

Magnesium deficiency and metabolic syndrome: stress and inflammation may reflect calcium activation

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Abstract. Magnesium (Mg) intake is inadequate in the western diet and metabolic syndrome is highly prevalent in populations around the world. Epidemiological studies suggest that high Mg intake may reduce the risk but the possibility of confounding factors exists, given the strong association between Mg and other beneficial nutrients (vegetables, fibers, cereals). The concept that metabolic syndrome is an inflammatory condition may explain the role of Mg.

Mg deficiency results in a stress effect and increased susceptibility to physiological damage produced by stress. Stress activates the hypothalamic-pituitary-adrenal axis (HPA) axis and the sympathetic nervous system. The activation of the renin-angiotensin-aldosterone system is a factor in the development of insulin resistance by increasing oxidative stress. In both humans and rats, aldosteronism results in an immunostimulatory state and leads to an inflammatory phenotype. Stress response induces the release of large quantities of excitatory amino acids and activates the nuclear factor NFκB, promoting translation of molecules involved in cell regulation, metabolism and apoptosis. The rise in neuropeptides is also well documented. Stress-induced HPA activation has been identified to play an important role in the preferential body fat accumulation but evidence that Mg is involved in body weight regulation is lacking. One of the earliest events in the acute response to stress is endothelial dysfunction. Endothelial cells actively contribute to inflammation by elaborating cytokines, synthesizing chemical mediators and expressing adhesion molecules. Experimental Mg deficiency in rats induces a clinical inflammatory syndrome characterized by leukocyte and macrophage activation, synthesis of inflammatory cytokines and acute phase proteins, extensive production of free radicals. An increase in extracellular Mg concentration decreases inflammatory effects, while reduction in extracellular Mg results in cell activation. The effect of Mg deficiency in the development of insulin resistance in the rat model is well documented. Inflammation occurring during experimental Mg deficiency is the mechanism that induces hypertriglyceridemia and pro-atherogenic changes in lipoprotein metabolism. The presence of endothelial dysfunction and dyslipidemia triggers platelet aggregability, thus increasing the risk of thrombotic events. Oxidative stress contributes to the elevation of blood pressure. The inflammatory syndrome induces activation of several factors, which are dependent on cytosolic Ca activation.

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Recent findings support the hypothesis that the Mg effect on intracellular Ca^{2+} homeostasis may be a common link between stress, inflammation and a possible relationship to metabolic syndrome.

Key words: magnesium, calcium, metabolic syndrome, inflammation

Adequate magnesium (Mg) intake is critical in maintaining Mg balance and normal Mg-dependent cellular reactions in the human body. Mg deficiency has been correlated with chronic diseases including metabolic syndrome [1-7]. A recent hypothesis is that an inflammatory syndrome induced by Mg deficiency may represent a triggering factor in the development of metabolic syndrome [2-7]. The aim of this review is to summarize recent findings on the role of Mg.

Metabolic syndrome: is Mg deficiency involved?

Interaction between genetic predisposition and a Western-type lifestyle contributes to the epidemic of metabolic syndrome. Profound changes in the environment occurred too fast for the genome to adjust. Hence the emergence of so-called diseases of civilization [1], first described in agriculture, and that can be extended in modern food industry. Intensive agriculture has been related to grass tetany in dairy cows, a disease due to an acute decrease of plasma magnesium. Similarly, food refining leads to a reduction of the micronutrient density and thereby induces a marginal magnesium intake, resulting in a higher prevalence of Mg deficiency in westernized populations [1]. Even if recent findings using balance studies suggest a lower Mg requirement than that estimated previously, the evidence suggests that the occidental diet is relatively deficient in Mg [1]. Moreover, a diet rich in animal foods and poor in vegetable foods induces acidosis and increased Mg urinary excretion [1]. Data from the National Health and Nutrition Examination Survey (NHANES) showed that the average intake of magnesium was 350 mg in men, below the RDA of 420 mg [8]. In the French Supplementation with antioxidants, vitamins and minerals study (SUVIMAX), 20% of subjects consumed less than 2/3 of the RDA [9]. Thus, Mg intake is inadequate in the Western diet, while a higher content of Mg intake is found in the "prudent diet" as compared to the Western diet because it also contains more cereals and vegetables [9]. Metabolic syndrome is a condition characterized by a cluster of several risk factors, including abdominal obesity, insulin resistance, dyslipidemia

and hypertension [2, 4, 5]. This syndrome and type 2 diabetes are occurring at epidemic rates with frightful consequences for human health worldwide. In the US, over 40% of people older than 60 years suffer from metabolic syndrome [10]. Many epidemiological studies have described inverse relationships between a Mg-rich food intake and diabetes, insulin resistance and metabolic syndrome [11]. For instance in the Boston study in young adults with 3 day food record, higher Mg intake was associated with a 31% lower risk of developing metabolic syndrome over 15 years of follow up [12]. In the Women's Health Study, high magnesium status was associated with a 27% of lower risk of metabolic syndrome [13]. Little is known about the possible role of Mg in populations in which dietary patterns differ from Western populations. However, the Shanghai Women's Health Study suggests that Ca and Mg may protect against the development of type 2 diabetes [14]. A limitation of observational studies showing the relationship between dietary Mg intake and metabolic syndrome is that it can be difficult to separate the effects of nutrients from those of foods. There is also a strong association between Mg and other beneficial nutrients: vegetables fibers, cereals. Thus, the possibility of confounding factors exists and not all studies confirm the relationship between the Mg and metabolic syndrome [6]. A recent study indicates that two common genetic variations in TRPM6 and TRMP7, which play a central role in Mg homeostasis, might confirm susceptibility to type 2 diabetes in women with low Mg intake [15]. Further studies are needed to investigate the metabolic consequences of genetic hypomagnesaemia. Many epidemiological studies indicate that lower serum Mg levels are associated with insulin resistance and various components of the metabolic syndrome [16]. Patients with metabolic syndrome had also lower intramononuclear cell Mg concentration (which may provide reliable information about intracellular Mg concentration) as compared to controls. Such findings are also well documented in patients with diabetes mellitus [17, 18]. Altogether, the results of these studies suggest plausibility for the relationship between Mg and metabolic syndrome. The concept that the metabolic syndrome is an inflammatory condition

and that there are stress-induced inflammation mechanisms [19] brings an exciting approach to the understanding of this syndrome. Markers including acute phase proteins, cytokines and mediators have been associated with endothelial activation [20]. White adipose tissue of obese individuals is characterized by increased production and secretion of a wide range of inflammatory molecules involved in macrophage infiltration, and insulin resistance contribution [20].

Magnesium deficiency modulates stress and inflammation

The effect of Mg on inflammation-related factors may explain the association between Mg intake and Mg status on metabolic syndrome [2, 21]. In humans, an inverse association between markers of chronic inflammation and Mg intake has been reported on serum levels [22-25]. The inverse association between Mg and C-reactive protein suggested that Mg deficiency might be involved in the development of low chronic inflammatory syndrome, which can modulate metabolic disorders; Mg supplementation has been shown to reduce CRP blood levels in patients with heart failure [6]. Even if, in epidemiological studies, the association between Mg and inflammatory markers is not always evidenced [6], in experimental

animals, there is now a strong biological plausibility for the direct impact of dietary Mg on inflammation [2, 3]. We will discuss below the interrelationship between magnesium deficiency, stress, inflammation and metabolic syndrome (*figure 1*).

Stress

There is much evidence that stress alone can cause an inflammatory response. Nervous and immune systems interact bi-directionally. It is interesting to consider whether stress alone in Mg deficiency can cause an inflammatory syndrome or whether dysfunction of the nervous system also participates in the progression of inflammation. Mg deficiency results in a stressor effect and increases susceptibility to the physiological damage produced by stress [26, 27]. Neuromuscular hyperexcitability is a well known consequence of experimental and clinical Mg-deficiency. Mg supply has been shown to attenuate the development of adverse stress reactions. Stress activates the hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic nervous system [28]. The activation products are mainly cortisol and catecholamines. The innervations of the kidney may result in the production of renin which initiates reactions whereby renin and angiotensin converting enzymes convert angiotensinogen to angiotensin II,

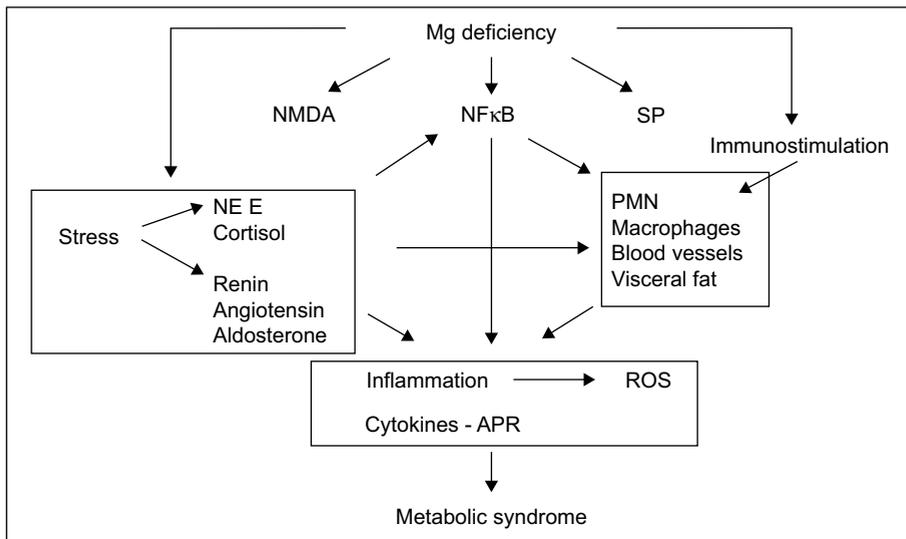


Figure 1. Interrelationship between magnesium deficiency, stress, inflammation and metabolic syndrome. APR: acute phase response; E: epinephrine; NE: norepinephrine; NFκB: nuclear factor κB; NMDA: N-methyl-D-aspartic acid; PMN: polymorphonuclear leukocytes; ROS: reactive oxygen species; SP: substance P.

a powerful vasoconstrictor that elevates the blood pressure and heart rate [29]. This response has indeed been documented in experimental animals following Mg deficiency [30]. Experimental Mg deficiency is accompanied by hyperaldosteronism [31]. There is growing evidence that enhanced activation of the renin-angiotensin-aldosterone system is a factor in the development of insulin resistance, by increasing oxidative stress. In both humans and rats, and in various diseases not directly related to Mg deficiency, aldosteronism results in an immunostimulatory state and leads to a proinflammatory phenotype [32]. Moreover, hyperaldosteronism has been associated with electrolyte disturbances, including hypomagnesaemia, in relation with TRPM7 downregulation [33]. Peripheral blood mononuclear activation is induced by a reduction in cytosolic free $[Mg^{2+}]$ and a subsequent Ca^{2+} loading that is translated into an oxidative/nitrosative stress. The latter activation is attenuated by Mg supplementation but also by preventing Ca loading by Ca channel blockers. Moreover, we have shown that the pro-inflammatory phenotype seen in experimental dietary Mg deficiency can be prevented when a Ca free diet is administered [34]. On the other hand, a stress response induces the release of large quantities of excitatory amino acids, such as aspartate and glutamate. Released glutamate can bind to different receptors, and N-methyl-D-aspartic acid (NMDA) activation also causes the mobilization of free cytosolic Ca. Mg deficiency in rats induces hyperalgesia involving NMDA receptors since MK801, a non-competitive NMDA receptor antagonist, prevents hyperalgesia in those animals [35]. Excess intracellular Ca concentration resulting from Mg deficiency activates Ca-dependent processes such as the release of cytokines during stress. At the molecular level, one of the earliest events in inflammatory stress is the activation of nuclear factor $\text{NF}\kappa\text{B}$ [29]. The association of stress with inflammation is strengthened by the results suggesting that substance P (SP) is also involved in the response to Mg deficiency [36, 37]. The rise in this neuropeptide, besides inducing a neurogenic inflammation, may activate cells including endothelial cells, mast cells, macrophages and circulating blood cells. Inhibition studies of SP receptor suggest that the neurogenic peptide contributes to the pathology of Mg deficiency.

Visceral fat

Visceral obesity represents another important risk factor associated with metabolic syndrome. Stress-induced HPA axis activation has been identified to

play an important role in this preferential body fat accumulation and to stimulate the proinflammatory cytokine secretion by adipocytes that hold a potentially important pathogenic role. A prominent feature of the inflammatory process in visceral fat is the presence of activated macrophages recruited from bone marrow. A number of activator molecules, such as cortisol, angiotensin II, leptin, and inhibitor ones, such as adiponectin, are able to promote the synthesis and secretion of adhesion molecules in endothelium cells [38]. Evidence that Mg is involved in body weight regulation is still controversial. Studies have reported either a negative or no relationship between magnesium and body mass index (BMI). However, a recent study indicates that men and women with the highest Mg intake were less likely to be overweight/obese than those with the lowest intakes – but because Mg is found in a whole range of foods, it may be the overall dietary pattern and not only Mg intake that contribute to a lower BMI or lower prevalence of abdominal adiposity [11].

Endothelium

One of the earliest events in the acute response to stress is endothelium dysfunction. Endothelial cells actively contribute to inflammation by elaborating cytokines, by synthesis of chemical mediators and by expressing adhesion molecules, which bind leukocytes. In addition, the endothelium cell is sensitive to cytokines. *In vitro*, low Mg stimulates the synthesis of IL-1 and IL-6, NO, a modulator of inflammatory responses, and of VCAM, which modulates monocyte/endothelial interactions. Mg may suppress inflammatory responses by human endothelial cells through the $\text{NF}\kappa\text{B}$ pathway [39-41].

Clinical symptoms and acute phase response

A characteristic allergy-like crisis with erythema, hyperemia and edema occurs spontaneously in Mg deficient rats. This response has been proposed as a model for the screening of anti-inflammatory substances [2, 3]. The greater spleen size is due to infiltration with phagocytic cells. An increased number of plasma PMN leukocytes is observed. One important observation during inflammation is the production levels of positive and negative acute phase proteins. The increased IL-6 concentrations are responsible for the synthesis of many acute phase proteins by the liver such as $\alpha 2$ macroglobulin, $\alpha 1$ acid glycoprotein, complement, fetoprotein, haptoglobulin and fibrinogen. The decrease of albumin,

apolipoprotein E, and retinol binding protein (RBP) concentrations is also the consequence of the acute phase response [2, 3].

Several studies have been performed to assess the activation of proinflammatory cells in Mg deficiency. Neutrophils and resident macrophages are more responsive to activation than those of controls, as shown by respiratory burst studies using chemiluminescence. Different gene expression analyses of stress proteins have confirmed the neutrophil activation. The majority of stress proteins were upregulated in neutrophils from Mg-deficient animals. Moreover, Mg-deficient rats are more sensitive to immune stress, as measured by TNF α response, following an endotoxin challenge. Increasing extracellular Mg concentration *in vivo* or *in vitro* decreased the inflammatory response as shown by chemiluminescence studies or cytokine production [2, 3]. Long-term Mg deficiency also results in inflammation and oxidative stress [42].

Mg deficiency induces metabolic syndrome in animal models

Stress and inflammation are involved in the induction and development of the metabolic syndrome. Several observations document the effect of Mg deficiency in the development of insulin resistance in the rat model. Of particular significance is the observation of the aggravating effect of Mg deficiency on metabolic syndrome in fructose-fed rats [2]. Increased Mg intake prevents hyperlipidemia and insulin resistance and reduces lipid peroxidation in that experimental model [43]. Mg deficiency contributes to other aspects of the metabolic syndrome: hyperlipidemia, elevated blood pressure, endothelial dysfunction and increased thrombosis tendency [2]. Inflammation occurring during experimental Mg deficiency is the mechanism that induces hypertriglyceridemia and the proatherogenic changes in lipid metabolism [3, 44, 45]. Several data indicate that hypertriglyceridemia, the effect of Mg deficiency, can be suppressed or reduced by decreasing the inflammatory response [34]. Metabolic syndrome also leads to enhanced blood clotting, likely resulting from endothelial dysfunction and dyslipidemia, which trigger platelet aggregation, thereby increasing the risk of thrombotic events [46, 47]. TRPM7 dysregulation, that may be a critical regulator of Mg homeostasis in vascular cells, is associated with endothelial dysfunction in mice selected for low intracellular Mg. Thus, low Mg intake or genetic hypomagnesemia may induce endothelial dysfunction

[48]. Epidemiological and experimental studies have demonstrated an inverse association between magnesium status and blood pressure [31]. Chronic dietary magnesium deficiency causes elevated blood pressure; initially a hypotension phase is observed, which is due to the release of inflammatory agents, the subsequent hypertension is a result of oxidative stress and structural modifications in the vascular system [31]. In fact, inactivation of nitric oxide contributes to hypertension during chronic Mg deficiency, by decreasing the relaxing property of nitric oxide [31].

Which mechanism is responsible?

A cellular Mg deficiency exaggerates Ca-induced cell stimulation and extracellular Mg acts as a non-specific antagonist of various Ca channels. Elevation of Ca^{2+} with a reciprocal decrease in Mg^{2+} is consistently seen in subjects with hypertension, obesity and NIDDM. According to Resnick [17], the clinical abnormalities associated with metabolic syndrome may be related to this ionic defect. The inflammatory response induces activation of several processes, which are dependent on cytosolic Ca^{2+} elevation.

Enhanced Ca^{2+} response of macrophages from Mg-deficient rats

Resident macrophages from Mg-deficient rats are activated and there is an enhanced Ca^{2+} response after *in vitro* stimulation. This occurs very early in the course of the deficiency [49].

Effect of Ca antagonists on neutrophil respiratory burst

Neutrophils from mice activated with PMA showed an increased respiratory burst when incubated in low Mg concentration (0.1 mM) as compared to normal Mg concentration (1 mM). We have investigated the effect of intracellular (TMB-8) and extracellular (verapamil) Ca channel blockers. These antagonists were more effective in decreasing oxidative burst when cells were incubated in low-Mg medium than in a medium with a normal concentration. Verapamil did not decrease the respiratory burst in normal medium. A decrease was observed when cells were treated with both verapamil and TMB-8. By contrast, both verapamil and TMB-8 reduced the oxidative burst of cells in low Mg medium. With the association verapamil plus TMB-8, the decrease was significantly more important than observed in normal medium. The effect of Ca antagonists supports the

hypothesis that Ca is the intracellular modulator involved in the inflammatory response induced by Mg-deficiency [50].

Inhibition of inflammatory responses by Ca intake modification *in vivo*

A recent study was performed *in vivo* to assess if an inflammatory effect of Mg is the consequence of a reduced extracellular Mg/Ca antagonism [34]. Adaptation to a low-Ca diet is impaired by Mg deficiency and hypocalcaemia is a manifestation of Mg deficiency when rats are fed a Ca-deficient diet. The inflammatory response (hyperemia, leukocytosis) is dramatically reduced in Mg deficient rats when there is a concomitant decrease in plasma Ca levels. On the other hand, recent observations suggesting that there is a relationship between parathyroid hormone, Ca intake and metabolic syndrome in human subjects is of particular interest [51].

Beneficial effect of Ca antagonists on mortality of Mg-deficient mice

It is known that Ca channel blockers can be used to treat patients with metabolic syndrome. The potential beneficial effect of Ca antagonists has been investigated in Mg-deficient mice [52]. About 65% of Mg-deficient mice died within 30 days, in contrast all the nifedipine-treated mice survived for 30 days. This experiment does not provide information on a potential anti-inflammatory effect of Ca antagonists in Mg deficiency but these findings suggest that a Ca antagonist may play a compensatory role for Mg as natural physiological blocker. In Mg-deficient rats, a decreased acute stress reaction has also been described after Ca antagonist treatment [52]. To conclude, a Mg effect on Ca²⁺ homeostasis may be a common link between stress, inflammation and metabolic syndrome.

Disclosure

None of the authors has any conflict of interest to disclose.

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