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ROLE OF CELLULAR MAGNESIUM IN HEALTH AND HUMAN DISEASE

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1. ABSTRACT

The aim of this paper is to discuss, on the basis of an extensive literature review, the role of magnesium in health and disease. Magnesium is an essential cation playing a crucial role in many physiological functions. It is critical in energy-requiring metabolic processes, in protein synthesis, membrane integrity, nervous tissue conduction, neuromuscular excitability, muscle contraction, hormone secretion, and in intermediary metabolism. Serum magnesium concentration is maintained within a narrow range by the small intestine and kidney which both increase their fractional magnesium absorption under conditions of magnesium deprivation. If magnesium depletion continues, the bone store helps to maintain serum magnesium concentration by exchanging part of its content with extracellular fluid. The abundance of magnesium within cells is consistent with its relevant role in regulating tissue and cell functions. Recent data suggest that large fluxes of magnesium can cross the cell plasma membrane in either direction following a variety of hormonal and non-hormonal stimuli, resulting in major changes in total and, to a lesser extent, in free magnesium content within tissues. Imbalances of magnesium are common and are associated with a great number of pathological situations responsible for human morbidity and mortality. A large part of the population may have an inadequate magnesium intake, and in particular elderly subjects and athletes may be prone to chronic latent magnesium deficiency. Magnesium deficit is frequently observed in alcoholics and diabetic patients, in whom a combination of factors contributes to its pathogenesis. We will discuss some of the aspects of the involvement of magnesium in the etiology of some pathological situations, such as cardiovascular diseases, diabetes, pre-eclampsia, eclampsia, sickle cell disease and chronic alcoholism.

2. INTRODUCTION

There is increasing interest in the role of magnesium in physiology, nutrition, and clinical medicine. The history of medicine teaches that the use of a substance in medical practice always precedes by many years the understanding of its biological mode of action. Magnesium salts were used as cathartic drugs during the Renaissance in Italy. In 1810, magnesium oxide was used to treat patients with uric acid stones. In 1869, Jolyet & Cahours carried out specific pharmacological studies with magnesium. In 1926, Jehan Leroy recognized magnesium as an essential ion, and many of the physiological properties of this cation in the rat were described by Mac Collum & Greenberg in the 1930's. Flink, in 1956, carried out the earliest clinical study clearly identifying the syndrome of magnesium deficiency in man. Aikawa published his first book about magnesium in 1963, but the understanding of the biology of magnesium was still very rudimentary due to the lack of a suitable analytical method. In 1968, Wacker published a review on

magnesium metabolism, which was a classic reference for several years. Meanwhile, Seelig in the United States, and Durlach in Europe were beginning to carry out clinical studies of magnesium metabolism. A series of clinical case reports in the early 1960's helped focus attention on the role of hypomagnesemia in various malabsorptive states and stimulated efforts to study magnesium depletion and its consequences under controlled conditions. The rapid progress made in recent years is clearly demonstrated by the regular occurrence of meetings on magnesium, papers in specific international journals such as *Magnesium Research*, and the already extinct *Magnesium*, and *Magnesium Bulletin*, and in innumerable biomedical journals. The aim of this review is to discuss the role of magnesium in health and disease (1,2).

3. MAGNESIUM NORMAL PHYSIOLOGY AND BIOCHEMISTRY

3.1. Role of magnesium in metabolic functions

Magnesium, the second most abundant intracellular cation, plays an important role in cellular function. It is involved in more than 300 enzymatic reactions in the body, participating in the metabolism of glucids, lipids, proteins, and nucleic acids, in the synthesis of H_2 transporters, and particularly in all reactions involving the formation and use of adenosine triphosphate (ATP) (2). Magnesium participates in such reactions by forming a chelate, i.e., a coordination complex. Kinetic studies of several enzymes requiring both magnesium and ATP, including fructokinase, creatine kinase, and hexokinase, indicated that enzyme activity depends on the ratio (as well as on the absolute concentrations), of the two cofactors and that magnesium chelates strongly with ATP. These observations led to the concept of the Mg(ATP) complex as the active substrate for enzyme action. Similarly, a magnesium complex with adenosine diphosphate (ADP) appears to be the active substrate for creatine kinase and other enzymes. Although complexation with nucleotides is by far the most common example of the involvement of magnesium in the formation of active substrates, the same principle may also operate in other situations. For example, the results of kinetic studies suggest that Mg(isocitrate) is the active substrate for isocitrate dehydrogenase (3). The second general mechanism of magnesium action is its direct binding to the enzyme protein and resultant allosteric activation. Examples of such enzymes are pyruvate kinase, phosphofructokinase, and pyruvate carboxilase. With some of these enzymes magnesium has a dual function, not only forming part of the reactive substrate but also activating the enzyme allosterically (4). However, in fundamental cellular functions magnesium may have opposite actions. For example, the Mg(ATP) complex stimulates the type-L calcium channels, and Mg^{2+} inhibits them (5).

At the cell membrane level, magnesium is known to altering both receptor sites and ion movements across the membrane. By making complexes with the phospholipids, magnesium stabilizes the membranes, reducing their fluidity and permeability. In magnesium deficit, intracellular concentrations of calcium and sodium increase, and concentrations of potassium and phosphorus decrease. Simultaneously, the membrane depolarizes (6, 7). These alterations may be the result of magnesium's direct effect on sodium, calcium, or potassium channels or its indirect effect on enzymes in the cell membrane that are involved in active transport, e.g., (Na^+K^+)–ATPase. Thus, magnesium is an important modulator of intracellular ion concentrations. Magnesium also regulates lipid and phosphoinositide-derived second messengers (8).

Within the cell, magnesium affects the function of organelles such as sarcoplasmatic reticulum, primarily by its ability to alter calcium flux (9-11), or mitochondria by altering their membrane's permeability to protons, which leads to alterations in the coupling of oxidative phosphorylation and electron transport chains, thus affecting the efficiency of ATP production (2).

Magnesium also acts as a calcium antagonist. In the neuromuscular system it reduces the electric excitability of the neurons and inhibits the release of acetylcholine by the nerve endings at the neuromuscular junction and blocks the effect of N-methil-D-aspartate, an excitatory neurotransmitter of the central nervous system (12). Magnesium also acts as a vasodilator, like other calcium antagonists, and inhibits the coagulation processes (13). In muscle contraction, both stimulation and the activity of the calcium transport system in the sarcoplasmatic reticulum membranes depend on the presence of magnesium ions (14). Troponin contains four calcium-binding sites, two of which have a high affinity for calcium and bind magnesium competitively. It appears that these calcium-magnesium-type sites are not directly involved in any rapid twitching mechanism, but that they play only a structural role in muscle (15). Magnesium bound to these sites may have several functions. One might be to maintain the protein permanently in a particular conformational state regardless of the fluctuation in calcium (assuming that both magnesium- and calcium-induced structural changes are essentially the same). In the case of troponin, this conformation may be a prerequisite for calcium activation via binding at the calcium-specific sites (16,17).

Compelling evidence shows that magnesium is directly correlated to proliferation in normal cells as magnesium stimulates DNA and protein synthesis (18).

These and many other cellular functions make magnesium a crucial element in living beings. It is easy to understand why disruptions in magnesium metabolism may be factors in the development of pathological conditions.

3.2. Magnesium distribution and regulation

In biological systems, magnesium ions exist in three different states: bound to proteins, complexed to anions, and free (Mg^{2+}). Only free magnesium has biological activity.

The adult human body contains approximately 24 g (1 mol) of magnesium in cells versus 280 mg in extracellular fluids (19). The skeleton represents the body's largest magnesium store (about 60% of total magnesium)

that can be divided into two subcompartments. The slow bone compartment corresponds to firmly apatite-bound magnesium that cannot be mobilized even under conditions of extreme depletion. The second is the mobile compartment comprising magnesium that is absorbed to the surface of mineral crystals; this fraction can be increased by increasing magnesium supply and is mobilized during hypomagnesemia. Thus, bone functions as a large magnesium reservoir that may help to stabilize its concentration in serum. About one fourth of total magnesium is located in skeletal muscle; the nervous system and other organs with high metabolic rates, namely the liver, myocardium, digestive tube, kidney etc., account for the remaining. In serum, about one third is bound to proteins, mainly albumin; the remaining two-thirds are ultrafiltrable, being approximately 92% free and 8% complexed to citrate, phosphate, and other compounds (20,21). The mean serum magnesium concentration is about 0.8 mmol/l (22). The concentration of magnesium in red blood cells (approximately 2.5 mmol/l) is genetically controlled, with the oldest cells having the least magnesium (23,24).

The intracellular free magnesium ($[Mg^{2+}]_i$) is the component essential for the regulation of a large number of cellular functions and can be controlled within a narrow range (4). Regulation of the intracellular magnesium compartmentalization by the organism is an important evolutionary development. Control of Mg^{2+} flow into and around cells is also fundamental, and specific mechanisms have been described for its regulation although they have not yet been fully elucidated: passive process; Mg-Mg exchange; and Na-Mg antiport (25-28). Nevertheless, Mg^{2+} seems to move rapidly between intracellular compartments and across the plasma membrane (29). Although a complete picture of its intracellular regulation is lacking, Mg^{2+} seems to enter cells by a passive mechanism due to the electrochemical gradient that results from the electronegative charge of the cells' interior and by facilitated diffusion (25); it is thought that Mg^{2+} is extruded from cells mainly via a Na-Mg exchanger (10,25,28). Data from Wolf and co-workers (30) confirmed in a tumor cell line that the Na-Mg antiporter is the main mechanism driving Mg^{2+} efflux and that this Na-Mg antiporter is both regulated by cAMP and is an active process strongly dependent on cell energy metabolism. Such an efflux has been described in several cell types with different stoichiometry; it seems to be the most widely diffused magnesium pump at the plasma membrane of eucharyotic cells (31). Although the transporter has been functionally demonstrated, it has not yet been structurally characterized.

Several hormones influence $[Mg^{2+}]_i$, with modes depending on the hormone and the tissue (32). Thus, insulin and taurine increase $[Mg^{2+}]_i$ and inversely adrenalin (possibly by beta-stimulation), decreases it (33). In 1996, Touyz & Schiffrin reported that angiotensin II modulates $[Mg^{2+}]_i$ in vascular smooth muscle cells and in platelets. More recently, the same authors demonstrated that in renal cells, angiotensin II mobilizes $[Mg^{2+}]_i$ through AT_1 receptor-mediated pathways. Angiotensin II decreases $[Mg^{2+}]_i$ by activating the Na-Mg exchanger to promote transmembrane Mg^{2+} transport in a Ca^{2+}-independent way (8). In isolated neurons, glutamate stimulation triggers a $[Mg^{2+}]_i$ increase of about one order of magnitude (29).

Like other intracellular cations, magnesium shows an intracellular compartmentalization that has not yet been fully characterized. Nuclei and mitochondria seem to store large amounts of magnesium, and an Mg-Ca exchange has been described in endoplasmatic reticulum (34).

From all the information available at present, it is clear that intracellular magnesium homeostasis is the result of several specific mechanisms working in different directions for the maintenance of constant cell magnesium content. These mechanisms must be very effective, since it has been found very difficult to identify physiological conditions where cytosolic free magnesium concentration is substantially modified. Nevertheless, these regulation mechanisms deserve further studies.

3.3. Magnesium balance: absorption, excretion, and homeostasis

Magnesium balance is achieved through intestinal absorption and renal excretion. Elimination by sweat is only important under extreme conditions with large sudation (35). In normal individuals, intestinal and renal conservation and excretory mechanisms permit homeostasis over a wide dietary intake (36).

In physiological conditions, 30 to 50% of magnesium uptake is absorbed (37), mainly by the small intestine. The net magnesium absorption results from the content of ingested foods and from retention of the magnesium in the digestive fluids (38). There are two mechanisms of absorption: one active and saturable and another passive (37). Passive absorption occurs by paracellular pathway following a favorable electrochemical gradient as a function of water and solutes movements and seems to be proportional to dietary intake. Regulated active transport operates only under conditions of low magnesium intake (39). Magnesium concentration in the digestive tube is the most important determinant of the amount of magnesium absorbed (38); nevertheless, food elements capable of influencing magnesium absorption should be taken into account in investigation of cases of low magnesium intake (40). Substances increasing magnesium solubility favor its absorption, opposite the action of substances that form insoluble complexes. Recent data show that no competition exist between magnesium and calcium for absorption, as calcium supplementation does not decrease magnesium absorption (40). Phosphates can inhibit magnesium absorption through the formation of insoluble magnesium complexes in the intestine (41). Several works have popularized the concept according to which diets rich in fiber have unfavorable effects on magnesium absorption (42,43). However, from recent studies it is clear that the effect of fiber on magnesium absorption depends on the nature (solubility and fermentability) and the amount of fiber and on the associated compounds in the meal such as phytates (44-47). Drinking water may be an important source of

magnesium that may be better absorbed than dietary magnesium (48).

Analyzing magnesium intake and urinary excretion, one can observe that there is on average an elimination of about one third of the dietary magnesium in the urine. When magnesium intake is severely restricted in humans with normal kidney function, output becomes very small. Supplementing a normal intake increases urinary excretion without altering normal serum levels, as long as renal function is normal and the amounts given are not excessive. So, the kidney conserves magnesium in response to a deficiency, and renal excretion increases in proportion to the load presented to the kidney. Approximately 70 to 80% of the serum magnesium is filtered through the glomerular membrane but only 20 to 30% of the filtered magnesium is reabsorbed along the proximal tubule. The primary site for magnesium reabsorption is the thick ascending limb of the loop of Henle, where about 65% of the filtered magnesium is reabsorbed. At this site, magnesium reabsorption is associated with NaCl cotransport. The increase in renal excretion that accompanies increased magnesium uptake seems to be a response to increased plasma magnesium. The decreasing urinary excretion that is observed in deficit situations seems to result from increased magnesium reabsorption in the loop of Henle. Factors decreasing NaCl reabsorption in the thick ascending limb of the loop of Henle, like osmotic diuretics, increase magnesium excretion (49).

Several studies indicate that glucagon, parathyroid hormone, calcitonin, and insulin increase magnesium reabsortion in the loop of Henle and proximal tubule. On the contrary, hypercalciuria and hypophosphatemia decrease tubular reabsorption. Metabolic acidosis also increases magnesium urinary excretion (40,50-52). Increases in catecholamine levels from any cause can induce hypomagnesemia that seems to be associated with an increase in magnesium excretion in the urine. Female sex hormones significantly affect magnesium metabolism (57). Serum magnesium levels of women of active reproductive age have been reported to be lower than levels of males of equal age. In addition, serum magnesium levels of women taking hormonal contraceptives were lower than in age-matched controls. Schlemmer and co-workers reported decreased urinary excretion in postmenopausal women treated with estrogens in cyclical combination with gestagens when compared with a group of postmenopausal given placebo (58). Female sex hormones also favor magnesium uptake into bone as part of the process of mineralization (59). Hypomagnesemia that frequently develops during the second half of pregnancy is probably caused by increased urinary magnesium losses (60).

In chronic magnesium deficit, magnesium decreases in plasma and tissues, and bone magnesium is mobilized to restore normal magnesemia (53). According to some authors, vitamin D plays a minor role in magnesium homeostasis (54), while for others, it has a direct action on magnesium intestinal absorption (55,56).

Thus, the mechanisms leading to hypomagnesemia may include decreased intake or absorption, internal redistribution, and/or increased renal or nonrenal loss.

3.4. Dietary magnesium intake

Until the mid 1960's, textbooks of biochemistry devoted only a few lines to the importance of magnesium in nutrition. One of the factors that helped to focus more attention to magnesium was the appearance, in 1964, of a monograph by Seelig emphasizing the inadequate dietary magnesium intake in the Western World. Since then, several investigators have observed a decreasing magnesium intake in different countries (61). This decrease has been attributed to a wide variety of factors, including a drop in the consumption of grain products, agricultural techniques of accelerated growth, decreased magnesium fixation by plants, use of magnesium-poor soil fertilizers, use of pesticides (inhibition of absorption), refining of foods (resulting in large losses of magnesium, especially in sugars and grains), and boiling of vegetables, which causes major losses in water etc. (19).

A large number of balance studies have been performed over the years in an effort to obtain quantitative data on magnesium requirements (62). These studies involved the measurement of daily dietary intake along with subtraction of daily excretory losses via urine and feces, yielding a positive or negative "balance". Based on long-term balance studies, Seelig and Durlach, in the early sixties, recommended a daily magnesium intake of 6 mg/kg/day (63,64). More recently, other authors have agreed with Seelig and Durlach's recommendation (40,65). However, when analyzing the results of the reported surveys in several populations, we conclude that this value is frequently not reached in developed countries (62,66-71).

As marginal magnesium intake may condition hypomagnesemia, which is observed in several pathological situations, more attention needs to be paid to magnesium intake. In some cases the need for supplementation should be considered. However, the use of supplementation should be carefully evaluated, as renal disturbances may lead to magnesium excess and pathological consequences. Populations with special needs, including pregnant women, individuals practicing exercise, alcoholics, elderly people, diabetics, etc., should pay extra attention to magnesium status.

4. MAGNESIUM DEFICIT

4.1. Etiological mechanisms of magnesium deficit

Primary magnesium deficit originates from two etiological mechanisms: deficiency and depletion. Deficiency is due to insufficient intake, and depletion is due to deregulation of factors controlling magnesium status such as intestinal magnesium hypoabsorption, urinary leakage, reduced magnesium bone uptake and mobilization, hyperglucocorticism, insulin-resistance and adrenergic hyporeceptivity. Secondary magnesium deficit results from various pathologies and treatments: non-insulin dependent *Diabetes mellitus*, alcoholism, or ingestion of hypermagnesuric diuretics (51), etc. Magnesium deficit may

contribute to neuromuscular, cardiovascular, renal and other pathologies (2).

4.2. Consequences of chronic magnesium deficit

The investigation of magnesium deficit has often involved the induction of magnesium deficiency in animals. These studies have shown several cellular alterations including mitochondrial disorders, with partial uncoupling of oxidative phosphorylation (72,73); impairment of electrolyte homeostasis with loss of potassium and phosphorus and gain of calcium and sodium as a result of the alteration in the fluidity and integrity of cellular and sarcoplasmic reticulum membranes; inhibition of glutathione biosynthesis (74,75); depletion of selenium and reduced glutathione peroxidase activity (76); increased cytokine concentrations; and alterations in iron metabolism (77,80). A study of magnesium supplementation in animals with oxidative stress diseases suggested its anti-peroxidant effect through a finding of decreased lipid peroxidation (81). Magnesium-deficient animals showed, among other pathological signs, anemia (82), myopathy (83), muscular weakness (84), tetany (16), cardiac rhythm disorders, and changes in blood pressure (85). These data should be treated with care in application to humans, since they were observed in severely magnesium-deficient animals.

The assessment of a patient's magnesium status is problematic, because there are no easily performed tests that reliably predict the intracellular concentration (2). The patient should be tested for clinical and paraclinical signs of neuromuscular hyperexcitability. The most significant is Chvostek's sign: careful auscultation of the heart reveals a systolic click and a mitral valve murmur. Paraclinical examination involves electromyogram (EMG), electroencephalogram (EEG), and echocardiogram (ECC), after which mitral valve prolapse is often identified. Clinical assessment also includes measurement of magnesium in plasma and red blood cells, plasma calcium levels, and urinary magnesium.

When analyzing the consequences of magnesium deficit in man, the best-documented clinical aspects are the nervous alterations resulting from primary chronic magnesium deficiency (2,86). Nervous hyperexcitability due to chronic insufficient magnesium intake results in a non-specific clinical pattern with an associated central and peripheral neuromuscular symptomatology, analogous to what has been described in latent tetany, chronic fatigue, asthenia and idiopathic Barlow's disease (87). Central nervous hyperexcitability may be associated with decreased energy and cationic gradients, along with disturbances in calcium distribution and increased susceptibility to peroxidation; or with increased activity of the excitatory neuromediators acetylcholine and catecholamines, and with decreased activity of the inhibitory neuromediators taurine and glutaurine (88).

Cardiovascular manifestations of magnesium deficit have also been described. They mainly include modifications of EMG, arrhythmias and increased sensitivity to cardiac glucosides. Nevertheless, these disturbances are similar to those described for potassium deficit, and may indeed be secondary to magnesium deficit (89). Magnesium deficit is often in the origin of a mitral diskinesia (prolapse) (90). Besides disturbances in the nervous and cardiac system, magnesium deficit can influence fibre and collagen structures, lipid profile, and the endocrine system (2,91). Additionally, there is much controversy about the role of magnesium deficit in the physiopathology of osteoporosis and immunodepression. (92,93).

4.3. Magnesium deficit and special populations

4.3.1. Magnesium and athletes

Primary magnesium deficit plays a role in the pathophysiology of physical exercise (94,95). Several studies have reported that athletes may be deficient in magnesium (96,97). The studies of Boggio and co-workers (98) showed that the food intake of athletes and sedentary subjects is very similar, apart from significantly different energy values in the intake of athletes. Two conditions contribute to frequent mineral deficits among athletes: they consume diets with inadequate mineral amounts, lower than the recommended dietary allowances (RDA) (99), and mineral losses in urine and sweat are more important during exercise that in basal conditions (100). During exercise, compartmental shifts of magnesium have been observed, but data to demonstrate the variations in magnesium levels after exercise are inconsistent. Inconsistencies may be related to differences in experimental designs, work intensity, work duration, timing of blood samples, etc.. With respect to exercise intensity, various authors have indicated that short-term maximal exercise leads to hypermagnesemia as a consequence of the decrease in plasma volume and the shift of cellular magnesium into the extracellular fluids that results from acidosis and muscle contraction (101,102). Data from Joborn and co-workers (103) suggests that releases of magnesium from muscles might contribute to rises in plasma magnesium, as is well known to be the case for potassium. These changes may depend on the relative contribution of anaerobic metabolism to the total energy expended during exercise. Submaximal exercise has also been reported to be accompanied by hypomagnesemia (104-110). Low plasma magnesium levels have been explained by several mechanisms: redistribution of magnesium to red blood cells, adipocytes, or myocytes (111); loss in urine due to increases in aldosterone, antidiuretic hormone, thyroid hormones, and acidosis, all of which reduce the tubular reabsorption of magnesium (102); and stress induced by exercise raises endogenous catecholamine levels, increasing lipolysis (112). Possibly magnesium is lowered by adrenaline indirectly via lipolysis, as suggested by Rayssiguier (113). Also, Elliot and Rizack (114) found increased magnesium content in plasma membrane vesicles derived from fat cells after adrenaline stimulation *in vitro*; hyperexcretion in sweat acquires real importance only in cases of intense activity under conditions of high temperature and humidity. Although some explanations have been offered for the compartmental shifts of magnesium, the precise mechanism remains to be clarified. It is important to determine whether there is only a transient fall in plasma magnesium concentration or whether participation in sustained exercise

may induce permanent alterations. Several studies indicate that there is a sustained fall in plasma magnesium after strenuous exercise and that hypomagnesemia persists during a season of athletic training (115-119).

Dissimilar findings are reported concerning magnesium variations in red blood cells with aerobic exercise. Córdova (101) investigated the effects of three high-intensity exercises on plasma and red blood cell magnesium levels in men. The latter were increased after all maximal exercises, in agreement with reports by other authors (102,120), and were related to the increased metabolic activity during exercise, which would induce a shift of the cation from the plasmatic compartment. However, Golf and co-workers (121) reported a significant reduction in red blood cell magnesium following vigorous cycloergometer exercise, which was in accordance both with our findings (107; 109) and those of Lijnen and co-workers (122). These inconsistencies may result from the measurement method for red blood cell magnesium content, the intensity and duration of the exercise test, and the metabolic pathways involved. Regarding submaximal exercises, Casoni and co-workers (96) observed an increase in red blood cell magnesium that they associated with the cell uptake required by increased glycolisis and NADPH production. However, several other studies indicated a decrease after strenuous exercise and found that low magnesium levels in red blood cells persist during a season of athletic training (117,118).

In view of these results it may be proposed that prolonged intensive exercise exposes subjects to direct magnesium depletion, which can only aggravate the consequences of a frequent marginal deficiency. So, during the practice of strenuous exercise, the conditions are prone to the decrease of magnesium *status*. We might highlight the importance of evaluating magnesium status in athletes, not only because its deficit may compromise performance, but also because the practice of exercise with a magnesium deficit may render the individual more susceptible to cellular damage.

4.3.2. Magnesium and elderly people

As in the population at large, magnesium deficit has been observed in elderly people (91) and may be of primary or secondary origin. Some studies of magnesium intake in elderly people have shown inadequate intake (123-128) especially among the institutionalized elderly (129). Nevertheless other authors suggested that healthy free-living elderly have adequate intakes (130,131). Additionally, in elderly people, magnesium depletion is frequently observed, attributed to deregulation of factors controlling magnesium metabolism (132). Magnesium absorption seems to decrease with age, and magnesium exchange pools are reduced in elderly people (133). Concerning magnesium leakage, results are controversial. Increased, decreased and normal values have been observed (124,134,135). Age-related hormonal changes might include hypothalamic alteration that may induce the disruption of circadian rhythm for plasma cortisol and melatonin (136). Several clinical observations confirm the frequency of hyperglucocorticism in the elderly. Thus, a chronic stressful state may be latent during the aging process. This deregulation may be an important factor in magnesium depletion among elderly persons (137), through the vicious cycle between magnesium and stress.

The pathological aspect of aging must also be considered in relation to secondary magnesium deficit. Elderly subjects present a high incidence of illness, which generally predisposes them to two types of magnesium deficit: pathologic magnesium deficit induced by the disease itself and magnesium deficit due to the side effects of the treatments. Among the various diseases that may induce secondary magnesium deficit is *Diabetes mellitus* (138). High consumption of drugs among aged people may also predispose to secondary magnesium deficit, e.g. diuretics. Long-term treatment with loop diuretics may induce magnesium depletion due to excessive urinary leakage (137).

Magnesium deficit may accelerate aging through its various effects on the neuromuscular, cardiovascular, and endocrine apparatus as well as the bone, kidney, immune, stress and anti-oxidant systems (51,139).

4.4. Magnesium deficit and disease

4.4.1. Magnesium and cardiovascular diseases

Magnesium deficit has been discussed as a possible contributing factor to the development of cardiovascular diseases (51, 61,140,141). Idiopathic mitral valve prolapse is the clinical expression in cardiac tissue of a wider form of primary magnesium deficit that includes the nervous system and the heart that encompasses even neuromuscular hyperexcitability (142). The symptomatology of the idiopathic mitral valve prolapse syndrome coincides in diversity and lack of specificity with the clinical features of latent tetany. Our group has observed the therapeutic efficacy of magnesium lactate in patients suffering from mitral valve prolapse and latent tetany. The results appear quite favorable, particularly in relation to the functional manifestations and regarding palpitations, atypical precordialgias, peripheral vascular spasms, muscular cramps, and lipothymias. However, a modification of the characteristic stethoscopic signs of mitral valve prolapse was not observed, and the same can be said for levels of magnesium in red blood cells: no notable alteration was detected with treatment. Serum magnesium levels at the end of the trial revealed a rise (143). Detailed randomized studies should be carried out in order to test the true efficacy of magnesium administration, the convenient duration of treatment, the maintenance dose, the influence of other factors at onset, and maintenance of clinical features.

Important changes in magnesium levels were observed in the acute phase of myocardial infarction, generally expressed as low serum magnesium levels (144-148). Experimental research on animals and postmortem examination of human hearts suggest a magnesium deficit in ischemic myocardium (149). Studies have also demonstrated that magnesium can suppress platelet activation by either inhibiting platelet stimulating factors such as thromboxane A_2, or by stimulating synthesis of

platelet inhibitory factors such as prostacyclin (PgI_2) (150-152). Hypomagnesemia also impairs the release of nitric oxide, a potent nitro-vasodilator and inhibitor of platelet aggregation, from the coronary endothelium (153). Intervention trials have shown that magnesium administration has a cytoprotective effect in myocardial reperfusion therapy (154,155). Shechter (156) observed the inhibition of thrombus formation, and several other investigators have observed a reduction in mortality among thrombosis patients with acute myocardial infarction treated with intravenous magnesium (LIMIT-2: Leicester intravenous magnesium intervention trial; 157). However, another large study (ISIS-4: International Study of Infarct Survival) showed no efficacy (158). In the LIMIT-2 study, the treatment started around 3 hours after the onset of symptoms, but in the ISIS-4 the treatment was administrated later (52). An early administration is probably important for favorable results (159). These observations led to the hypothesis that magnesium modulates vasoconstriction and platelet-dependent thrombosis. The molecular basis for these effects probably involves the reduction of intracellular calcium mobilization.

Acute myocardial infarction represents an oxidative stress, with the production of oxygen free radicals responsible for peroxidative damage to myocardial tissue, which becomes more evident during reperfusion (160,161). To determine whether magnesium sulfate administration may have antioxidant effects, levels of plasma tiobarbituric reactive substances, low-density lipoproteins (LDL) susceptibility to peroxidation, and plasma epinephrine oxidase activity were measured at several moments during the evolution of an acute myocardial infarction. Magnesium was intravenously administered early after the onset of symptoms (162,163). Plasma thiobarbituric reactive substances and epinephrine oxidase activity were increased very early, suggesting the production of reactive oxygen species (162,163). However, the group given magnesium sulfate showed significantly smaller increases of plasma tiobarbituric reactive substances and epinephrine oxidase activity when compared with the group given placebo (classic thrombolitic therapy). The increase in LDL susceptibility to peroxidation was not significantly different between groups. These results suggest an antioxidant protective effect for magnesium, although further studies are needed.

There is also growing evidence that magnesium deficiency may affect lipoprotein metabolism, increase lipoprotein peroxidation, and induce an inflammatory response which may contribute to the development of atherosclerosis (140). However, in opposition to animal models, the effect of magnesium deficit on atherogenesis in humans is very difficult to determine. Although epidemiological and clinical data suggest an association between the two situations, there is not yet a consensus.

Increasing evidence also suggests an important physiological role for magnesium in blood pressure regulation and a pathophysiological role in hypertension, although the mechanisms are unclear (164,165). At the cellular level, intracellular free magnesium concentration $[Mg^{2+}]_i$ is decreased in essential and experimental hypertension (166,167). It has been proposed that the ability of magnesium to influence blood pressure could be related to its calcium-antagonistic actions in cardiovascular cells (168, 169). Angiotensin II, a vasoactive agent that has been implicated in the pathogenesis of hypertension (169), has been shown to induce a significantly greater response in vascular smooth muscle cells from spontaneously hypertensive rats than in those from normotensive Wistar Kyoto rats (170).

Touyz and co-workers have suggested that in vascular smooth muscle cells, $[Mg^{2+}]_i$ is regulated by angiotensin II and that magnesium may be an important second messenger in angiotensin II signaling in these cells from hypertensive rats (8). The use of oral magnesium supplementation showed no hypotensor effect; nevertheless experimental and clinical studies have shown that intravenous magnesium administration lowers blood pressure through vasodilatory action (171).

4.4.2. Magnesium and insulin resistance associated with obesity, hypertension and *Diabetes mellitus*

Since the seventies, it has been widely recognized that diabetic patients, both type I and II, have low levels of magnesium in plasma (172-177). At the same time, the role of magnesium in hypertensive disease and its clinical subtypes was also described (173,178-180). These and other studies established the relationship between magnesium and calcium physiopathology, mainly concerning the stimulus of contraction coupling in vessels smooth muscle cells (178,181,182). Ferranini and co-workers (183) and Reaven and Hoffman (184) described the syndrome X (now called plurimetabolic syndrome), which establishes the connection between the result of a hemodynamic dysfunction such as hypertension and pure metabolic situations like insulin resistance or diabetes type II (185). Magnesium's function as a predominant "metabolic" cation partially explains that relationship, in conjunction with calcium in terms of cation physiopathology. Low levels of magnesium were associated with insulin resistance, both in normotensive and hypertensive people (186-192). Basal glucose levels also seem to modulate the response of cells to the regulation of intracellular magnesium levels by insulin (186,193-195). To corroborate these findings there is evidence of magnesium depletion in poorly controlled patients with both type I and II diabetes (172,185). The explanation for the increasingly lower levels of magnesium in poorly controlled type I and II diabetes could be the high magnesium excretion secondary to higher levels of glucose, which inhibits tubular reabsortion of magnesium (196,197). Those mechanisms can explain the vicious cycle of insulin resistance followed by hyperglicemia that aggravates insulin resistance, because magnesium depletion can induce a defective tyrosine phosphorylation of insulin receptors (198). The mechanisms of magnesium loss through urine can also involve hypergluco- and/or hypermineralo-corticism, both of which are more accentuated in aged people (199-201).

4.4.3. Magnesium and preeclampsia, eclampsia and convulsive disorders

Pregnancy is one of the physiological conditions which courses with hypomagnesiemia inversely correlated with gestational age even when magnesium concentration in plasma is corrected for hemodilution and protein levels. Additionally, intracellular levels of magnesium in the myometrium are lower in pregnant than in non-pregnant women. A higher urinary magnesium excretion is correlated with magnesium levels in the myometrium (202,203). Preeclampsia and one of its major complication's that courses with convulsions, eclampsia, are pathologic situations very responsive to parenteral magnesium treatment. The rationale of this therapy is based on blocking of acetylcholine at the neuromuscular junction, inhibition of release of catecholamines, and enhancement of the effect of the prostacycline/thomboxane ratio (204-206). Two reports studying women with hypertensive disorders of pregnancy from different ethnic backgrounds confirmed previous findings of low levels of magnesium, both intracellular and extracellular (207,208).

4.4.4. Magnesium and sickle cell disease

There is ample evidence of a differential distribution of inter-erythrocyte magnesium between free and ATP or 2,3-DPG chelates in erythrocytes of different ages (209,210). Total magnesium is elevated in the least dense fraction of erythrocytes, where the youngest circulating cells are localized (210-212). The erythrocytes with higher density from homozygotic sickle cell patients (SS) have low levels of magnesium, particularly the free magnesium, that are correlated with higher efflux through the Gardos channel (213). Also, when magnesium content in erythrocytes is increased, K-Cl cotransporter is inhibited, and consequently this inhibits red blood cell dehydratation. Two clinical trials demonstrate that administration of magnesium salt can both hydrate SS erythrocytes and inhibit K-Cl cotransporter (214,215).

4.4.5. Magnesium and chronic alcoholism

In chronic alcoholics, the tendency to develop magnesium deficit is due to several factors: these individuals are frequently mal nourished, have increased losses of intestinal contents through vomiting, and may have increased urine volume as the result of inhibition of the antidiuretic hormone (ADH) by ethanol. Other factors such as hypophosphatemia, hyperaldosteronemia secondary to hepatic lesion, and hypoparathyroidism can also contribute to hypermagnesuria (216- 218). In addition, these patients may often be treated with diuretics, which increase the loss of magnesium. Respiratory alkalosis may also induce a magnesium efflux from cells (216). Sometimes, when ethanol is stopped and a balanced diet is furnished, a fast recovery follows (217,219).

5. CONCLUSIONS AND PERSPECTIVES

The Authors have focused on reviewing the major aspects of magnesium physiology, which may explain the physiopathology of several pathologic situations where magnesium deregulation is observed and have given examples of some of these diseases. It is clear that magnesium alterations are associated with various diseases. However, we do not know the precise mechanism for these relationships. Several gaps in our knowledge of magnesium metabolism still challenge various investigators. Major areas in question are the relationship between calcium and magnesium, especially the pure metabolic effects as opposed to the biophysical aspects as well as the molecular identification and characterization of systems involved in magnesium metabolism and transport. In the future we expect that the increasing knowledge about the human genome will allow the formulation of informed hypothesis regarding the above mentioned issues in addition to allowing the identification of new molecules and their physiological functions. Advances in this field of research may lead to the possibility of linking specific molecular defects to clinical disorders and provide the molecular basis for development of new selective drugs for better and more targeted treatment. In the coming years, controlled randomized clinical trials will also be necessary to characterize the role of magnesium in the physiopathology of disease and to determine whether magnesium supplementation can alter the natural history of diseases such as cardiovascular diseases, diabetes, pre-eclampsia, eclampsia, and sickle cell disease.

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7. REFERENCES

1. Birch, N.J: Magnesium in Biology and Medicine. An Overview. In: *Metal ions in biology systems* Eds: Sigel H. & Sigel A., Marcel Dekker, New York, pp. 105-117 (1990)
2. Durlach, J & M. Bara: *Le Magnésium en Biologie et en Médecine.* Editions médicales internationales, 2nd ed., Cachan, França (2000)
3. Heaton, F.W: Role of Magnesium in enzyme systems. In: *Metal ions in biological systems*. Eds: Sigel, H. & A. Sigel, Marcel Dekker, New York, Vol. 26, pp. 119-141 (1990)
4. Reinhart, R.A: Magnesium metabolism. *Wis Med J* 89(10), 579-583 (1990)
5. Beyenbach, K.W: The physiology of intracellular magnesium. In: *Magnesium and Physical Activity*, Ed: Vecchiet L., The Parthenon Publishing Group, New-York, pp. 93-116 (1995)
6. Bara, M: Mg et membranes. *Annés biologique*, 16, 290-315 (1977)
7. Günther, T: The biochemical basis of drug interactions with magnesium. *Magnesium Bull* 9, 55-61 (1987)
8. Touyz, R.M, C. Mercure & T.L. Reudelhuber: Angiotensin II type I receptor modulates intracellular free Mg^{2+} in renally derived cells via Na^{+}-dependent Ca^{2+}-independent mechanisms. *J Biol Chem* 276(17), 13657-13663 (2001)
9. Chiese, M. & G. Inesi: Mg^{++} and Mn^{++} modulation of Ca^{++} transport and ATPase activity in sarcoplasmic reticulum vesicles. *Arch Biochem Biophys* 208, 586-592 (1981)
10. Yago, M.D, M. Manas & J. Singh: Intracellular magnesium: transport and regulation in epithelial secretory cells. *Front Biosci* 1(5), D602-D618 (2000)

11. White, R.E & H.C. Hartzell: Effects of intracellular free magnesium on calcium current in isolated myocytes. *Science* 239, 778-780 (1988)
12. Vink, R & I. Cernak: Regulation of intracellular free magnesium in central nervous system injury. *Front Biosci* 1(5), D656-D665 (2000)
13. Sneddon, J.M: Divalent cations and the blood platelet release reaction. Nature Lond 236, 103-104 (1972)
14. Lamb, G.D & D.G. Stephenson: Effects of intracellular pH and [Mg^{2+}] on excitation-contraction coupling in skeletal muscle fibres of the rat. *J Physiol* 478, 331-339 (1994)
15. Potter, J.D & J. Gergely: The Calcium and Magnesium Binding Sites on Troponin and Their Role in the Regulation of Myofibrillar Adenosine Triphosphatase. *J Biol Chem* 250, 4628-4633 (1975)
16. Potter, J.D, S.P. Robertson & J.D. Johnson: Magnesium and the Regulation of Muscle Contraction. *Federation Proc* 40, 2653-2656 (1981)
17. Seamon, K.B, D.J. Hartshorne, & A.A. Bothner-By: Ca^{2+} and Mg^{2+} dependent conformations of troponin C as determined by ^{1}H and ^{19}F nuclear magnetic resonance. *Biochemistry* 16, 4039-4046 (1977)
18. Wolf, F.I & A. Cittadini: Magnesium in cell proliferation and differentiation. *Front Biosci* 1(4), D607-D617 (1999)
19. Halpern, M.J: Magnesium Physiopathology. I. Normal Physiology and Biochesmistry. In: *Magnesium deficiency.* Eds: Halpern M.J & J. Durlach, Karger, Basel, Gernany, pp. 1-8 (1985).
20. Reinhart, R.A: Magnesium metabolism. A review with special reference to the relationship between intracellular content and serum levels. *Arch Intern Med* 148, 2415-2420 (1988)
21. Wacker, W.E.C & A.F. Parisi: Magnesium metabolism. *New Engl J Med* 278, 712-717 (1968)
22. Rousselet, F & J. Durlach: Techniques analytiques et explorations pratiques du métabolisme magnésique en clinique humaine. In: 1er symposium International sur le deficit magnésique en pathologie humaine. Ed: Durlach, J., SGEMV, Vittel, França, Vol. I, pp. 65-90 (1971)
23. Henrotte, J.G: Facteurs génétiques de régulation du métabolisme magnésique chez l'homme. *Magnesium Bull* 3, 237-248 (1981)
24. Henrotte, J.G: Genetic regulation of cellular magnesium contents. In: *Magnesium and the Cell*. Ed: Birch, N.J. Academic Press, London, UK, pp. 177-195 (1993)
25. Flatman, P.M: Mechanisms of magnesium transport. *Ann Rev Physiol* 53, 259-71 (1991)
26. Flatman, P.M: The role of magnesium regulating ion transport. In: *Magnesium and the Cell*. Ed: Birch, N.J., Academic Press, London, UK, pp. 137-155 (1993)
27. Günther, T. & J. Vormann: Probable role of protein phosphorylation in the regulation of Mg^{2+} efflux via Na^{+}/Mg^{2+} antiport. *Magnesium Bull* 8, 307-309 (1986)
28. Günther, T: Putative mechanism of Mg^{2+}/Mg^{2+} exchange and Mg^{2+}/Mg^{2+} antiport. *Magnesium Bull* 18, 2-6 (1996)
29. Quamme, G.A, L.J. Dai, Ç. Krieger & S.W. Rabkin: Intracellular $[Mg^{2+}]_i$ concentration following metabolic inhibition in opossum kidney cells. *Biochim Biophys Acta* 1181, 307-315 (1993)
30. Wolf, F.I, A. Di Francesco, V. Covacci & A. Cittadani: Regulation of Na-dependent magnesium efflux from intact tumor cells. In: *Advances in Magnesium Research: 1 Magnesium in Cardiology*. Ed: Smetana R., John Libbey & Company Ltd., London, UK, pp. 490-496 (1995)
31. Günther, T. & R.M. Förster: Reversibility of Na^{+}/Mg^{2+} antiport in HL 60 cells. *Magnesium Bull* 22, 10-14 (2000)
32. Romani, A.M & A. Scarpa: Regulation of cellular magnesium. *Front Biosci* 1(5), D720-D734 (2000)
33. Maguire, M.E: Mg – a regulated and regulatory cation. In: *Metal ions in Biological Systems*. Eds: Sigel H. & A. Sigel, Marcel Dekker, New York, Vol. 26, pp. 135-153 (1990)
34. Romani, A & A. Scarpa: Regulation of cell magnesium. *Arch Biochem Biophy* 298, 1-12 (1992)
35. Beller, G.A, J.T. Maher & L.H. Hartley: Changes in serum and sweat magnesium levels during work in the heat. *Aviat Space Environ Med* 46(5), 709-712 (1975).
36. Schweigel, M & H. Martens: Magnesium transport in the gastrointestinal tract. *Front Biosci* 5, D666-D677 (2000)
37. Fine, K.D, C.A. Santa-Ana, J.L. Porter & J.S. Fordtran: Intestinal absorption of magnesium from food and supplements. *J Clin Invest* 88(2), 396-402 (1991)
38. Kayne, L. & D.B. Lee: Intestinal magnesium absorption. *Miner Electrolyte Metab* 19, 210-217 (1993)
39. Brink, E.J & A.C. Beynem: Nutrition and magnesium absorption: a review. *Prog Food Nutr Sci* 16, 125-162 (1992)
40. Rayssiguier, Y, Y. Boirie & J. Durlach: Magnésium. In: *Apports nutritionnels. Conseillés pour la population française*, 3rd ed., Ed: Martin, A., Editions Tec & Doc, Paris, France, pp. 146-149 (2001)
41. Bunce, G.F, H.E. Sauberlich, P.G. Reeves, T.S. Oba: Dietary phosphorus and magnesium deficiency in the rat. *J Nutr* 86, 406-413 (1965)
42. Drew, L.M, C. Kies & H.M. Fox: Effect of dietary fiber on cooper, zinc, and magnesium utilization by adolescent boys. *Am J Clin* 32, 1893-1897 (1979)
43. Kelsay , J.L, K.M. Behall & E.S. Prather: Effect of fiber from fruits and vegetables on metabolic responses of human subjects. II. Calcium, magnesium, iron, and silicom balances. *Am J Clin Nutr* 32 1876-1880 (1979)
44. Coudray, C, J. Bellanger, C. Castiglia-Delavaud, C. Remesy, M. Vermorel & Y. Rayssignuier: Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur J Clin Nutr 51(6), 375-380 (1997)
45. Coudray, C & Y. Rayssiguier: Impact of Vegetable Products on Intake, Intestinal Absorption and Status of Magnesium. In: Advances in Magnesium Research: Nutrition and Health. Eds: Rayssiguier, Y., A. Mazur & J. Durlach John Libbey & Company Ltd., London, UK, pp. 115-123 (2001)
46. Lopez, H.W, C. Coudray, M. Levrat-Verny, C. Feillet-Coudray, C. Demigne & C. Remesy: Fructo oligosaccharides enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats. J Nutr Biochem 11(10), 500-508 (2000)
47. Tahiri, M, J.C. Tressol, J. Arnaud, F. Bornet, C. Bouteloup-Demange, C. Feillet-Coudray, V. Ducros, D. Pépin, F. Brouns, A.M. Roussel, Y. Rayssiguier & C. Coudray: The ingestion of short-chain fructo-

oligosaccharides increases intestinal magnesium absorption in post-menopausal womem. In: *Advances in Magnesium Research: Nutrition and Health*. Eds: Rayssiguier, Y., A. Mazur & J. Durlach, John Libbey & Company Ltd. London, UK, pp. 125-129 (2001)
48. Hankin, J.H, S. Margen, & N.F. Goldsmith: Contribution of hard water to calcium and magnesium intakes of adults. *J Am Dietet Assoc* 56, 212-224 (1970)
49. Quamme, G.A & D.E.C. Cole: Physiology and pathophysiology of renal magnesium handling. In: *Advances in Magnesium Research. Nutrition and Health*. Eds: Rayssiguier, Y., A. Mazur, J. Durlach, John Libbey & Company Ltd., London, UK, pp. 155-162 (2001)
50. Bailly, C & C. Amiel: Effect of glucagon on Mg renal reabsorption in the rat. *Pflueger Archiv* 392(4), 360-365 (1982)
51. Durlach, J: *Magnesium in Clinical Practice*. Ed: Durlach, J., John Libbey Eurotext, Paris, France (1988)
52. Rayssiguier, Y, J. Durlach & Y. Boire: Métabolisme du magnesium et son role en pathologie. *Encyclopédie Médico-Cirurgicale* 10-357-A-10 (2000)
53. Wallach, S: Availability of body magnesium during magnesium deficiency. *Magnesium* 7, 262-270 (1988)
54. Davila, P.F & J.M.T. Roma: Fisiopatologia del magnésio. *Med Clin* 69, 163-171 (1977)
55. Brickman, A.S, J.W. Coburn, K. Kurokawa, N.E. Bethune & A.W. Norman: Action of 1,25-dihydrocholecalciferol in patients with hypophosphatemia, vitamin D-resistant rickets. *New Engl J Med* 289, 495-498 (1973)
56. Hardwick, L.L, M.R. Jones, N. Brautbar & D.B. Lee: Magnesium absorption: mechanisms and the influence of vitamin D, calcium and phosphate. *J Nutr* 121, 13-23 (1991)
57. Classen, H.G., S. Baier, H.F. Schimatschek & C.U. Classen: Interrelations between magnesium and hormones in health humans. In: Advances in Magnesium Research: 1 Magnesium in Cardiology. Ed: R. Smetana, John Libbey & Company Ltd., London, UK, pp. 381-385 (1995)
58. Schlemmer, A, J. Podenphant, B.J. Riis & C. Chistiansen: Urinary magnesium in early postmenopausal women. Influence of hormone therapy and calcium. Magnesium Trace Elem 10, 34-39 (1992)
59. Muneyyirci-Delale, O, V.L. Nacharaju, M. Dalloul, B.M. Altura & B.T. Altura: Serum ionized magnesium and calcium in women after menopause: inverse relation of estrogen with ionized magnesium. Fertil Steril 71(5), 869-872 (1999)
60. Spätling, L: The role of Mg in pregnancy, for the newborn and in children diseases. In: Metal ions in biological systems. Eds: Sigel H. & A. Sigel, Marcel Dekker, New York, Vol. 26, pp. 513-529 (1990)
61. Seelig, M.S: Epidemiologic data on magnesium deficiency-associated cardiovascular disease and osteoporosis: consideration of risks of current recommendations for high calcium intakes. In: Advances in Magnesium Research. Nutrition and Health. Eds: Rayssiguier, Y., A. Mazur, J. Durlach, John Libbey & Company Ltd., London, UK, pp. 177-190 (2001)
62. Marier, J.R: Dietary magnesium and drinking water, effects on human health status. In: Metal ions in biological *systems*. Eds: Sigel H. & A. Sigel, Marcel Dekker, New York, Vol. 26, pp. 85-100 (1990)
63. Durlach, J, V. Durlach, M. Bara & A. Guiet-Bara: Pour une réévaluation des apports quotidiens recommendés en magnesium. *Méd Nutr* 28, 9-15 (1992)
64. Seelig, M.S: The requirement of magnesium by the normal adult. Summary and analysis of published data. *Am J Clin Nutr* 14(6), 342-390 (1964)
65. Institute of Medicine, Food and Nutrition Board: Dietary references intakes: calcium, phosphorous, magnesium, vitamin D and fluoride. National Academy Press, Washington D.C. (1999)
66. Alencar, L.E, A. Martinez, C. Fernandez, M. Garaulet, F. Perez-Llamas & S. Zamora: Dietary intake in adolescents from south-east Spain and its relationship with physical activity. *J Am Diet Assoc* 100(11), 1334-1340 (2000)
67. Durlach, J: Recommended dietary amounts of Mg. Mg RDA. *Magnesium Res* 2, 195-204 (1989)
68. Giddens, J.B, S.K. Krug, R.C. Tsang, S. Guo, M. Miodovnik & J.A. Prada: Pregnant adolescent and adult women have similarly low intakes of selected nutrients. *J Am Diet Assoc* 100(11), 1334-1340 (2000)
69. Hellenbrand, W, G. Bauer, H. Boeing, A. Seidler & B.P. Robra: Diet in residents of East and West Germany in 1991-1992 as ascertained by a retrospective food frequency questionnaire. *Soz Praventivmed* 45(1), 13-24 (2000)
70. Wolter, R, D. Grandjean, J.P. Valette, R. Mateo, J. Marion & B. Paragon: Nutrition magnésique et effort. Aplications à l'animal de sport. Interêt en nutrition comparée (Fl-37). In: CAAA, Le point sur le magnésium et le soufre en alimentation animale. Paris: INA, Paris-Grignon. (1984)
71. Wynn, M & A. Wynn: New nutrient intake recommendations are needed for childbearing. *Nutr Health* 13(4), 199-211 (2000)
72. Heaton, F.W: Magnesium in intermediary metabolism In: *Magnesium in Health and Diseases*. Eds: Cantin, M. & M.S. Seelig, Sp. Medical Sci. Books, New York, pp. 43-45 (1980)
73. Heaton, F.W & Y. Rayssiguier: Magnesium deficiency and membrane properties. In: *Magnesium in cellular processes and medicine*. Eds: Altura, B., J. Durlach & M.S. Seelig, Karger, Basel, Germany, pp. 121-130 (1987)
74. Mak, I.T, R.E. Stafford & W.B. Weglicki: Loss of red cell glutathione during Mg deficiency: prevention by vitamin E, D-propranolol, and chloroquine. *Am J Physiol* 267, C1366-C1370 (1994)
75. Mills, B.J, R.D. Lindeman & C.A. Lang: Magnesium deficiency inhibits biosynthesis of blood glutathione and tumour growth in the rat. *Proc Soc Exp Biol Med* 181, 326-332 (1986)
76. Zhu, Z, M. Kimura & Y. Itokawa: Selenium concentration and glutathione peroxidase activity in selenium and magnesium deficient rats. *Biol Trace Elem Res* 37, 209-217 (1993)
77. Weglicki, W.B, T.M. Phillips, A.M. Freedman, M.M. Cassidy, & B.F. Dickens: Magnesium deficiency elevates circulating levels of inflammatory cytokines and endothelin. *Mol Cell Biochem* 110, 169-173 (1992)
78. Weglicki, W.B, T.M. Phillips, I.T. Mak, M.M. Cassidy, B.F. Dickens, R. Stafford & J.H. Kramer: Cytokines, neuropeptides, and reperfusion injury during magnesium deficiency. *Ann N Y Acad Sci* 264, 246-257 (1994)
79. Weglicki, W.B, B.F. Dickens, T.L. Wagner, J.J. Chmielinska & T.M. Philips: Immunoregulation by

neuropeptides in magnesium deficient: *ex vivo* effect on enhanced substance P production on circulating T lymphocytes from magnesium-deficient mice. *Magnesium Res* 9, 3-11 (1996)
80. Weglicki, W.B, J.H. Kramer, I.T. Mak, B.F. Dickens, A.M. Komarov & T.M. Philips: Proinflammatory neuropeptides in magnesium deficiency. In: *Metal Ions in Biology and Medicine*. Eds: Centeno, J.A., Ph. Collery, G. Vernet, R.B. Finkelman, H. Gibb & J.C. Etienne, John Libbey Eurotext, Paris, France, Vol. 6, pp. 472-474 (2000)
81. Chugh, S.N, T. Kolley, R. Kakkar, K. Chugh, & A. Sharma: A critical evaluation of anti-peroxidant effect of intravenous magnesium in acute aluminium phosphate poisoning. *Magnesium Res* 10, 225-230 (1997)
82. Tongyai, S, Y. Rayssiguier, C. Motta, E. Gueux, P. Maurois & F.W. Heaton: Mechanism of increased erythrocyte membrane fluidity during magnesium deficiency in weanling rats. *Am J Physiol* 257 (Cell Physiol 26), 270-276 (1989)
83. Heggtveit, A: Myopathy in experimental magnesium deficiency. *Ann Acad Sci* NY 162, 758 (1969)
84. Cronin, R.E, E.R. Ferguson, W.A. Shannon & J.P. Knochel: Skeletal muscle injury after magnesium depletion in the dog. *Am J Physiol* 243(2), 113-120 (1982)
85. Luthringer, C, Y. Rayssiguier, E. Gueux, & A. Dirthelot: Effect of moderate magnesium deficiency on serum lipids, blood pressure and cardiovascular reactivity in normotensive rats. *Br J Nutr* 59, 243-250 (1988)
86. Durlach, J: Aetiology and pathophysiology of central nervous hyperexcitability due to various types of magnesium deficit. Magnesium deficiency and magnesium depletion. In: *Magnesium/Mg and interaction of Mg with trace elements*. Ed: Kiss S.A., Hungarian Chem. Soc., Budapest, Hungary, pp. 11-16 (1998)
87. Durlach, J: Importance and clinical forms of chronic primary magnesium deficiency in human beings. In: *Advances in Magnesium Research. Nutrition and Health*. Eds: Rayssiguier, Y., A. Mazur & J. Durlach, John Libbey & Company Ltd., London, UK, pp. 13-20 (2001)
88. Durlach, J, M. Bara, A. Guiet-Bara & P. Ringard: Taurine and Mg homeostasis: new data and recent advances. In: *Mg in cellular processes and medicine*. Eds: Altura, B. & J. Durlach, Karger, Basel, Germany, pp. 219-238 (1985)
89. Douban, S, M.A. Brodsky, D.D. Whang & R. Whang: Significance of magnesium in congestive heart failure. *Am Heart J* 132(3), 664-671 (1996)
90. Halpern, M.J: Magnesium Physiopathology. II. Magnesium Deficiency and Depletion. In: *Magnesium deficiency.* Eds: Halpern M.J & J. Durlach, Karger, Basel, Germany, pp. 9-23 (1985)
91. Durlach, J, P. Bac, V. Durlach, M. Bara & A. Guiet-Bara: Neurotic, neuromuscular and autonomic nervous form of magnesium imbalance. *Magnesium Res* 10, 169-195 (1997)
92. Rude, R.K: Magnesium Deficiency: A Possible Risk Factor for Osteoporosis. In: *Advances in Magnesium Research. Nutrition and Health*. Eds: Rayssiguier, Y., A. Mazur, J. Durlach, John Libbey & Company Ltd., London, UK, pp. 391-398 (2001)
93. Stendig-Lindberg, G, R. Tepper, & I. Leichter Trabecular bone density in a two year controlled trial of peroral magnesium in osteoporosis. *Magnesium Res* 6(2), 155-163 (1993)
94. Laires, M.J. & C.P. Monteiro: Magnesium status: Influence on the regulation of exercise induced oxidative *stress* and immune function in athelets. In: *Advances in Magnesium Research. Nutrition and Health*. Eds: Rayssiguier, Y., A. Mazur, J. Durlach, John Libbey & Company Ltd., London, UK, pp. 433-441 (2001)
95. Rayssiguier, Y, C.Y. Guezennec & J. Durlach: New experimental and clinical data on the relationship between magnesium and sport. Magnesium Res 3(2), 93-102 (1990)
96. Casoni, I, C. Guglielmini, L. Graziano, M.G. Reali, D. Mazzotta, & V. Abbasciano: Changes of magnesium concentrations in endurance athletes. Int J Sports Med 11, 234-237 (1990)
97. Seelig, M.S: Consequences of magnesium deficiency on the enhancement of stress reactions: Preventive and therapeutic implications. J Am Coll Nutr 13, 429-446 (1994)
98. Boggio, V, J.C. Guilland, D. Moreau, J. Durlach, & J. Klepping: Dietary Magnesium Intake Among Male Athletes. Magnesium Bull 8, 275 (1986)
99. Córdova, A, F.J. Navas, M. Gómez-Carraminãna & H. Rodríguez: Evaluation of magnesium intake in elite sportsmen. Magnesium Bull 16, 59-63 (1994)
100. Fischer, P.W.F & A. Giroux: An evaluation of plasma and erythrocyte magnesium concentration and the activities of alkaline phosphatase and creatine kinase as indicators of magnesium status. Clin Biochem 24, 215-218 (1991)
101. Córdova, A Changes on plasmatic and erythrocytic magnesium levels after high intensity exercises in men. Physiol Behav 52, 819-821 (1992)
102. Deuster, P.A, E. Doley, S.B. Kyle, R.A. Anderson & E.B. Schoomaker: Magnesium homeostasis during high-intensity anaerobic exercise in men. J Appl Physiol 62, 545-550 (1987)
103. Joborn, H, G. Akerstrom & S. Ljunghall: Effects of exogenous catecholamines and exercise on plasma magnesium concentrations. Clinical Endocrinol 23, 219-226 (1985)
104. Franz, K.B, H. Rüddel, G.L. Todd, T.A. Dorheim, J.C. Buell & R.S. Elliot: Physiology changes during a marathon with special reference to magnesium. J Am Coll Nutr 4, 187-194 (1985)
105. Guerra, M, A. Monje, R. Perez-Beriain, A. García de Jalón, J. Villanueva, A. Herrera & J.F. Escanero: Ionic magnesium and selenium in serum after a cycle-ergometric test in football-players. In: Metal Ions in Biology and Medicine. Eds: Centeno, J.A, Ph. Collery, G. Vernet, R.B. Finkelman, H. Gibb & J.C. Etienne, John Libbey Eurotext, Paris, France, Vol. 6, pp. 501-504 (2000)
106. Laires, M.J, F. Alves & M.J. Halpern: Changes in Serum and Erythrocyte Magnesium and Blood Lipids after Distance Swimming. Magnesium Res 1, 219-222. (1988)
107. Laires, M.J, F. Madeira, J. Sérgio, C. Colaço, C. Vaz, G.M. Felisberto, I. Neto, L. Breitenfeld, M. Bicho & C. Manso: Preliminary study of the relationship between plasma and erythrocyte magnesium variations and some circulating pro-oxidant and antioxidant indices in a standardized physical effort. Magnesium Res 6, 233-238 (1993)
108. Laires, M.J & F. Alves: Changes in plasma, erythrocyte and urinary magnesium with prolonged swimming exercise. *Magnesium Res* 4(2), 119-122 (1991)
109. Pereira, D., M.J. Laires, C. Monteiro, C. Rabaçal, H. Ribeiro, G. Felisberto, C. Mendonça, M. Dias, L. Nuno, E.

Carvalho, J. Afonso & J.S. Fernandes: Oral Mg supplementation in heavily trained football players. Impact on exercise capacity and lipoperoxidation. In. Current Research in Magnesium, London. Eds: Halpern, M.J. & J. Durlach, John Libbey & Company, Ltd., London, UK, pp. 237-241 (1996)
110. Rose, L.I, D.R. Caroll, S.L. Lowe, E.W. Peterson & K.H. Cooper: Serum electrolyte changes after marathon running. J Appl Physiol 29, 449-451 (1970)
111. Haymes, E: Vitamin and mineral supplementation to athletes. Int J Sport Nutr 1, 146-169 (1991)
112. Rayssiguier, Y & P. Larvor: Hypomagnesemia following stimulation of lipolysis in ewes: effects of cold exposure and fasting. Magnesium in Health and Disease, 9, 68-72 (1980)
113. Rayssiguier, Y: Hypomagnesemia Resulting from Adrenaline Infusion in Ewes. Its Relation to Lipolysis. Horm Metab Res 9, 309-314 (1977)
114. Elliot, D.A & M.A. Rizack: Epinephrine and adrenocorticotropic hormone-stimulated magnesium accumulation in adipocytes and their plasma membranes. J Biol Chem 249, 3985-3990 (1974)
115. Golf, V.S, V. Graef, J.J. Gerlach & K.E. Seim: Veänderunger der Serum-CK- und Serum-CK-MB Aktivatäten in Abhängigkeit von einer Magnesium-substitution bein Leistungs Sportlerinnen. Magnesium Bull 2, 43-46 (1983)
116. Huet, F, J. Keppling, J. Marajo, M. Giaoui, H. Legrand & C.Y. Guezennec: Comportement nutritionnel du coureur de demi-fond. Aspects qualitatifs et quantitatifs. Rapports avec la depense energétique de l'entraînement. Sci Sports 3, 17-28 (1988)
117. Monteiro, C.P, A. Palmeira, G.M. Felisberto, C. Vaz, A. Rodrigues, J.T. Barata & M.J. Laires: Magnesium, Calcium, Trace Elements and Lipid Profile in Trained Volleyball Players: Influence of Training. In: Current Research in Magnesium. Eds: Halpern, M.J. & J. Durlach, John Libbey & Company Ltd., London, UK, pp. 231-235 (1996)
118. Resina, A, M. Brettoni, L. Gatteschi, P. Galvan, F. Orsi & M.G. Rubenni: Changes in the concentrations of plasma and erythrocyte magnesium and of 2,3-diphosphoglycerate during a period of aerobic training. Eur J Appl Physiol 68, 390-394 (1994)
119. Stendig-Lindberg, G, W.E.C. Wacker & Y. Shapiro: Long Term Effects of Peak Strenuous Effort on Serum Magnesium, Lipids, and Blood Sugar in Apparently Healthy Young Men. Magnesium Res 4, 59-65 (1991)
120. Stromme, S.B, I.E. Stenwold, H.D. Meen, & H.E. Refsum: Magnesium metabolism during prolonged heavy exercise. In: Metabolic adaptation to prolonged physical exercise. Eds: Howald, H. & J.R. Poortmans, Birkhauser, Basel, Germany, pp. 361-366 (1975)
121. Golf, S.W, O. Happel & V. Graef: Plasma aldosterone, cortisol and electrolyte concentration in physical exercise after magnesium supplementation. J Clin Chem Biochem 22, 717-721 (1984)
122. Lijnen, P, P. Hespel, R. Fagard, R. Lysens, E. Vanden Eynde, M. Goris, W. Goossens & A. Amery: Erythrocyte 2,3-Diphosphoglycerate Concentration Before and After a Marathon in Men. Eur J Appl Physiol 57, 452-455 (1988)
123. Costello, R.B & P.B. Moser-Veillon: A review of magnesium intake in the elderly. A cause for concern? *Magnesium Res* 5(1), 61-7 (1992)
124. Durlach, J, V. Durlach, P. Bac, Y. Rayssiguier, M. Bara & A. Guiet-Bara: Magnesium and ageing. II. Clinical data. Aetiological mechanisms and pathophysiological consequences of magnesium deficit in the elderly. *Magnesium Res* 6(4), 379-394 (1993)
125. Dweyer, J.T, A.M. Magarey & L.A. Daniels: Evaluation of micronutrient intakes of older Australians: The National Nutrition Survey. *J Nutr Health Aging* 5(4), 243-247 (1995)
126. Gullestad, L, M. Nes, R. Ronneberg, K. Midtvedt, D. Falch, & J. Kjekshus: Magnesium status in healthy free-living elderly Norwegians. *J Am Coll Nutr* 13(1), 45-50 (1994)
127. Kafatos, A, A. Diacatou, D. Labadarios, D. Kounali, J. Apostolaki, J. Vlachonikolis, G. Mamalakis & S. Megremis: Nutrition status of the elderly in Anogia, Crete, Greece. *J Am Coll Nutr* 12(6), 685-692 (1993)
128. Lee, J.S & E.A. Jr. Frongillo: Nutritional and health consequences are associated with food insecurity among U.S. elderly persons. *J Nutr* 131(5), 1503-1509 (2001)
129. Vir, S.C & A.H.G. Love: Nutritional status of institutionalized aged in Belfast. *Am J Clin* 32, 1934-1937 (1979)
130. Del Corso, L, F. Pastine, M.A. Protti, A.M. Romanelli, D. Moruzzo, L. Ruocco, & F. Pentimone: Blood zinc, copper and magnesium in aging. A study in healthy home-living elderly. *Panminerva*, 42(4), 273-277 (2000)
131. Torres, M.D, J.E. Campillo, G. Perez & A.J. Rodriguez: Vitamins and mineral intake in elderly people from extremadura. *Nutr Health Aging* 6(1), 55-56 (2002)
132. Durlach, J, P. Bac, V. Durlach, A. Durlach, M. Bara & A. Guiet-Bara: Magnesium status and ageing. An update. *Magnesium Res* 11(1), 25-42 (1997)
133. Alfray, A.C, N.L. Miller & D. Butkus: Evaluation of body Mg stores. *J Lab Clin Invest* 84, 153-162 (1974)
134. Mountokalakis, T.D: Effect of ageing, chronic disease and multiple supplements on magnesium requirements. *Magnesium* 6, 5-11 (1987)
135. Singh, R.B, M.A. Niaz, S. Ghosh & V. Rastogi: Epidemiological study of magnesium status and risk of coronary artery disease in elderly rural and urban populations of North India. *Magesium Res* 8, 165-172 (1996)
136. Olsson, T, M. Vittanen, E. Hagg, K. Asplund, K. Grankvist, S. Erikson, & Y. Gustafson: Hormones in "young" and "old" elderly pituitary thyroid and pituitary-adrenal axes. *Gerontology* 35, 144-152 (1989)
137. Durlach, J: Secondary magnesium deficits. In: *Magnesium in Clinical Practice.* Ed. Durlach, J, John Libbey Eurotext, Paris, France, pp. 114-194 (1988)
138. Durlach, J: Magnesium deficit in diabetes mellitus. In: *Magnesium in Clinical Practice*, Ed. Durlach, J, John Libbey Eurotext, Paris, France, pp. 156-169 (1988)
139. Durlach, J, P. Bac, V. Durlach, A. Durlach, M. Bara & A. Guiet-Bara: Are age-related neurodegenerative diseases linked with various types of magnesium depletion? *Magnesium Res* 10, 339-353 (1997)
140. Rayssiguier, Y, C. Malpuech, W. Nowacki, E. Rock, E. Gueux: & A. Mazur: Inflammatory response in magnesium deficiency. In: *Advances in Magnesium Research: 1 Magnesium in Cardiology.* Ed: Smetana R.

John Libbey & Company Ltd., London, UK, pp. 415-421 (1997)
141. Smetana, R: Cardiovascular medicine – the importance of magnesium in coronary artery disease and acute myocardial infarction. In: Advances in Magnesium Research: 1 Magnesium in Cardiology. Ed: Smetana R,. John Libbey & Company Ltd., London, UK, pp. 5-11 (1997)
142. Durlach, J: Déficit magnésique, tétanie et dystonie neurovégétative: donnés nouvelles sur les formes neuro-musculaires du déficit magnésique chronique. Magnesium Bull 3, 121-136 (1981)
143. Fernandes, J.S, T. Pereira, A. Alcazar, A. França, R. Andrade, J.N. Pereira, J. Pessoa, J.C. Rodrigues, L. Guerra, M.J. Laires & M.J. Halpern: Idiopatic Mitral Valve Prolapse and the Neuromuscular Form of Primary Magnesium Deficit. In: Mg Deficiency. Physiopathology and Treatment Implications. Eds: Halpern, M.J. & Durlach, J., Basel, Karger, Germany, pp. 120-121 (1985)
144. Abraham, A, U. Eylath, H. Weinstein & E. Czaczkes: Serum Mg levels in patients with acute myocardial infarction. New Engl J Med 296, 862-863 (1977)
145. Cohen, L., A. Laor & R. Kitzes: Serum Mg in propanol-treated patients with acute myocardial infarction. Magnesium 3, 138-144 (1984)
146. Dycker, T: Serum Mg in acute myocardial infarction. Acta Med Scan 207, 59-66. (1980).
147. França, A., J. Nogueira Pereira, M.J. Laires, T. Pereira, C. Rabaçal, M.J. Halpern & J. Simões Fernandes Serum Magnesium Levels in Patients after Myocardial Infarction or Unstable Angina Treated with Nitrites and Calcium Antagonists. Magnesium 4, 291-294 (1985)
148. Pereira, J.N, M.J. Laires, A. França, A. Alcazar, R. Andrade, J.C. Rodrigues, P.P. Jacinto, T. Pereira, M.J. Halpern & J. Simões Fernandes: Hypomagnesemia and Coronary Ischemic Disease. Experience of a Coronary Follow-up Service. In: Mg Deficiency. Physiopathology and Treatment Implications. Eds: Halpern, M.J. & J. Durlach, Karger, Basel, Germany, pp. 132-134 (1985)
149. Freedman, H.S: Coronary vasospasm and its relationship to Mg deficiency. Magnesium I, 81-83 (1982)
150. Frandsen, N.J, K. Winther, F. Pedersen, I. Christiansen, & P. McNair: Magnesium and platelet function: In vivo influence on aggregation and alpha-granule release in healthy volunteers. Magnesium Bull 17, 37M0 (1995)
151. Gawaz, M, I. Ott, A.J. Reininger & F.J. Neumann: Effects of magnesium on platelet aggregation and adhesion. Magnesium modulates surface expression of glycoproteins on platelets in vitro and ex vivo. Thromb Haemost 72, 912-918 (1994)
152. Nadler, J.L, S. Goodson & R.K. Rude: Evidence that prostacyclin mediates the vascular action of magnesium in humans. Hypertension 9, 379-383 (1987)
153. Pearson, P.J, P.R. Evora, J.F. Seccombe & H.V. Schaff: Hypomagnesemia inhibits nitric oxide release from coronary endothelium: protective role of magnesium infusion after cardiac operations. Ann Thor Surg 65, 967-972 (1998)
154. Ferrari, R, A. Curello, C. Ceconi, F. Di Lisa, R. Raddino & O. Visioli: Myocardial recovery during post-ischaemic reperfusion: effects of nifedipine, calcium and magnesium. J Mol Cell. Cardiol 18, 487-498 (1986)
155. Lareau, S, A. Boyle, R. Déslauries, W.J. Keon, T. Kroft, & R.S. Labow: Magnesium enhances function of postischaemic human myocardial infarction tissue. *Cardiovasc Res* 27, 1009-1014 (1993)
156. Shechter, M: The role of Magnesium as Antithrombotic Therapy. In: *Advances in Magnesium Research: Nutrition and Health*. Eds: Rayssiguier, Y., A. Mazur & J. Durlach, John Libbey & Company Ltd., London, UK, pp. 333-340 (2001)
157. Woods, K.L, S. Fletcher, C. Roffe & Y. Haider: Intravenous magnesium sulphate in suspected acute myocardial infarction: result of the second Leicester intravenous magnesium intervention trial (LIMIT 2). *Lancet* 339, 1553-1558 (1992)
158. ISIS: A randomised factorial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulfhate in 58,058 patients with suspected acute myocardial infarction. ISIS-4 (fourth international study of infarct survival collaborative group). *Lancet* 345, 669-685 (1995)
159. Ravn, H.B, S.D. Kristensen, V.E. Hjortdal, K. Thygesen & S.E. Husted: Early administration of intravenous magnesium inhibits arterial thrombus formation. *Arterioscler Thromb Vasc Biol* 17, 3620-3625 (1997)
160. Giardina, B, M. Penco, G. Lazzarino, S. Romano, B. Tavazzi, F. Fedele, D. Di Pierro & A. Dagianti: Effectiveness of thrombolysis is associated with a time-dependent increase of malondialdehyde in peripheral blood of patients with acute myocardial infarction. *Am J Cardiol* 71, 788-793 (1993)
161. Tavazzi, B, G. Lazzarini, D. Di Pierro & B. Giardina: Malondialdehyde production and ascorbate decrease are associated to the reperfusion of the postischaemic rat heart. *Free Radic Biol Med* 13, 75-78 (1992)
162. Mendonça, C, M.J. Laires, H. Ribeiro, C.P. Monteiro, L. Nuno, D. Pereira, C. Rabaçal, E. Carvalho, J. Sieuve Afonso & J. Simões Fernandes: Influence of Intravenous Magnesium Administration on Lipid Peroxidation During the Acute Phase of Myocardial Infarction - preliminary results. In: *Current Research in Magnesium*. Eds: Halpern M.J. & J. Durlach John Libbey & Company, Ltd., London, pp. 261-263 (1996)
163. Mendonça, C, H. Ribeiro, C.P. Monteiro, L. Nuno, D. Pereira, C. Rabaçal, E. Carvalho, J. Sieve Afonso, M.J. Laires, M. Bicho & J. Simões Fernandes: Aspects of Lipid Peroxidation and Epinephrine Oxidase Activity of Plasma During the Acute Phase of Myocardial Infarction. Influence of Intravenous Magnesium Administration. In: *Advances in Magnesium Research: 1 Magnesium in Cardiology.* Ed: Smetana R., John Libbey & Company Ltd., London, UK, pp. 46-51 (1997)
164. Laurant, P. & A. Berthelot: Influence of Magnesium on Vascular Functions and Blood Pressure in Rats. In: *Advances in Magnesium Research. Nutrition and Health*. Eds: Rayssiguier, Y., A. Mazur, J. Durlach, John Libbey & Company Ltd., London, UK, pp. 327-332 (2001)
165. Laurant, P & R.M. Touyz: Physiological and pathophysiological role of magnesium in the cardiovascular system: implications in hypertension. *J Hypertens* 18, 1177-1191 (2000)
166. Resnick, L.M: Cellular calcium and magnesium metabolism in the pathophysiology and treatment of hypertension and related metabolic disorders. *Am J Med* 93 (2A), 2A-115-2A-205 (1992)

167. Touyz, R.M, F.J. Milne, & P.R. Marshall: Altered cations and muscle membrane ATPase activity in SHR with benign and malignant hypertension. J Hypertens 9, 737-750 (1991)
168. Altura. B.M, B.T. Altura, A. Carella, A. Gebrewold, T. Murakawa & A. Nishio: Mg2+-Ca2+ interaction in contractility of vascular smooth muscle: Mg2+ versus organic calcium channel blockers on myogenic tone and agonist-induced responsiveness of blood vessels. Can J Physiol Pharmacol 65(4), 729-745 (1987)
169. Touyz, R.M: Cellular and molecular mechanisms regulating vascular function and structure - implications in the pathogenesis of hypertension. Can J Cardiol 16(9), 1137-1146 (2000)
170. Touyz, R.M: Magnesium Responsiveness to Angiotensine II and Endothelin-1 in Vascular Smooth Muscle Cells From Spontaneously Hypertensive Rats. In: Advances in Magnesium Research. Nutrition and Health. Eds: Rayssiguier, Y., A. Mazur, J. Durlach, John Libbey & Company Ltd., London, UK, pp. 341-346 (2001)
171. Altura, B.M, A. Zhang & B.T. Altura: Magnesium, hypertensive vascular diseases, atherogenesis, subcellular compartmentation of Ca2+ and Mg2+ and vascular contractility. Miner Electrolyte Metab 19(4-5), 323-336 (1993)
172. Lima, M.L, T. Cruz, J.C. Pousada, L.E. Rodrigues, K. Barbosa & V. Cangucu: The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes. Diabetes Care 21(5), 682-6 (1998)
173. Muneyyirci-Delale, O, V.L. Nacharaju, M. Dalloule, S. Jalou, M. Rahman, B.M. Altura & B.T. Altura: Divalent cations in women with PCOS: implications for cardiovascular diseases. Gynecol Endocrinol 15(3), 198-201 (2001)
174. Paolisso, G., A. Scheen, F. D'Onofrio & P.J. Lefebvre: Magnesium and glucose homeostasis. Diabetologia 33, 511-514 (1990)
175. Sjorgen, A, C.H. Floren, & A. Nilsom: Magnesium, potassium and zinc deficiency in subjects with type II diabetes mellitus. Acta Med Scand 224, 461-465 (1988)
176. de Valk, H.W: Magnesium in diabetes mellitus. *Neth J Med* 54(4), 139-146 (1999)
177. Vanroellen, W.F, L.F. van Gaal, P. van Rooy & I.H. De Leeuw: Serum and erythrocyte magnesium levels in type I and type II diabetes. Acta Diabetol 22, 185-190 (1985)
178. Altura, B.M, B.T. Altura, A. Grebrewold, H. Ising & T. Günther: Magnesium deficiency and hypertension: correlation between magnesium deficient diets and microcirculatory changes in situ. Science 223, 1315-1317 (1984)
179. Chen, M.D. & W.H. Sheu: Plasma status of selected minerals in hypertensive men with and without insulin resistance. J Trace Elem Med Biol 14(4), 228-231 (2001)
180. Delva, P, C. Pastori, G. Montesi, M. Degan, R. Micciolo, F. Paluani & A. Lechi: Intralymphocyte free magnesium and calcium and insulin tolerance test in a group of essential hypertensive patients. Life Sci 63(16), 1405-1415 (1998)
181. Altura, B.M & B.T. Altura: Magnesium and contractions of arterial smooth muscle. Microvasc Res 7, 145-155 (1974)
182. Altura, B.M & B.T. Altura: Magnesium ions and contractions on vascular smooth muscles: relationship to some vascular diseases. *Fed Proc* 40, 2672-2679 (1981)
183. Ferranini, E, G. Buzzigoli, R. Bonadonna, M.A. Giorico, M. Oleggini, L. Graziadei, R. Pedrinelli, L. Brandi & S. Bevilacqua: Insulin resistance in essential hypertension. *N Eng J Med* 317, 350-357 (1987)
184. Reaven, G.M & B.B. Hoffman: A role of insulin in the aetiology and course of hypertension. *Lancet* ii, 435-437 (1987)
185. Paolisso, G & M. Barbagallo: Hypertension, diabetes mellitus, and insulin resistance. The role of intracellular magnesium. *Am J Hypertens* 10(3), 346-355 (1997)
186. Alzaid, A.A, S.F. Dinneen, T.P. Moyer & R.A. Rizza: Effects of insulin on plasma magnesium in noninsulin dependent diabetes mellitus: evidence for insulin resistance. *J Clin Endocrinol Metab* 80(4), 1376-1381 (1995)
187. Barbagallo, M, R.K. Gupta, O. Bardicef, M. Bardicef & L.M. Renick,: Altered ionic effects of insulin in hypertension: role of basal ion levels in determining cellular responsiveness. *J Clin Endocrinol Metab* 82(6), 1761-1765 (1997)
188. Corica, F, A. Allegra, R. Ientile, M. Buemi, A. Corsonello, S. Bonanzinga, R. D'Agelo, T. De Gregorio & D. Ceruso: Changes in platelet, erythrocyte and plasma magnesium levels in normotensive and hypertensive obeses during oral glucose tolerance test. In: *Magnesium: Current Status and New Developments*. Eds: Theophanides T. & Anastassopoulou J., Kluwer Acad. Publ., Dordrecht, Germany, pp. 251-252 (1997)
189. Dominguez, L.J, M. Barbagallo, J.R. Sowers & L.M. Resnick: Magnesium responsiveness to insulin and insulin-like growth factor I in erythrocytes from normotensive hypertensive subjects. J Clin Endocrinol Metab 83(12), 4402-4407 (1998)
190. Durlach, J, P. Bac, V. Durlach, Y. Raysiguier, M. Bara, & A. Guiet-Bara: Magnesium status and ageing: an update. Magnesium Res 11(1), 25-42 (1998)
191. Humphries, S, H. Kushner & B. Falkner: Low dietary magnesium is associated with insulin resistance in a sample of young, nondiabetic Black americans. Am J Hypertens 12(8), 747-56 (1999)
192. Paolisso, G. & E. Ravussin: Intracellular magnesium and insulin resistance: results in Pima Indians and Caucasians. J Clin Endocrinol Metab 80(4), 1382-1385 (1995)
193. Barbagallo, M., L.J. Dominguez, O. Bardicef & L.M. Resnick: Altered cellular magnesium responsiveness to hyperglycemia in hypertensive subjects. Hypertens 38(2), 612-615 (2001)
194. Rosolova, H, O.Jr. Mayer & G. Reaven: Effect of variations in plasma magnesium concentration on resistance to insulin-mediated glucose disposal in nondiabetic subjects. J Clin Endocrinol Metab 82(11), 3783-3785 (1997)
195. Rosolova, H, O.Jr. Mayer & G. Reaven: Insulin-mediated glucose disposal is decreased in normal subjects with relatively low plasma magnesium concentrations. Metabolism 49(3), 418-420 (2000)
196. Ponder, S.W, B.H. Brouhard & L.B. Travis: Hyperphosphaturia and hypermagnesuris in children with IDDM. *Diabetes Care* 13, 437-441 (1990)
197. Sanders, G.T, H.J. Huijgen & R. Sanders Magnesium in disease: a review with special emphasis on the serum ionized magnesium. *Clin Chem Lab Med* 37(11-12), 1011-1033 (1999)
198. Suarez, A, N. Pulido, A. Casla, B. Casanova, F.J. Arrieta & A. Rovira: Impaired tyrosine-kinase activity of muscle insulin receptors from hypomagnesaemic rats. *Diabetologia* 38(11), 1262-1270 (1995)

199. Barbagallo, M., L.M. Resnick, L.J. Dominguez & G. Licata: Diabetes mellitus, hypertension and ageing: the ionic hypothesis of ageing and cardiovascular - metabolic disease. *Diabetes Metab* 23(4), 281-294 (1997)
200. Haenni, A, R. Reneland, L. Lind & H. Lithell: Serum aldosterone changes during hyperinsulinemia are correlated to body mass index and insulin sensitivity in patients with essential hypertension. *J Hypertens* 19(1), 107-112 (2001)
201. Resnick, L: The cellular ionic basis of hypertension and allied clinical conditions. *Prog Cardiov Dis* 42(1), 1-22 (1999)
202. Altura, B.M, S.M. Handwerker & B.T. Altura: Serum ionized Mg^{2+} in antenatal and perinatal period. In: Advances in Magnesium Research: 1 Magnesium in Cardiology. Ed: R. Smetana, John Libbey & Company Ltd. London, UK, pp. 249-255 (1997)
203. Spätling, L, G. Disch & H.G. Glassen: Magnesiun in pregnant women and the newborn. *Magnesium Res* 2(4), 271-280 (1989)
204. Engback L: Pharmacodynamics of neuromuscular blocking agents. Inhibitory ion: Magnesium. In: *Neuromuscular blocking and stimulating agents 1*. Ed: Cheymol, J., Perg. Press, Oxford, pp. 391-405 (1972)
205. James, M.F.M, K.R.L. Huddle, A.D. Owen, & B.W. van der Veen: Use of magnesium sulphate in the anaesthetic management of phaechromocytoma in pregnancy. *Can J Anaesth* 35, 178-182 (1988)
206. Watson, K.V, C.F. Molow, P.L. Ogburn & H.S. Jacob: Magnesium sulphat: rationale for its use in preeclampsia. *Proc Natl Acad Sci USA*, 83, 1075-1078 (1986)
207. Khedun, S.M, D. Ngotho, J. Moodley & T. Naicker: Plasma and red cell magnesium levels in black african women with hypertensive disorders of pregnancy. *Hypertens in Pregnancy* 17(2), 125-134 (1998)
208. Kisters, K, J. Körner, F. Louwen, R. Witteler, C. Spieker, W. Zidek, M. Barenbrock & K.H. Rahn: Plasma, intracellular, and membrane Mg^{2+} concentrations in normal pregnancy and preeclampsia. *Hypertens in Pregnancy* 17(2), 169-178 (1998)
209. Flatman, P.W & V.L. Lew: Magnesium buffering in intact human red blood cells measured using the ionophore A23187. *J Physiol* (London) 305, 13-30 (1980)
210. Watson, W.S, T.D. Lyon & T.E. Hilditch: Red cell magnesium as a function of cell age. *Metabolism* 29, 397-399 (1980)
211. Bang, O & S.L. Orskow: The magnesium content of the erythrocytes in pernicious and some other anemias. *J Clin Invest* 18, 497 (1939)
212. Erlandson, M.E, J. Golubow, J. Wehman & C.H. Smith: Divalent cations in homozigous thalassemia. *J Pediatr* 66, 637 (1965)
213. Brugnara, C & D. Tosteson: Inhibition of K transport by divalent cations in sickle erythrocytes. *Blood* 70(6), 1810-1815 (1987)
214. De Franceschi, L, D. Bachir, F. Galacteros, G. Tchernia, T. Cynober, S. Alper, O. Platt, Y. Beuzard & C. Brugnara: Oral magnesium supplements reduce erythrocyte dehydratation in patients with sickle cell disease. *J Clin Invest* 100(7), 1847-1852 (1997)
215. De Franceschi, L, D. Bachir, F. Galacteros, G. Tchernia, T. Cynober, D. Neuberg, Y. Beuzard & C. Brugnara: Oral magnesium pidolate: effects of long-term administration in patients with sickle cell disease. *Br J Haematol* 108(2), 284-289 (2000)
216. De Marchi, S, E. Cecchin, A.Basile, A. Bertotti, R. Nardini, E. Bartoli: Renal tubular dysfunction in chronic alcohol abuse. Effect of abstinence. *N Engl J Med* 329(26), 1927-1934 (1993)
217. Flink, E.B, M. Omar & S.R. Shane: Alcoholism, liver disease and magnesium. *Magnesium Bull* 1, 209-216 (1981)
218. Sullivan, J.E, H.C. Lankford, M.J. Swartz & C. Farrel: Magnesium metabolism in alcoholism. *Am J Clin Nutr* 13, 297-303 (1963)
219. Laires, M.J, M. Lucilia de Mello, O. Maia, J. Ruas da Silva, T. Pereira & M.J. Halpern: Hypomagnesemia in Chronic Alcoholism. In: *Magnesium Deficiency*. Eds: Halpern, M.J. & J. Durlach, Karger, Basel, Germany, pp. 138-141 (1985)

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