Magnesium homeostasis and aging

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Abstract. Aging is very often associated with magnesium (Mg) deficit. Total plasma magnesium concentrations are remarkably constant in healthy subjects throughout life, while total body Mg and Mg in the intracellular compartment tend to decrease with age. Dietary Mg deficiencies are common in the elderly population. Other frequent causes of Mg deficits in the elderly include reduced Mg intestinal absorption, reduced Mg bone stores, and excess urinary loss. Secondary Mg deficit in aging may result from different conditions and diseases often observed in the elderly (*i.e.* insulin resistance and/or type 2 diabetes mellitus) and drugs (*i.e.* use of hypermagnesuric diuretics). Chronic Mg deficits have been linked to an increased risk of numerous preclinical and clinical outcomes, mostly observed in the elderly population, including hypertension, stroke, atherosclerosis, ischemic heart disease, cardiac arrhythmias, glucose intolerance, insulin resistance, type 2 diabetes mellitus, endothelial dysfunction, vascular remodeling, alterations in lipid metabolism, platelet aggregation/thrombosis, inflammation, oxidative stress, cardiovascular mortality, asthma, chronic fatigue, as well as depression and other neuropsychiatric disorders. Both aging and Mg deficiency have been associated to excessive production of oxygen-derived free radicals and low-grade inflammation. Chronic inflammation and oxidative stress are also present in several age-related diseases, such as many vascular and metabolic conditions, as well as frailty, muscle loss and sarcopenia, and altered immune responses, among others. Mg deficit associated to aging may be at least one of the pathophysiological links that may help to explain the interactions between inflammation and oxidative stress with the aging process and many age-related diseases.

Key words: magnesium, aging, Mg deficiency, anti-aging, oxidative stress, chronic inflammation, diabetes, hypertension, dementia

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Aging represents a major risk factor for magnesium (Mg) deficit. Several alterations of Mg status have been identified in the elderly [1-13]. Total body Mg content tends to decrease with age, with bone being the main storage compartment of body Mg. Of the 21-28 g of Mg present in the adult human body, about 55-65% is in the mineral phase in the skeleton, 34-44% in the intracellular space, and only 1% in the extracellular fluid [14]. Although the Mg stored in the bone is not easily exchanged, the age-related reduction of bone mass is associated to a reduction of total body mineral and Mg content *(figure 1)*. Despite its importance, there is still insufficient information available regarding the distribution and turnover of exchangeable Mg in humans. There is a lot of variability in Mg intake, absorption, conservation and excretion. Alterations of Mg metabolism that have been associated to aging include a reduction of Mg intake and intestinal absorption, and an increase of Mg urinary and fecal excretion *(figure 1)*, all these changes indicating a tendency to a Mg deficit with aging.

An age-related decline in the capacity of the intestine to absorb dietary Mg has been suggested but is not well documented. In rats, several results indicate that the apparent Mg absorption is not altered

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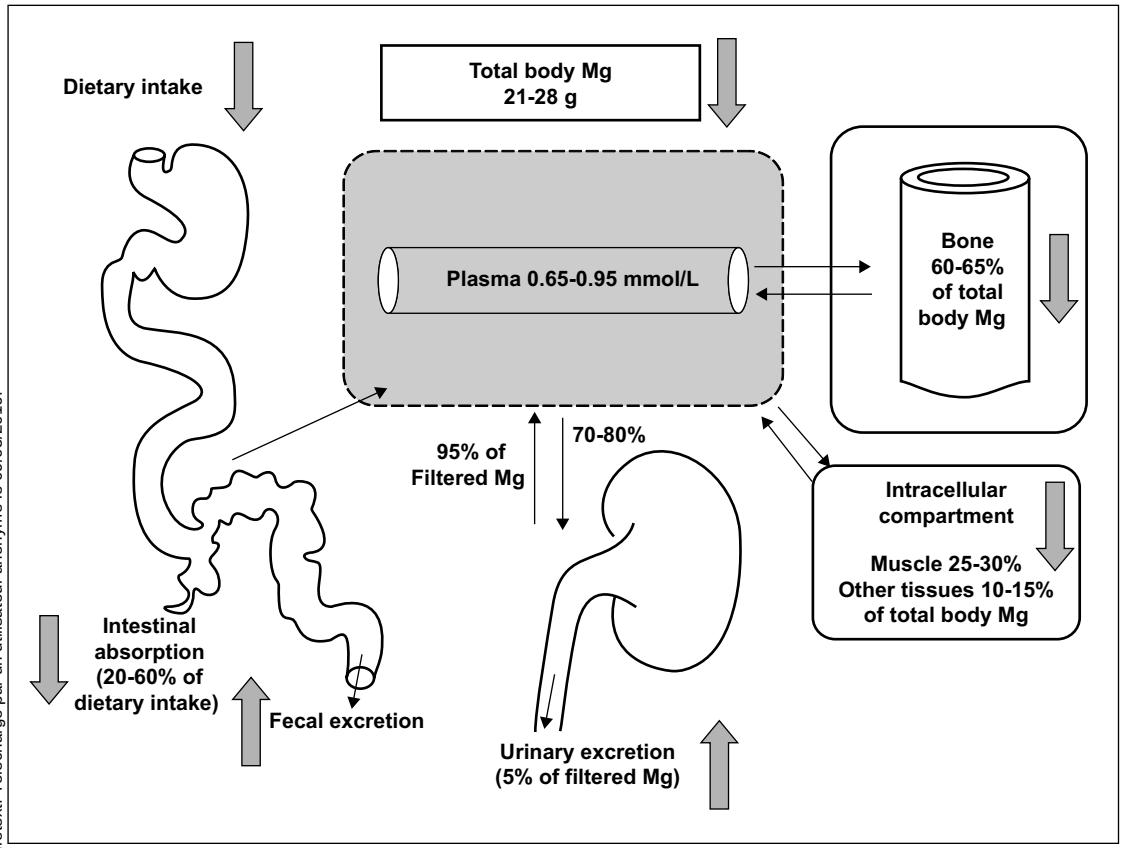

Figure 1. Mg homeostasis with age (arrows indicate possible sites of alteration with aging).

with aging [15], but more recent studies using stable isotopes suggest that Mg absorption decreases moderately with age [16].

Plasma and cellular equilibrium of Mg homeostasis as well as Mg concentrations are tightly regulated [17-19], and changes in plasma Mg can occur only in the presence of a significant long lasting Mg depletion. Although no known hormonal factor is specifically involved in the regulation of Mg metabolism, many hormones are known to affect Mg balance and transport, such as parathyroid hormone (PTH), calcitonin, vitamin D, catecholamines, and insulin. In particular, there is an important link between Mg and calciotropic hormones, since not only PTH and vitamin D may regulate Mg homeostasis, but Mg itself is essential for the normal function of the parathyroid glands, vitamin D metabolism, and to ensure an adequate sensitivity of target tissues to PTH and active vitamin D metabolites [20, 21]. It is thus likely that the modifications with age of these regulating hormones (decrease in vitamin D status and increase in PTH levels) [22, 23] may affect the Mg homeostasis in the elderly, although these aspects have not been completely elucidated. In particular, although vitamin D is an important regulator of calcium transport in the intestine, the importance of vitamin D for Mg absorption remains uncertain. In humans, the results of experiments on the effect of vitamin D and Mg absorption have been conflicting. The effect of vitamin D-stimulated Mg absorption remains uncertain given the increase in urinary excretion of Mg that has been associated with vitamin D administration.

Total plasma Mg concentrations (MgT), in relation to this tight control, are remarkably constant in healthy subjects throughout life and do not tend to change with aging [1, 6] *(figure 2)*. MgT concentration ranges from 0.65 to 0.95 mmol/L. In the serum Mg exists in 3 forms: a protein-bound fraction

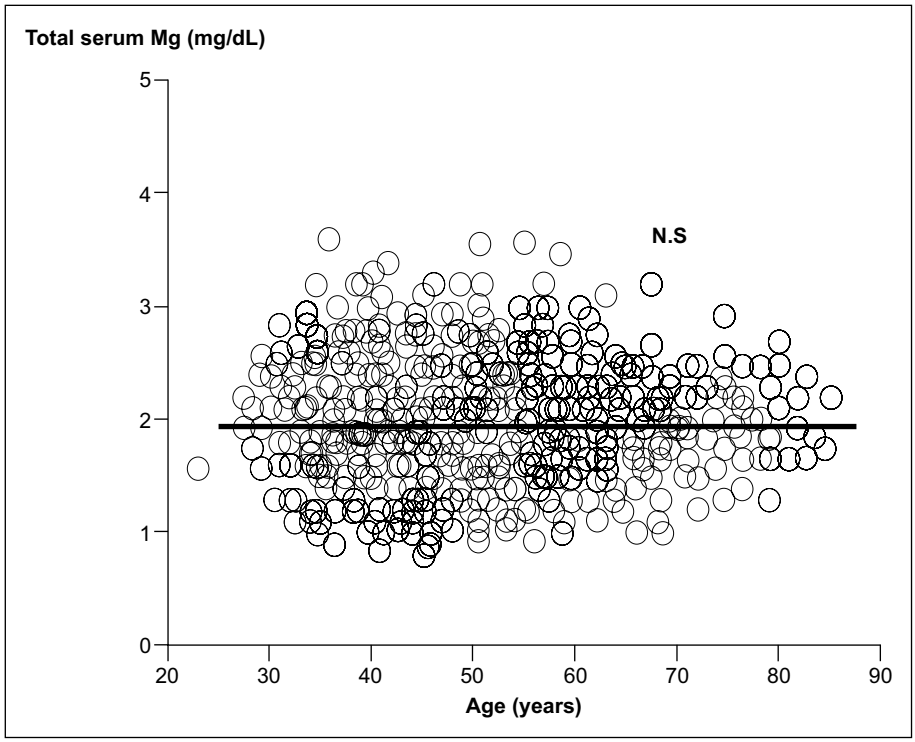

Figure 2. Relationship of total serum Mg concentration with aging.

(25% bound to albumin and 8% bound to globulins), a chelated fraction (12%), and the metabolically active ionized fraction (Mg-ion: 55%) [1, 14, 17, 18]. MgT, probably because of the large part bound to proteins or chelated, is not very sensitive in detecting subclinical Mg deficiencies. Possible changes may depend mainly on age-related diseases, therapies and age-related changes in renal function; 24-hour Mg retention studies have revealed an increased Mg retention in the elderly, suggesting a significant subclinical Mg deficit, not easily detected by total serum Mg [10]. The use of an ion-selective electrode (ISE), Mg-selective electrode to measure the active ionized free Mg (Mg-ion), has been suggested to be of help in detecting some of these subclinical Mg deficits. A close direct relationship was found between Mg-ions and the intracellular Mg measurement [24]. In clinical practice, the measurement of active ionized free Mg in the serum may allow a higher sensitivity than MgT in detecting subclinical Mg deficits in several clinical conditions, including aging. In preliminary data in healthy elderly (> 65 years old) subjects, we found a slight but significant reduction in Mg-ions compared to young controls (< 65 years old), changes not detected by the measurement of total serum Mg *(table 1)*.

Mg in the intracellular compartment also tends to be reduced with aging. Intracellular free Mg (Mgi) has been found to be significantly decreased in healthy elderly (> 65 years old) compared to young controls (< 65 years old) [11, 12]. We have specifically studied the behavior of intracellular Mg content with age, using ^{31}P-NMR spectroscopy, in peripheral red blood cells in healthy subjects and have shown a continuous age-dependent fall of intracellular Mg levels in healthy elderly subjects [12], without significant changes in total serum Mg

Table 1. Ionized (Mg ion) and Total (Mg Tot) Magnesium in the elderly (> 65 y) *vs* younger (< 65 y) subjects.

Group	Mg Tot (mmol/L)	Mg ion (mmol/L)
Younger (< 65 y)	0.82 ± 0.2	0.521 ± 0.01
Old (> 65 y)	0.78 (0.2)	0.496 (0.02)*

* $p < 0.001$ *vs* young subjects.

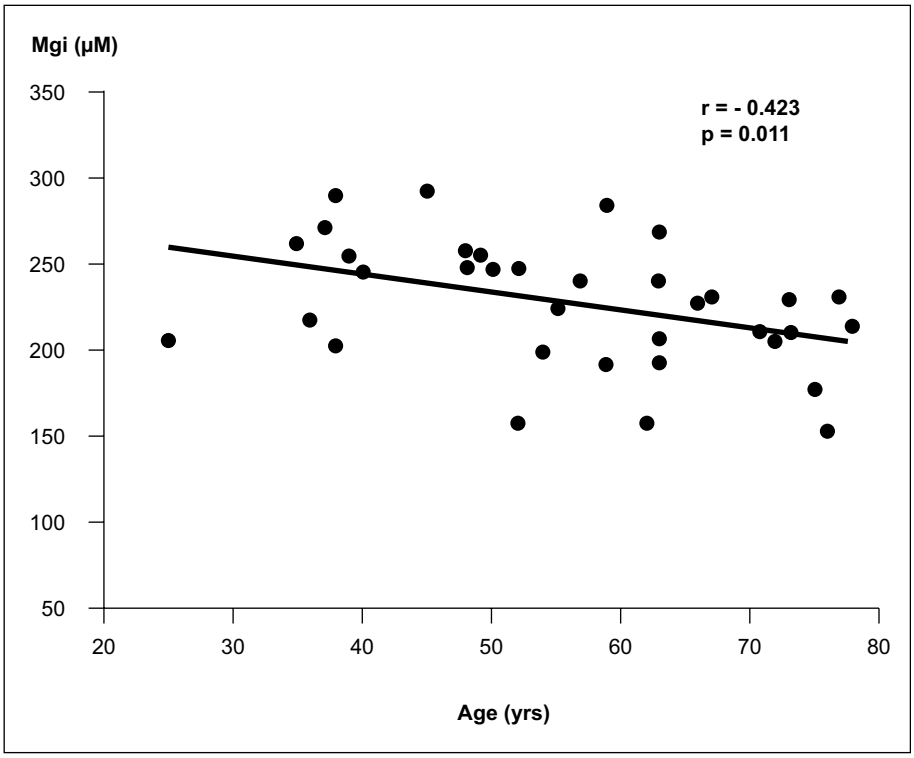

Figure 3. Relationship of intracellular free Mg concentration with aging (reproduced from [12] with permission).

(figures 3, 4). Thus, at least in conditions associated to a subclinical Mg deficit, the initial compartments that seems to be involved are the intracellular compartment and the ionized fraction of serum Mg, while a reduction of the bound and complexed total serum Mg (hypomagnesemia) may appear only at a later stage, in relation to more considerable and long-lasting Mg depletion.

Mechanisms of Mg deficits with age

The most common mechanisms that may cause Mg deficits with aging are summarized in *table 2*. A decreased intake of Mg has been suggested to have a primary role in age-related Mg deficit. Epidemiological data have shown that Mg intake in western countries tends to decrease with aging [25-30]. This is probably because the elderly tend to consume more processed foods and less whole grains and green vegetables. Although it has been shown that Mg requirements do not change with age [30], dietary Mg deficiency in the elderly is more prevalent than generally suspected. Data from the National Health and Nutrition Examination Survey (NHANES) III found that the Mg daily intake progressively decreases with age, independently of sex and race [25]. Older adults, affected by chronic conditions and on chronic drug treatment, are less likely than younger adults to consume enough Mg to meet their needs.

Analyses from the same NHANES III survey have shown that Mg intake in the older US population is well below the recommended daily allowance (RDA, average of 225 and 166 mg/day *vs* recommended 420 and 320 mg/day for men and women, respectively) [25]. Among US adults, 68% consume less than the RDA for Mg, 45% consume less than 75% of the RDA, and 19% consume less than 50% of the RDA [31]. In Europe, the Suppléments en Vitamines et Minéraux Antioxidants (SU.VI.MAX) study showed that 77% of women and 72% of men have dietary Mg intakes lower than RDA; 23% of women and 18% of men consumed less than 2/3 of these RDA [26].

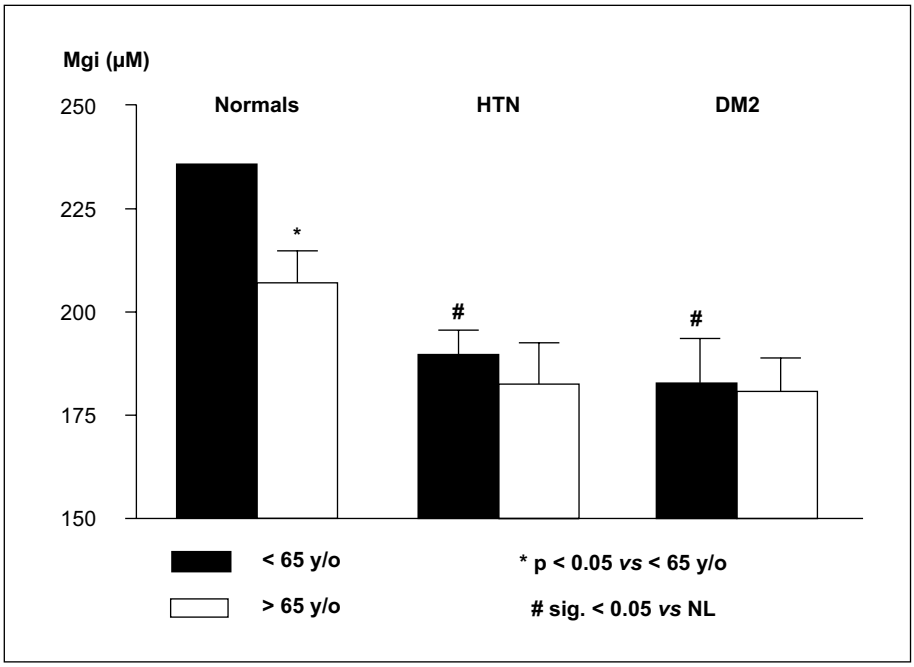

Figure 4. Effect of age on cytosolic free magnesium (Mg_i) levels in healthy normal, hypertensive (HTN) and in type 2 diabetic subjects (DM2) subjects (reproduced from [12] with permission). Full bars indicate data from young-middle aged subjects (< 65 years old) and empty bars indicate data from elderly subjects (> 65 years old). * $p < 0.05$ *vs* young-middle age subjects; # $p < 0.05$ *vs* normal (NL) subjects.

Other possible pathogenetic factors that may contribute to a Mg depletion with age (in addition to the inadequate dietary intake) are a decreased Mg absorption and/or an increased urinary Mg loss, and/or multiple drug use. The efficiency of Mg absorption declines with age. Mg is absorbed by both passive and active processes, mostly in the duodenum and in the ileum. A reduction of the absorption of Mg from the intestines in the elderly may be influenced by the reduction of vitamin D metabolism with age [1-3].

Renal active reabsorption of Mg takes place in the loop of Henle, in the proximal convoluted tubule, and is influenced by both the urinary concentration of sodium, and urinary pH. An increase of renal Mg excretion may also contribute to the Mg deficit and is linked to a reduced tubular reabsorption, associated with a reduction of the renal function that is a common condition in the elderly. Drug use (*i.e.* long-term treatment with loop diuretics, digitalis) and/or pathological conditions associated to aging (*i.e.* type 2 diabetes mellitus, hyperadrenoglucocorticism, insulin resistance, alcoholism, acute myocardial infarction, stroke, among others) are also associated to secondary Mg deficiencies [2, 3, 5, 6].

Table 2. Mechanisms of magnesium deficits with aging.

Primary Mg deficit
- Inadequate Mg nutrient intake.
- Reduced efficiency of Mg absorption (associated to reduced vitamin D levels)?
- Increased urinary excretion of Mg (associated to age-dependent reduction of kidney function and of Mg tubular reabsorption)
Secondary Mg deficiency
- Associated to age-related diseases and comorbidities
- Increased urinary Mg loss secondary to drugs (*i.e.* diuretics) used in the elderly subjects

Aging, Mg and inflammation

A chronic, low-grade inflammation [32] and oxidative stress have been proposed to be underlying conditions present in many age-related diseases,

and to be involved in the aging process itself. Inflammatory processes, particularly those mediating chronic inflammation, have been implicated as predictors or initiators of, or contributors to, chronic diseases and conditions primarily associated with aging, including cardiovascular disease, osteoarthritis, osteoporosis, Alzheimer's disease, insulin resistance and diabetes, muscle wasting, and frailty. Recent studies have shown that inflammatory changes are associated with aging per se. Although the literature provides evidence connecting inflammation or inflammatory mediators with aging and with chronic disease(s), most of these studies are correlative, and the underlying biology connecting mediators of inflammation with these various disease processes is unclear. Because the direct effects of aging on inflammatory responses and disease physiology are poorly understood, it is not surprising that a direct causal role of inflammation in the diseases of aging has yet to be demonstrated. Recent data suggest Mg may have a role in this age-related activation of a low-grade inflammatory process. Hypomagnesemia has been associated with inflammation and increased production of free oxygen radicals. Poor magnesium status may trigger the development of a proinflammatory state but the sequence of events leading to the inflammatory response remains unclear. The mechanisms that may explain the proinflammatory effect of Mg deficiency includes a stimulation of the production and circulating levels of inflammatory cytokines while a rise in circulating substance P levels and proinflammatory neuropeptides remains controversial because not all investigators have detected this event during dietary Mg restriction [33]. Malpuech-Brugere *et al.*, in Mg-deficient rats, demonstrated a significant elevation of circulating interleukin-6 (IL-6) plasma levels, accompanied by an increase in the plasma levels of acute phase proteins (alpha2-macroglobulin and alpha1-acid glycoprotein), leukocyte and macrophage activation, plasma fibrinogen, a liver increase in the level of mRNA coding for these proteins, without plasma elevation of substance P [34]. Because magnesium acts as a natural calcium antagonist, the molecular basis for the inflammatory response may also be the result of a modulation of the intracellular calcium concentration. Potential mechanisms include the priming of phagocytic cells, the opening of calcium channels, activation of N-methyl-D-aspartate (NMDA) receptors, the activation of nuclear factor-kappaB (NFkB) and activation of the renin-angiotensin system.

In animals, several studies have shown that Mg deprivation causes excessive production and release of proinflammatory molecules tumor necrosis factor (TNF)-α, IL-1β, IL-6, vascular cell adhesion molecule (VCAM)-1, and plasminogen activator inhibitor (PAI)-1, increased circulating inflammatory cells, and increased hepatic production and release of acute phase proteins (i.e. complement, α2-macroblobulin, fibrinogen) [33-41]. Experimental studies in rats have shown that Mg deficiency induces a chronic impairment of the redox status associated with inflammation, which could contribute to increased oxidized lipids, and may promote hypertension and vascular disorders [38].

In humans, clinical data have shown that low serum Mg levels as well as inadequate dietary Mg are strongly related to low-grade systemic inflammation [31, 42, 43]. Data from the Women's Health Study, have shown that Mg intake is inversely related to systemic inflammation, measured by serum C-reactive protein (CRP) concentrations, and with the prevalence of the metabolic syndrome in adult women [43]. Using the 1999–2002 NHANES database, King *et al.* found that dietary Mg intake was inversely related to CRP levels. Among the 70% of the population not taking supplements, Mg intake below the RDA was significantly associated with a higher risk of having elevated CRP [44]. Several other studies have confirmed an inverse relationship among Mg intake, serum Mg and TNF-α, IL-6, and CRP levels [44-46]. In a cross-sectional study, a higher TNF-α concentration was inversely correlated with serum Mg and in multivariate analysis, those with the lowest serum Mg were 80% more likely to have higher circulating levels of TNF-α [46].

Mg deficiency has been associated, both in experimental animal models and in humans, with increased oxidative stress and decreased antioxidant defense due, at least in part, to increased inflammation parameters [39, 47, 48]. Previous studies have convincingly shown that Mg deficiency results in increased production of oxygen-derived free radicals in various tissues, increased free radical-elicited oxidative tissue damage, increased production of superoxide anion by inflammatory cells, decreased antioxidant enzyme expression and activity, decreased cellular and tissue antioxidant levels, and increased oxygen peroxide production [2, 38, 49, 50]. Mg may also prevent oxygen radical formation by scavenging free radicals and by inhibiting xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [51].

There is also evidence that magnesium may play a role in the immune response as a co-factor for immunoglobulin (Ig) synthesis, C'3 convertase, immune cell adherence, antibody-dependent cytolysis, IgM lymphocyte binding, macrophage response to lymphokines, and T helper-β cell adherence [52, 53].

Mg and age-related cardiovascular and metabolic diseases

Mg imbalances in elderly people and consequent defective membrane function, inflammation, increased oxidative stress and immune dysfunction may cause an increased vulnerability to several age-related diseases. Among them the link between Mg alterations and type 2 diabetes/cardio-metabolic diseases is well known, also because both conditions have been associated with Mg alterations, independently of age.

The role of Mg in the regulation of cellular glucose metabolism, insulin action and sensitivity, as well as in the modulation of vascular smooth muscle tone, and blood pressure homeostasis is well established [12, 14, 54, 55]. Chronic Mg deficits have been linked to an increased risk of cardiovascular and metabolic diseases, including hypertension, stroke, atherosclerosis, ischemic heart disease, cardiac arrhythmias, glucose intolerance, insulin resistance, type 2 diabetes mellitus, endothelial dysfunction, vascular remodeling, alterations in lipid metabolism, platelet aggregation/thrombosis, inflammation, oxidative stress, cardiovascular mortality, asthma, chronic fatigue, as well as depression and other neuropsychiatric disorders [56-62], all conditions mostly observed in the elderly population.

At the cellular level, cytosolic free Mg levels are consistently reduced in subjects with type 2 diabetes mellitus. Using gold standard NMR techniques, our group has shown significantly lower steady-state Mgi and reciprocally increased Cai levels in subjects with type 2 diabetes, compared with young non-diabetic subjects [12, 63]. Mgi depletion in diabetes has been shown to be clinically and pathophysiologically significant, since Mgi levels quantitatively and inversely predict the fasting and post glucose levels of hyperinsulinemia, as well as peripheral insulin sensitivity, and both systolic and diastolic blood pressures [12, 14, 54, 55, 63]. A continuous fall in Mg with increasing age was observed in peripheral blood cells. The previously described age-dependent alterations in cytosolic free magnesium levels were indistinguishable from those present in essential hypertension or type 2 diabetes, independently of age. Thus, both type 2 diabetes and hypertension display the same ionic changes (lower intracellular Mg and higher intracellular calcium) at all ages, and might therefore help to explain the age-related increased incidence of these diseases. In addition, the old clinical concept of diabetes being a disease of accelerated vascular aging is literally true, referring to Mg status, since diabetic patients display the same intracellular ionic changes at all ages *(figure 4)*.

In diabetic subjects, both low Mg intake and increased Mg urinary losses have been associated with Mg deficits [54, 55]. Hyperglycemia and hyperinsulinemia may both have a role in the increased urinary Mg excretion contributing to Mg depletion. A depletion of Mg seems to be a cofactor for a further derangement of insulin resistance. A Mg-deficient diet is associated with a significant impairment of insulin-mediated glucose uptake, and to an increased risk of developing glucose intolerance and diabetes [64].

Recent epidemiologic data have shown a significant inverse association between Mg intake and diabetes risk. A deficient Mg status may both be a secondary consequence or may precede and cause insulin resistance and altered glucose tolerance, and even diabetes [62, 65-68].

Inflammation and oxidative stress have been proposed to be the link between Mg deficit and insulin resistance/metabolic syndrome [44-46]. More generally, chronic hypomagnesaemia and conditions commonly associated with Mg deficiency, such as type 2 diabetes mellitus and aging, are all associated with an increase in free radical formation with subsequent damage to cellular processes [1, 2, 44-46]. We have shown that the effects of antioxidant therapies with vitamin E and glutathione to improve insulin sensitivity and whole body glucose disposal are, at least in part, mediated by their action to improve cellular Mg homeostasis [69-71].

Altogether, these data are consistent with a role of Mg deficiency in promoting oxidative stress and inflammation, hence, the development of insulin resistance, vascular remodeling, atherosclerosis, type 2 diabetes and cardio-metabolic syndrome.

Mg and age-related sarcopenia

Older age is frequently characterized by loss of skeletal muscle mass and function (sarcopenia) [72]. Mg depletion may play a role in this phenomenon causing muscle cells alterations through

increased oxidative stress and impaired intracellular calcium homeostasis [73]. Thus, it has been suggested that Mg status may affect muscle performance, probably due to Mg's key role in energetic metabolism, transmembrane transport and muscle contraction and relaxation [14, 17].

Mg supplementation (up to 8 mg/kg daily) enhanced muscle strength in young untrained individuals [74]. Similarly, physically active young subjects experienced improved endurance performance and decreased oxygen use during submaximal exercise after Mg supplementation [75]. Using data from the InCHIANTI study, a well-characterized representative sample of older men and women, a significant, independent and strong relationship between circulating Mg and muscle performance was found, which was consistent across several muscle parameters for both men and women [76]. These data are consistent: a) with the relation of Mg status to muscle ATP and the role of Mg in energetic metabolism; b) the increased reactive oxygen species (ROS) production in Mg deficiency; and, c) the proinflammatory effect of Mg depletion.

Mg and osteoporosis

Although it is impossible to discuss all the possible contributions of Mg deficit to the aging process and vulnerability to age related diseases, it is important to mention that bone fragility increases with Mg deficiency [77]. Epidemiological studies have linked dietary Mg deficiency to bone loss and osteoporosis. Severe Mg deficiency in the rat causes impaired bone growth, osteopenia and skeletal fragility. Potential mechanisms for bone loss in Mg deficiency includes impaired production of PTH and 1-25vit D, which may contribute to reduced bone formation, and elevated inflammatory cytokines that may increase osteoclastic bone resorption. A decrease in osteoprotegerin (OPG), and an increase in RANKL favoring an increase in bone resorption has also been suggested, all these data supporting a possible role of Mg deficit in impairing bone and mineral metabolism and in increasing the risk for osteoporosis.

Mg and the aging process

Mg alterations associated to aging may have a role in accelerating the aging process itself. Magnesium is an essential cofactor in cell proliferation and differentiation and in all steps of nucleotide excision repair and is involved in base excision repair and mismatch repair [78-81]. DNA is continuously damaged by environmental mutagens and by endogenous processes. Mg is required for the removal of DNA damage generated by environmental mutagens, endogenous processes, and DNA replication [78-80, 82]. In cellular systems, Mg, at physiologically relevant concentrations, is highly required to maintain genomic stability. Mg has a stabilizing effect on DNA and chromatin structure, and is an essential cofactor in almost all enzymatic systems involved in DNA processing [78]. Intracellular free Mg is a "second messenger" for downstream events in apoptosis. Thus, levels of free intracellular Mg increase in cells undergoing apoptosis. This increase is an early event in apoptosis, preceding DNA fragmentation and externalization of phosphatidylserine, and is likely due to a mobilization of Mg from mitochondria [82]. There is increasing evidence from animal experiments and epidemiological studies, that Mg deficiency may decrease membrane integrity and membrane function, increasing the susceptibility to oxidative stress, cardiovascular heart diseases, as well as accelerated aging.

Several studies have reported alterations in cell physiology with senescence features during Mg deficiency in different cell types. Mg related alterations may include reduced oxidative stress defense, cell cycle progression, culture growth, cellular viability [36, 50, 81, 83, 84], and activation of proto-oncogene (i.e. c-fos, c-jun) and transcription factor expressions (*i.e.* NF-κB) [85]. Recent data have shown that Mg deficiency may accelerate cellular senescence in cultured human fibroblasts [86]. Continuous culture of primary fibroblasts in magnesium-deficient media resulted in loss of replicative capacity with an accelerated expression of senescence-associated biomarkers. A marked decrease in the replicative lifespan was seen compared to fibroblast populations cultured in standard Mg media conditions. Human fibroblast populations cultured in Mg-deficient conditions also showed an increased senescence-associated β-galactosidase activity. Additionally, activation of cellular aging (p53 and pRb) pathways by Mg-deficient conditions also increased the expression of proteins associated with cellular senescence, including p16INK4a and p21WAF1. Telomere attrition was found to be accelerated in cell populations from Mg-deficient cultures, suggesting that the long-term consequence of inadequate Mg availability in human fibroblast cultures is an accelerated cellular senescence [86].

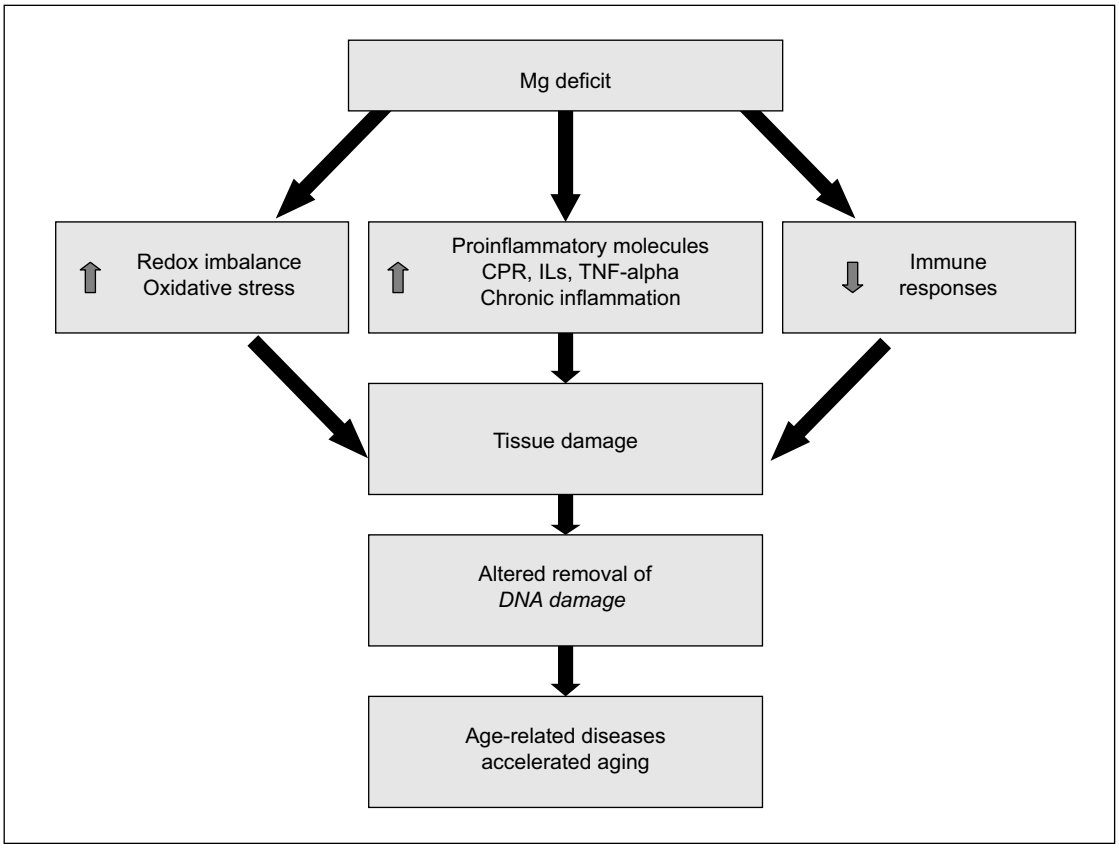

Figure 5. Overall hypothesis in which chronic Mg deficits has been proposed as one of the physiopathological links that may help to explain the interactions among inflammation, oxidative stress, and altered immune responses with the aging process and age-related diseases.

Conclusion

The above mentioned reasons confirm that the availability of an adequate quantity of Mg is a critical factor for normal cellular and body homeostasis. Aging is very often associated with Mg inadequacy. Chronic Mg deficiency is associated with inflammation and oxidative stress, as well as with an increased incidence of chronic diseases associated to aging. A chronic, low-grade inflammation and oxidative stress are underlying conditions present in many age-related diseases, and have been proposed to be involved in the aging process itself. We suggest that chronic Mg deficits may be at least one missing link activating the inflammatory process with age and connecting inflammation with the aging process and many age-related diseases *(figure 5)*.

The possibility that maintaining an optimal Mg balance throughout life might help in preventing or significantly retarding the inflammation process and manifestations of chronic diseases, is a working hypothesis that needs to be tested in prospective studies.

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