+} levels.

4 Magnesium Content and Distribution

The total body magnesium content in adults is approximately 24g. Of this amount, 99% is intracellularly stored in bone, muscle, and non-muscular soft tissue (Table 2), with only 1% stored in the extracellular space [20]. Approximately 50–60% of magnesium resides as surface substituents of the hydroxyapatite mineral component of bone [21]. Most of the remaining magnesium is contained in skeletal muscle and soft tissue. The magnesium content of bone decreases with age, and magnesium stored in this way is not completely bioavailable during magnesium deprivation [22]. Nonetheless, bone provides a large exchangeable pool to buffer acute changes in serum magnesium concentration [13]. Overall, one third of skeletal magnesium is exchangeable, serving as a reservoir for maintaining physiological extracellular magnesium levels [13]. Intracellular magnesium concentrations range from 5 to 20mmol/L; 1–5% is ionized, the remainder is bound to proteins, negatively charged molecules and ATP [21].

Extracellular magnesium accounts for ~1% of total body magnesium which is primarily found in serum and red blood cells (RBCs). Normal total serum magnesium concentration is in the range of 0.7–1.1 mmol/l, 1.4–2.2 mEq/l, or 1.7–2.6 mg/dl [19]. Serum magnesium can be categorized into three fractions; ionized, protein-bound and complexed

magnesium. Of the three fractions in plasma, ionized magnesium (Mg^{2+}) has the greatest biological activity. About 55-70 percent of serum magnesium exists in the ionized, free, and physiologically active form; whereas 20-30 percent of serum magnesium is protein-bound and 5-15 percent complexes with anions such as phosphate, bicarbonate and citrate or sulphate **[**13**]**.

Table 2 Distribution of magnesium in the adult human

Tissue	Body weight (kg wet weight)	Concentration (mmol/kg wet weight)	Content (mmol)	% of total body Magnesium
Serum	3.0	0.85	2.6	0.3
Red blood cells	2.0	2.5	5.0	0.5
Soft tissue	22.7	8.5	193.0	19.3
Muscle	30.0	9.0	270.0	27.0
Bone	12.3	43.2	530.1	52.9
Total	70.0	64.05	1000.7	100.0

5 Magnesium Homeostasis

Many studies have shown that intestinal Mg^{2+} absorption is balanced against renal Mg^{2+} excretion [23]. In times of a temporary Mg^{2+} deficit, the body depends on the availability of Mg^{2+} in bone to maintain constant serum levels [24]. Therefore, magnesium homeostasis depends on three organs: the intestine, which facilitates Mg^{2+} uptake; bone, the Mg^{2+} storage system of the body and the kidneys, which are responsible for Mg^{2+} excretion.

5.1 Intestinal magnesium uptake

Approximately 30–50% of dietary Mg^{2+} is absorbed by the intestine (Figure 1). Magnesium absorption takes place mainly in the distal small intestine and in the colon [25]. Therefore shortening of ileum, results in a substantial decrease of Mg^{2+} absorption [26]. Two Mg^{2+}-absorbing pathways have been identified in the mammalian intestine (Figure 2). Paracellular transport involves the absorption of Mg^{2+} through the small spaces between the intestinal epithelial cells and is a passive mechanism. Secondly, the transcellular pathway involves the active transport of Mg^{2+} to the blood through the interior of the epithelial cell mediated by apical channels; transient receptor potential channel melastatin members 6 and 7 (TRPM6/TRPM7). The transcellular pathway of Mg^{2+} transport is subject to tight regulation since the ions have to pass through two cell membranes. Paracellular Mg^{2+} absorption is responsible for 80–90% of intestinal Mg^{2+} uptake. The paracellular pathway is regulated by proteins comprising the tight junction, including claudins, occludin, and zona-occludens-1 [19]. The driving force behind this passive Mg^{2+} transport is supplied by the high luminal Mg^{2+} concentration, which ranges between 1.0 and 5.0 mmol/L, and the lumen-positive transepithelial voltage of ~15 mV [27]. Paracellular Mg^{2+} absorption relies on tight junction permeability. Tight junction assembly and function can be modulated by a number of signaling molecules that alter the phosphorylation state of the tight junctional proteins and the ionic permeability of the paracellular pathway. The ileum and distal parts of the jejunum are known to be the most permeable for ions because of the relatively low expression of 'tightening' claudins 1, 3, 4, 5 and 8 [28]. As such, paracellular Mg^{2+} transport seems mainly restricted to these areas that lack the 'tightening' claudins. Claudins 16 and 19, known to be involved in Mg^{2+} permeability, are not expressed in the intestine [28, 29]. The exact mechanism facilitating paracellular Mg^{2+} absorption, therefore, remains unknown.

Transient receptor potential channel melastatin 6 (TRPM6) and TRPM7 Mg^{2+} channels mediate transcellular absorption. Whereas TRPM7 is ubiquitously expressed, intestinal TRPM6 expression is mainly detected in the distal small intestine and colon in murine tissue [30]. Both TRPM6 and TRPM7 expression is restricted to the luminal membrane of the enterocytes (Figure 2). The basolateral Mg^{2+} extrusion mechanism is unknown, but several studies have suggested that basolateral Mg^{2+} transport is coupled to the Na^+ gradient, sodium concentrations being lower in the cytoplasm than the blood owing to the action of basolateral Na^+/K^+-ATPase [31]. This hypothesis, however, remains to be confirmed by the identification of the basolateral Mg^{2+} transporter. Intestinal absorption is regulated by a variety of factors. Magnesium absorption is altered by dietary Mg^{2+} intake, as demonstrated by Mg^{2+} uptake studies [32, 33]. When dietary magnesium intake is normal, transcellular transport mediates 30% of intestinal Mg^{2+} absorption due to changes in TRPM6 expression in the colon [30]. This fraction increases up to ~80% when dietary magnesium intake is lower

[19]. When dietary magnesium is higher, then the majority of intestinal absorption occurs via the paracellular pathway owing to changes in the electrochemical gradient. Furthermore, it has been shown that 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] stimulates intestinal Mg^{2+} absorption [34]. Indeed, patients with chronic renal disease often associated with hypomagnesemia have low 1,25(OH)$_2$D levels. However, TRPM6 expression in the kidneys is not regulated by 1,25(OH)$_2$D$_3$[35]. TRPM6 expression in the colon in response to 1,25(OH)$_2$D remains to be determined. Claudins 2 and 12, which are involved in paracellular Ca^{2+} transport, are regulated by 1,25(OH)$_2$D. It is therefore hypothesized that these claudins may be involved in paracellular Mg^{2+} absorption. Experiments in mice showed that low and high dietary Mg^{2+} affects Ca^{2+} balance via the kidney (i.e. increased reabsorption and elimination, respectively) [30]. The mechanisms responsible for these phenomena are unknown, but a regulatory role of calcium-sensing receptor (CaSR) in the interaction between Mg^{2+} and Ca^{2+} has been proposed.

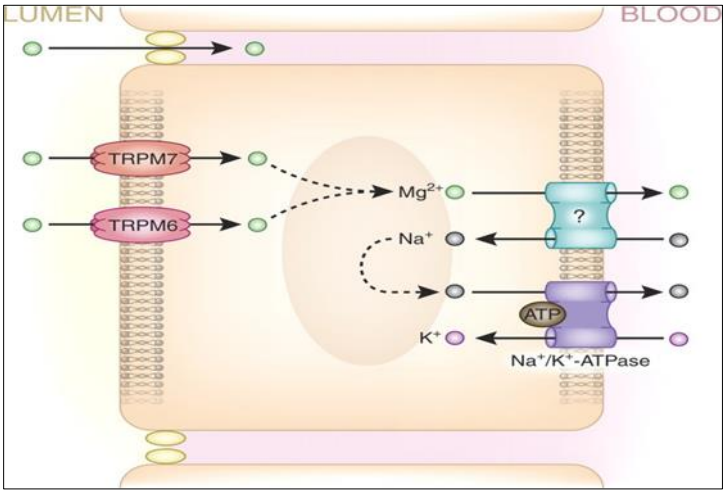


Figure 2. Pathways for **Mg^{2+}** absorption across the intestinal epithelium; (Source: de Baaij *et al.,* 2012)

5.2 Magnesium storage

Magnesium can be stored in muscle fibres, where it plays an important role in the regulation of muscle contraction by antagonizing the action of Ca^{2+}. At least 50% of the total body magnesium content resides in bone as hydroxyapatite crystals, where it also contributes to the density and strength of the skeleton [32, 33]. Dietary magnesium restriction causes decreased bone magnesium content [36]. Depletion of magnesium is, therefore, a risk factor for osteoporosis. A model of Mg^{2+}-induced bone loss has been proposed in which low blood plasma magnesium concentrations lead to activation of bone resorption by osteoclasts and decreased osteoblast bone formation [35]. Moreover, bone surface magnesium concentrations (of ~30%) are closely related to serum magnesium concentrations, indicating a continuous exchange of magnesium between bone and blood [35]. Although the bone magnesium stores are dynamic, the transporters that mediate magnesium flux in and out of bone have not yet been determined. PTH stimulates the release of magnesium from bone.

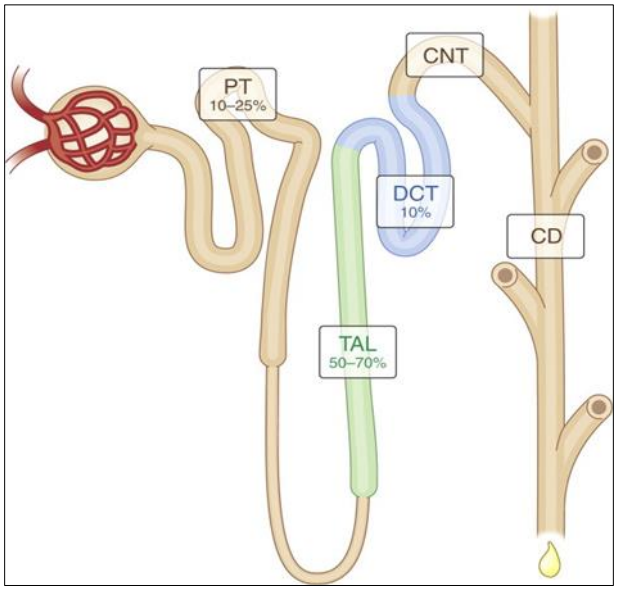
5.3 Renal magnesium elimination

Approximately 2400 mg of Mg^{2+} is filtered daily by the glomeruli. Along the nephron, 90–95% of Mg^{2+} is reabsorbed; while the remaining 100 mg leaves the body via the urine (Figure 1). The specific roles of the various parts of the nephron are considered in the following sections.

Little Mg^{2+} is reabsorbed in the proximal tubule in comparison with other electrolytes such as Na^+, $K^+$and Cl^- (Figure 3). Luminal Mg^{2+} concentrations increase as water is reabsorbed by Na^+ gradient driven transport. At this high concentration gradient, Mg^{2+} reabsorption occurs via passive paracellular transport, leading to the reabsorption of 10–25% of Mg^{2+} [35].

The majority of filtered Mg^{2+} is reabsorbed in the loop of Henle, mostly in the thick ascending limb (TAL) which accounts for up to 70% of total Mg^{2+} reabsorption (Figure 3). In fact, Mg^{2+} is the only bulk ion transported in the TAL of the loop of Henle. Magnesium reabsorption in the proximal tubule and TAL is the opposite of the reabsorption of Na^+ and K^+, which occurs mainly in the proximal tubule rather than in the TAL. The transepithelial voltage gradient is the driving force behind the passive paracellular transport of Mg^{2+} in the TAL (voltages in the tubular lumen are positive relative to blood). Claudins 16 and 19 form a cation-selective tight junction, facilitating the paracellular transport of Mg^{2+} in the

TAL [29]. Sodium entry and exit are mediated via the apical Na^+–K^+–2Cl cotransporter (NKCC2) and the Na+/K+-ATPase, respectively. NKCC2 mediates apical absorption of Na^+, K^+, and Cl^- into the TAL cells (Figure 4). Na^+/K^+ -ATPase mediates Na^+ exit through the basolateral membrane and generates the Na^+ gradient for Na^+ absorption. The ATP-dependent renal outer medullarly K^+ (ROMK) channels which represent the first member out of 7 subfamilies of the inward-rectifying K^+ channels (Kir1 to Kir7) mediates apical recycling of K^+ back to the tubular lumen which generates lumen-positive voltage that drives paracellular Mg^{2+} transport. The chloride channels expressed together with barttin polypeptide (ClC-Ka/ClC-Kb): predominantly localized in the cells of loop of Henle and inner ear, mediates chloride exit via ClC-Kb at the basolateral membrane of TAL cells.

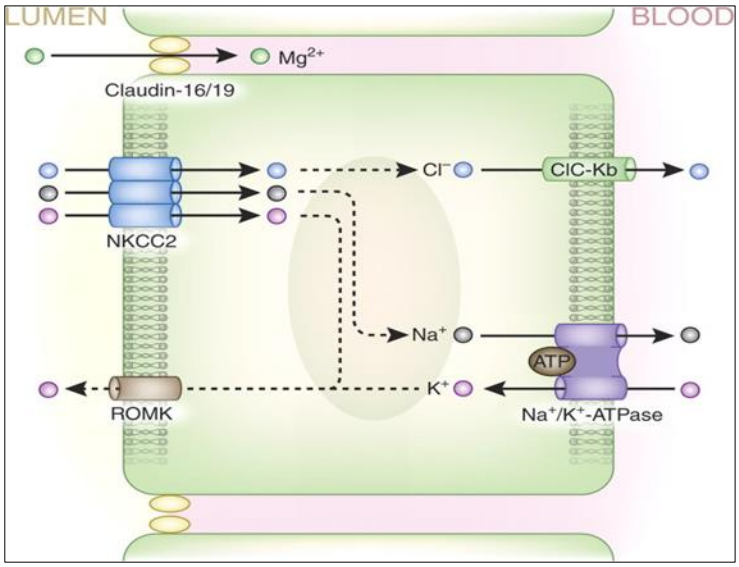


Proximal tubule (PT), thick ascending limb (TAL) of the loop of Henle, distal convoluted tubule (DCT), connecting tubule (CNT), collecting duct (CD).)

Figure 3 Magnesium reabsorption along the nephron; (Source: Source: de Baaij et al., 2012)

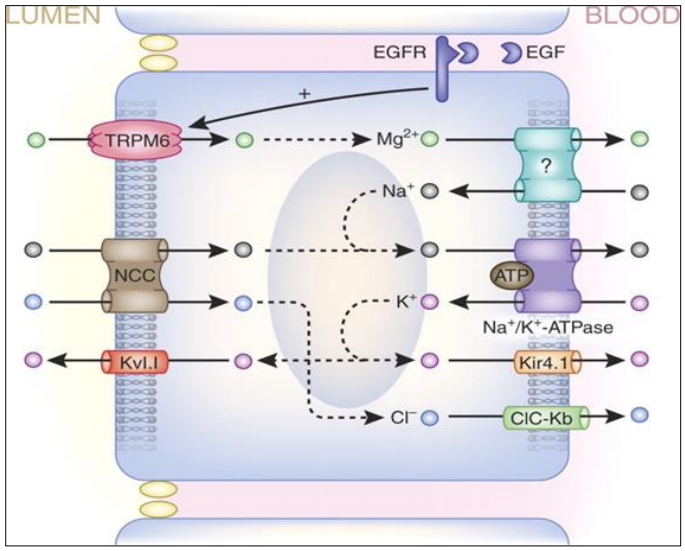
Altogether, apical NKCC2 and ROMK work in concert with basolateral CIC-Kb and Na^+/K^+ -ATPase to enhance electrogenic transcellular NaCl reabsorbtion, which subsequently generates the transepithelial voltage that drives paracellular absorption of Mg^{2+}. The Ca^{2+}/Mg^{2+} -sensing receptor (CaSR) is an important regulator of magnesium homeostasis [37]. The CaSR is located in the basolateral membrane of TAL cells and in the distal convoluted tubule (DCT) as well as in the plasma membrane of cells in the parathyroid gland [37]. CaSR senses ionized serum Ca^{2+} and Mg^{2+} concentrations and is involved in renal Ca^{2+} and Mg^{2+} reabsorption as well as in PTH secretion [38]. Upon stimulation or activation of the CaSR, renal Mg^{2+} reabsorption is decreased. Basolateral CaSR activation inhibits apical ROMK channels and possibly NKCC2 in the TAL [19]. This inhibition diminishes the transepithelial voltage and in turn inhibits the passive transport of magnesium within the cortical TAL.

The 'fine-tuning' of Mg^{2+} reabsorption takes place along the DCT, where ~10% of filtered Mg^{2+} is reabsorbed (Figure 3). In the DCT, Mg^{2+} reabsorption is an active transcellular process that is tightly regulated by several factors, each playing an important role in Mg^{2+} homeostasis (Figure 5) [39]. EGF acts as an autocrine/paracrine magnesiotropic hormone by activating the basolateral EGF receptor, causing an increase in luminal TRPM6 activity and enhanced luminal magnesium uptake in the DCT. The apical potassium voltage-gated channel subfamily A member 1 (Kv1.1) maintains transmembrane voltage which is the driving force for TRPM6-mediated magnesium absorption by establishing favorable luminal potential. Kir4.1 is responsible for recycling of K^+ at the basolateral site of the cell. The key molecule at the basolateral membrane is the Na^+/K^+ -ATPase, whose expression is regulated by transcription factor HNF1B. The Na^+/K^+-ATPase activity is stimulated by its gamma–subunit which is encoded by the FXYD2 gene. Basolateral Kir4.1 and the gamma-subunit of Na^+/K^+-ATPase also increase magnesium reabsorption by generating a sodium gradient, making it possible for Na-Cl cotransporter (NCC) to facilitate sodium transport from the apical lumen to the cytosol (Figure 5). The basolateral Mg^{2+} transporter remains to be identified. It has been proposed that absorbed Mg^{2+} is extruded via a magnesium/sodium exchanger SLC41A1 family across the basolateral membrane [19]. NCC and ClC-Kb are responsible for Na^+ and Cl^+ transport in the distal convoluted tubule. The divalent metal cation transporter cyclin M2 (CNNM2) has been identified to be involved in renal Mg^{2+} transport. In the kidney, CNNM2 is predominantly localized along the basolateral membrane of distal tubular segments involved in Mg^{2+} reabsorption [19].



Abbreviations: ClC-Ka and ClC-Kb, renal chloride channels; NKCC2, Na+–K+–2Cl– cotransporter; ROMK, renal outer medulla potassium channel.

Figure 4 Schematic overview of Mg^{2+} transport pathways in the thick ascending limb of the loop of Henle; (Source: de Baaij *et al.,* 2012)



Abbreviations: CLC-Kb, basolateral chloride channel; EGF, epidermal growth factor; NCCT, sodium–chloride cotransporter; pro-EGF, epidermal growth factor precursor protein; TRPM6, Transient receptor potential channel melastatin member 6.

Figure 5 Magnesium ion transport pathways in the distal convoluted tubule; (Source: de Baaij et al., 2012)

Factors that alter renal regulation of Mg^{2+} are presented in table 3. Epidermal growth factor (EGF) regulates TRPM6 activity and plasma membrane availability. Basolaterally expressed pro-EGF is almost exclusively found in the DCT and pro-EGF is cleaved to EGF which binds EGFR to regulate TRPM6 activity [40]. Estrogen has also been shown to stimulate TRPM6 expression [30]. Thus, estrogen substitution therapy is used to normalize hypermagnesuria, which occurs frequently in postmenopausal women [41]. It is demonstrated that TRPM6 expression is regulated by plasma magnesium levels and estrogens, but not by $1,25(OH)_2D$ or PTH action [30]. PTH directly induces renal tubular reabsorption of Mg^{2+}. Hypercalcemia interferes with the role of PTH in magnesium metabolism.

Table 3 Factors that alter renal regulation of magnesium

Increase Mg^{2+} absorption	Decrease Mg^{2+} absorption
Dietary magnesium restriction Parathyroid hormone Glucagon Calcitonin Vasopressin Aldosterone Insulin Amiloride Metabolic alkalosis Epidermal growth factor Estrogen	Hypermagnesemia Metabolic acidosis Hypercalcemia Phosphate depletion Potassium depletion Diuretics (loop and thiazide) Antibiotics (aminoglycosides) Antifungals (amphotericin B) Antivirals (foscarnet) Chemotherapy agents (cisplatin) Immunosuppressants (tacrolimus, cyclosporine, rapamycin) EGF receptor antagonists FHHNC caused by mutations in claudin-16 and claudin-19 HSH caused by mutations in TRPM6 Bartter's syndrome caused by mutations in NKCC2 (type 1), ROMK (type II), ClC-Kb (type III), or CaSR (type V) Dominant hypomagnesemia caused by mutations of FXYD2, HNF1B, or CNNM2 genes Isolated dominant hypomagnesemia caused by mutations of Kv1.1 Isolated recessive hypomagnesemia caused by mutations of pro-EGF Gitelman's syndrome caused by mutations of NCC EAST/SeSAME caused by mutations in Kir4.1

FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HSH, hypomagnesemia with secondary hypocalcemia; TRPM6, transient receptor potential melastatin 6; NKCC2, Na-K-2Cl cotransporter; ROMK, renal outer medullary potassium channel; CLC-Kb,Chloride channel Kb; CaSR, calcium sensing receptor; FXYD2, sodium/potassium ATPase gamma chain gene; HFN1B, hepatocyte nuclear factor 1 beta; CNNM2, cyclin M2; NCC, Na-Cl co-transporter; EAST (epilepsy, ataxia, sensorineural deafness, and tubulopathy): SeSAME (seizures, sensineural deafness, ataxia, mental retardation, and electrolyte imbalance).

6 Assessment of Body Magnesium Status

As magnesium is mainly an intracellular ion, assessing its status is difficult. At present, there is no simple, rapid, and accurate laboratory test to indicate the total body magnesium status. The major approaches available for clinical testing are presented in table 4 [3]. No single method is satisfactory to assess magnesium status. The simplest, most useful and readily available methods include the measurement of serum total magnesium and the magnesium tolerance test [5, 42]. Ionized magnesium measurement may become more readily available with the development of reliable analyzers.

Table 4 Assessment of magnesium status

Serum magnesium Concentration	Total magnesium Ultrafiltrable magnesium Ionised magnesium
Intracellular magnesium content	Red blood cells[a] Mononuclear blood cells[b] Skeletal muscle[c]
Physiological tests	Metabolic balance studies Renal excretion of magnesium Magnesium loading test

	Isotope balance studies
Intracellular free magnesium ion concentration	Fluorescent probes [d] Ion-selective electrodes[e] AAS spectroscopy[f] NMR spectroscopy[g]
Others	Hair or tooth magnesium Functional assays

a Red blood cell magnesium concentration does not correlate well with total body magnesium status; b Magnesium content of mononuclear cells may be a better predictor of skeletal and cardiac muscle magnesium content; c Muscle is an appropriate tissue for the assessment of magnesium status but it is an invasive and expensive procedure requiring special expertise; d Intracellular free magnesium concentration can be determined by using fluorescent probes. However, the application of fluorescent dyes is limited because the major fluorescent dye for magnesium (mag-fura 2) has a higher affinity for calcium than magnesium: e Ion-specific microelectrodes can be used to measure the internal free ion concentration of cells and organelles. Major advantages are that readings can be made over long time spans. In contrast to dyes, very little extra ion buffering capacity has to be added to the cells, and direct measurement of the ion flux across the membrane of a cell is possible with every ion passing across the membrane contributing to the result. Nonetheless, ion-selective electrodes for magnesium are not entirely selective for ionized magnesium. A correction is applied based on the ionized calcium concentration: f Total magnesium content of a biological sample can be determined by using flame atomic absorption spectroscopy (AAS). However, this technique is destructive and, for optimal accuracy, sample volume has to add up to ~2 mL with a concentration ranging from 0.1 to 0.4 l mol/L. With this technique, only content, but not uptake, can be quantified: g Nuclear magnetic resonance may be used to measure intracellular free magnesium concentration .

6.1 Serum magnesium concentration

The most commonly used method for assessing magnesium status is the total serum magnesium concentration. It is the most practicable and inexpensive approach for the detection of acute changes in magnesium status [20]. Serum total magnesium levels have been measured by various techniques including photometry, fluorometry, flame emission spectroscopy, and the atomic absorption spectrometry which is the reference method,. The spectrophotometric method, xylidyl blue is the most widely used in determining serum Mg^{2+} in many laboratories [43]. Magnesium concentration is preferably measured in serum rather than plasma as the anticoagulant used for collection of plasma could be contaminated with magnesium or could affect the assay procedure. For instance, citrate binds not only calcium but also magnesium and affects the fluorometric and colorimetric methodology [3]. In addition, hemolysis, bilirubin, lipemia, high phosphate levels and delays in serum separation may influence magnesium measurements [44]. Magnesium concentration in red cells is approximately three times greater than that of serum and it has been estimated that serum magnesium concentration will increase by 0.05 mmol/l for each gram/l of hemoglobin produced by haemolysis [45].The magnesium concentration is even higher in recticulocytes (immature RBCs), which might be particularly relevant in patients receiving erythropoietin. Thus, when measuring magnesium serum levels, it is important to avoid haemolysis to prevent misinterpretation [20]. Serum magnesium may be influenced by changes in serum albumin, other anionic ligands, and pH; however, correction for changes due to these factors is seldom done [46]. Spectrophotometric methods commonly used for the measurement of magnesium have relatively poor analytic performance in relation to the biologic variation [43]. As with many reference values, laboratory parameters will also vary from laboratory to laboratory resulting in slightly varying ranges for the 'healthy' populations evaluated. What is considered the 'normal level' might actually be slightly too low, representing a mild magnesium deficit present in the normal population [20]. In adults, serum magnesium concentration is not influenced by sex or age, except in the very elderly where it may be slightly higher. Variation in serum magnesium concentration between individuals (inter-individual) is between 5.9–7.5% and within individuals (intra-individual) is 3.4–4.7%. Moreover, serum magnesium might be higher in vegetarians and vegans than in those with omnivorous diets. The same applies to levels after short periods of maximal exercise as lower serum levels are observed after endurance exercises and also during the third trimester of pregnancy [44].

In healthy individuals, serum magnesium concentration is closely maintained within the physiological range. This reference range is 0.65–1.05 mmol/L for total magnesium concentrations in adult blood serum and 0.55–0.75 mmol/L for ionized magnesium [45, 47]. The extracellular fluid space contains only 2% of total body magnesium, and levels in serum may not always accurately reflect the intracellular magnesium status. Also only 1% of total body magnesium is present in extracellular fluids and only 0.3% of total body magnesium is found in serum, and serum total magnesium may not accurately reflect total body magnesium status [17, 5, 48]. Despite this limitation, serum magnesium concentration is still used as the standard for evaluating magnesium status in patients [49]. Also, since serum magnesium reflects interstitial fluid and bone magnesium pool, it has proven helpful in detecting rapid extracellular changes [17].

6.2 Intracellular magnesium content

Intracellular magnesium plays a critical role in enzyme activation within the cell, and measurement of intracellular magnesium has been considered more relevant physiologically. [50]. Magnesium content in red blood cells, skeletal muscle, and mononuclear cells have been investigated as an index of magnesium status [2]. Normal erythrocytes contain a high concentration of magnesium ions that are essential for ATP function and other metabolic processes [51]. Therefore magnesium concentration in red blood cells could serve as an early indicator of magnesium deficiency. Total red blood cell magnesium concentration can be determined directly or indirectly using total magnesium concentration of whole blood and hematocrit [52]. The indirect method is reproducible, reliable, accurate and easy to perform. However, it does not seem to correlate well with total body magnesium status. The magnesium content of mononuclear cells is a better predictor of total magnesium, but the method is technically more difficult than either red cell magnesium or serum magnesium and intra individual variation is high (12-22%). Platelet total magnesium and ionized magnesium can be measured, but the value of this test against other methods has not yet been properly evaluated. As muscle contains nearly 30% of the total body magnesium, research reports suggest that measurements of muscle magnesium may be a reliable indicator of magnesium status [53]. However, its routine use in the clinical laboratory is limited due to the need of invasive sampling via muscle biopsies coupled with the high cost of special technical skill.

6.3 Hair and bone magnesium

The potential use of hair and nails to assess magnesium status is an attractive idea as it is the least invasive sampling procedure, and samples can be taken over a long period of time and stored until the analysis is performed. Bone and teeth have also been experimented in the assessment of magnesium status [54]. However, the involvement of invasive sampling and the heterogeneous distribution of magnesium in bone limit its use in the clinical laboratory.

6.4 Intracellular free magnesium ion concentration

Free or ionized magnesium is measured with ion-selective electrodes, fluorescent probes, AAS, NMR which identifies and quantifies the biologically active serum Mg^{2+} [2]. Ion selective electrodes for Mg^{2+} have been developed and several commercial analyzers are now available for the measurement of ionized magnesium concentration. Measurement of Mg^{2+} has been found to be useful in several clinical situations. Ionized magnesium is more accurate, especially in critically ill patients with rapid changes in hemodynamics. It is also not affected by low serum albumin levels. Results obtained from different instruments are not always in agreement as the electrodes are not entirely selective for Mg^{2+} concentration, and a correction factor is required based on the ionized calcium concentration [3, 46]. Only a few studies have been conducted to assess the validity of this approach [55]. Ionized magnesium has also been analyzed in human blood plasma using NMR which involves the addition of a ligand so that free and bound magnesium have different resonances. Report shows that the magnetic resonance spectroscopy methods give higher values of Mg^{2+} than values obtained by ion-selective electrodes [56].

6.5 Physiological Tests

These are test that determine the metabolic fate of either endogenous or exogenously administered magnesium. Physiological test include metabolic balance studies, 24 hour urine magnesium and exogenous magnesium loading test. Balance studies are time consuming, labor intensive and need well trained staff. They are often performed in a metabolic unit and require complete urine and fecal collections; therefore it is not a method that can be applied as a routine test for the evaluation of magnesium status.

6.5.1 24-hour urine or fractional excretion of magnesium

Another approach for the assessment of magnesium status is urinary Mg^{2+} excretion. The 24 hour urine test is cumbersome, especially in the elderly, since it requires at least a reliable and complete 24-h time frame [13]. As a circadian rhythm underlies renal Mg^{2+} excretion, it is important to collect a 24-h urine specimen to assess Mg^{2+} excretion and absorption accurately. This test is particularly valuable for assessing magnesium wasting by the kidneys owing to medication or patients' physiological status [13]. The results provide etiological information: while a high urinary excretion indicates renal wasting of Mg^{2+}, a low value suggests an inadequate intake or absorption [13]. Thus this test helps in differentiating renal wasting of magnesium from inadequate intake or poor absorption as an etiology for hypomagnesemia. In the steady state, a 24-hour urine excretion of magnesium reflects intestinal absorption and it is valuable in determining whether magnesium wasting is via the renal route.

Normal 24 hour urine Mg^{2+} excretion stands at 24 mg/day (0.24g/day). Magnesium excretion > 1 mmol/day is suggestive of renal magnesium wasting. On the other hand, Mg^{2+} excretion < 0.5 mmol/day is suggestive of magnesium deficiency [57]. Fractional excretion of magnesium (FEMg) referred as magnesium clearance: creatinine clearance ratio

and defined by equation 1 have a reference value of 2 to 4 % (0.02 to 0.04). The reference values of urine magnesium creatinine ratio are age dependent and presented in table 5

FEMg=[(Urine/Plasma Mg) / (Urine/Plasma Cr)] * 100 …….. 1

Table 5 Reference values urine magnesium creatinine ratio

Age	Reference range (mg/mg creat)
1 - <12 months	0.10-0.48
12 - <24 months	0.09-0.37
24 months-<3 years	0.07-0.34
3 - <5 years	0.07-0.29
5 - <7 years	0.06-0.21
7 - <10 years	0.05-0.18
10 - <14 years	0.05-0.15
14 - <18 years	0.05-0.13
18 - 83 years	0.04-0.12

Reference values have not been established for patients who are less than 1 month of age and greater than 83 years of age.

Urinary magnesium excretion must be interpreted with caution during periods of intravenous magnesium infusion and should be interpreted in concert with serum concentrations. In the presence of hypomagnesemia, a 24-hour urine Mg^{2+} above 24 mg/day or fractional excretion above 0.5% suggests renal magnesium wasting. Lower values suggest inadequate magnesium intake and/or gastrointestinal losses. In the presence of hypermagnesemia, urinary Mg^{2+} levels provide an indication of current magnesium intake. Lower urinary magnesium excretion increases urinary calcium oxalate and calcium phosphate supersaturation and could contribute to kidney stone risk [58, 59, 60].

6.5.2 Magnesium loading test (magnesium retention or tolerance test)

Loading tests are simplified balance studies where absorption is supposed not to be disturbed when magnesium is given orally so that body retention is calculated from urine elimination. Magnesium administration during a loading test can be either oral or intravenous and it is important that the subjects have normal kidney function. Urine is collected for 24 hours following administration of the magnesium load as magnesium excretion by the kidney has been shown to have a circadian rhythm [32]. Under these conditions, the loading test is supposed to be a reliable indicator of magnesium status [53]. Intravenous magnesium tolerance test includes the intravenous infusion of 0.1mmol magnesium/kg body weight in 50 ml of 5% dextrose over 4 hours; the urinary excretion of Mg^{2+} over the next 24 hours (starting with the infusion) is determined and the percentage of magnesium retained is calculated.

The magnesium retention test is a very sensitive method to detect magnesium deficiency. Magnesium deficiency is assessed by the measurement of magnesium retention after acute magnesium loading. This test quantifies the major exchangeable pool of magnesium, providing a more sensitive index of magnesium deficiency than simply measuring serum magnesium concentration. The magnesium 'loading test' may serve for identification or differentiation of patients with hypomagnesemic magnesium deficiencies and normomagnesemic magnesium deficiencies. Clinical studies in patients with cardiovascular or neuromuscular abnormalities, diabetes mellitus, alcoholism, or malabsorption syndromes have demonstrated normomagnesemic magnesium deficiencies [61, 62]. In this clinical situation, a patient may be intracellularly magnesium depleted and exhibit signs of magnesium deficiency, yet have normal serum levels of magnesium. In this setting, serum magnesium tends to remain within the normal range due to recruitment of intracellular stores, until the point where the intracellular stores cannot keep up. Therefore magnesium retention test serve as a surrogate test to measure magnesium at the intracellular level, where the major physiologic role of magnesium occurs [13]. In magnesium deficiency the percentage of magnesium excreted is decreased (< 80% over 24 h) after infused magnesium load (2.4 mg/kg of lean body weight given over the initial 4 h). In other words, the percentage of magnesium retained is increased (greater than 20%) in cases of magnesium deficiency [61]. This method

is useful only when the clinical suggestion of magnesium deficiency is strong but serum magnesium levels are normal. It is important to note that the magnesium retention test depends on normal renal function and it may be of limited value in patients with poor renal function or those in whom there is increased renal magnesium loss as seen with diuretics. Patients with malnutrition, cirrhosis, diarrhea, typically have a positive result, whether or not they have signs or symptoms referable to magnesium depletion.

The magnesium retention test is also used to assess intestinal Mg^{2+} absorption, chronic losses and bone status. Changes in serum magnesium concentration and excretion following an oral magnesium load reflect intestinal Mg^{2+} absorption [63]. The test indirectly assesses bone status of magnesium since magnesium retained during this test is actually retained in bone. The percentage of magnesium retained is inversely correlated with the concentration of magnesium in bone [61]. This implies that the lower the bone magnesium content, the higher the magnesium retention in this test [64].The magnesium tolerance test is also of value in determining whether renal magnesium excretion is appropriate. A urinary excretion of >80% of the magnesium load suggests that magnesium depletion is unlikely. Standardization of this test, however, is lacking [57].

6.5.3 Isotopic balance studies

This test allows for a means of tracking the metabolic fate of an exogenously administered "dose" of the element upon ingestion or injection into the body without the harmful emissions associated with radioisotopes. Plasma, urine and fecal samples obtained from subjects administered with the tracer isotope undergo preparation [65]. Sample preparation involves the chelation of isotopic magnesium, extraction and recovery, then introduction via solid probe into mass spectrometer for analysis. Isotopic magnesium analysis allows a precise evaluation of intestinal Mg^{2+} absorption, fecal excretion, and body retention. In addition, using kinetic data obtained from the analysis of blood samples, it is possible to determine pool sizes and turnover rates. Stable isotopes are routinely used to study gastrointestinal functions. Isotopic analysis of magnesium in the assessment of gastrointestinal absorption is reserved for research purposes due to limitations of availability and cost of mass spectrometry measurements. Using stable isotopes, an in vitro blood magnesium load test could be performed by isolating blood cells from whole blood samples, incubating with magnesium and uptake determined with mass spectrometer. A high uptake would be triggered by magnesium deficiency.

7 Conclusion

Magnesium has several physiologic roles and functions mostly in enzymatic reactions. The maintenance of a balance of magnesium between body compartments is necessary for the optimal function of this cation. Normal bone metabolism, intestinal and renal function is necessary for the maintenance of this balance. So far, no method is available that accurately determines total body magnesium. However, a good knowledge of magnesium metabolism is key to the development of more accurate methods that will determine magnesium in both clinical and research settings.

Compliance with ethical standards

Acknowledgments

I appreciate the efforts of all the medical laboratory scientists of the chemical pathology department, federal medical center Makurdi, Nigeria, involved in the testing of serum electrolytes.

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