High fructose consumption combined with low dietary magnesium intake may increase the incidence of the metabolic syndrome by inducing inflammation*

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Abstract. The metabolic syndrome is a cluster of common pathologies: abdominal obesity linked to an excess of visceral fat, insulin resistance, dyslipidemia and hypertension. This syndrome is occurring at epidemic rates, with dramatic consequences for human health worldwide, and appears to have emerged largely from changes in our diet and reduced physical activity. An important but not well-appreciated dietary change has been the substantial increase in fructose intake, which appears to be an important causative factor in the metabolic syndrome. There is also experimental and clinical evidence that the amount of magnesium in the western diet is insufficient to meet individual needs and that magnesium deficiency may contribute to insulin resistance. In recent years, several studies have been published that implicate subclinical chronic inflammation as an important pathogenic factor in the development of metabolic syndrome. Pro-inflammatory molecules produced by adipose tissue have been implicated in the development of insulin resistance. The present review will discuss experimental evidence showing that the metabolic syndrome, high fructose intake and low magnesium diet may all be linked to the inflammatory response. In many ways, fructose-fed rats display the changes observed in the metabolic syndrome and recent studies indicate that high-fructose feeding is associated with NADPH oxidase and renin-angiotensin activation. The production of reactive oxygen species results in the initiation and development of insulin resistance, hyperlipemia and high blood pressure in this model. In this rat model, a few days of experimental magnesium deficiency produces a clinical inflammatory syndrome characterized by leukocyte and macrophage activation, release of inflammatory cytokines, appearance of the acute phase proteins and excessive production of free radicals. Because magnesium acts as a natural calcium antagonist, the molecular basis for the inflammatory response is probably 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. Since magnesium deficiency has a pro-inflammatory effect, the expected consequence would be an increased risk of developing insulin resistance when magnesium deficiency is combined with a high-fructose diet. Accordingly, magnesium deficiency combined with a high-fructose diet induces insulin resistance, hypertension, dyslipidemia, endothelial activation and prothrombic changes in combination with the upregulation of markers of inflammation and oxidative stress.

Key words: magnesium, fructose, metabolic syndrome, inflammation

The metabolic syndrome is a cluster of common pathologies, including abdominal obesity linked to an excess of visceral fat, insulin resistance, dyslipidemia and hypertension. This syndrome and type 2 diabetes are occurring at epidemic rates with frightful consequences for human health worldwide [1]. The increased prevalence of insulin resistance is linked to the western diet and reduced physical activity. In the past, diets high in saturated fats have been shown to induce insulin resistance and hyperlipidemia. However, recent studies suggest that a high intake of refined carbohydrates may also contribute to the risk of developing insulin resistance. In animal models, diets high in fructose have specifically been shown to contribute to a metabolic disturbance leading to insulin resistance. A significant change in the western diet is the substantial increase in dietary fructose consumption, which is due to a high intake of sucrose and high fructose corn syrup, a common sweetener used in the food industry [1]. Moreover, an increase in the consumption of empty calorie foods contributes to a decrease in the total mineral and vitamin density per unit of energy consumed and consequently, the marginal magnesium intake induces a high prevalence of marginal magnesium deficiency. A healthy magnesium intake supports efficient insulin function, whereas magnesium deficiency contributes to insulin resistance [2-6]. In industrialized countries, magnesium intake has been reduced, while fructose consumption has been rapidly increasing, and the aim of this review is to emphasize the consequences of this eating pattern, particularly in the development of the metabolic syndrome and to discuss the pathogenic role of inflammation.

Role of inflammation in the metabolic syndrome

In recent years, several studies have demonstrated that subclinical chronic inflammation is an important pathogenic factor in the development of insulin resistance and cardiovascular diseases [7, 8]. Markers for this inflammatory response include acute phase proteins, cytokines and mediators associated with endothelial activation. In obese individuals, the white adipose tissue contains an increased number of macrophages, when compared with lean individuals, and the macrophages appear to be activated. The adipocytes and macrophages produce leptin and other factors, which upregulate the number of adhesion molecules on endothelial cells. This leads to transmigration of monocytes, an increase in white

adipose tissue-resident macrophages and consequently, cytokines are released in large amounts from the adipose tissue [9]. According to this hypothesis, an excess energy intake leads to obesity and hyperglycemia, which can cause oxidative stress and inflammatory changes (nuclear factor-kappaB [NFkB] activation, increased levels of tumour necrosis factor [TNF] alpha and interleukin [IL] [6]). These inflammatory changes inhibit insulin signalling and can lead to insulin resistance. Moreover, the inflammatory state induces beta cells dysfunction, which in combination with insulin resistance leads to type 2 diabetes [10, 11].

High-fructose diet and metabolic syndrome

The fructose fed rat represents a model for the metabolic syndrome, including insulin resistance, hypertension and dyslipidemia, and growing evidence suggests a role for inflammation and oxidative stress in this model [12]. High fructose feeding of rats is associated with a rapid increase in reactive oxygen species (ROS) production by polymorphonuclear leukocytes (PMN) and tissues. This overproduction of ROS is dependent on NADPH oxidase activation and leads to an oxidative stress response as evidenced by elevated concentrations of plasma and urinary thiobarbituric acid reactive substances (TBARS). In rats, the fructose diet, when compared with a starch diet, lowers the plasma vitamin E/triglycerides (TG) ratio, which results in an increase in TG-rich lipoprotein susceptibility to lipid peroxidation, and hearts are less protected against *in vitro* peroxidation [13, 14]. Recent findings suggest that the pro-oxidant effect induced by a high fructose diet can be decreased by consumption of fermentable carbohydrates such as oligofructose [15]. The link between inflammation, oxidative stress and metabolic syndrome is supported by the fact that females, which are protected against the pro-oxidant effects of a high-fructose diet when compared with males, do not develop insulin resistance [16]. Diets rich in fructose can alter cellular metabolism via several pathways, thereby accelerating oxidative stress. Fructose feeding results in the activation of the renin-angiotensin system and it is well-established that angiotensin 2 production is associated with oxidative stress. This oxidative stress is characterized by overproduction of ROS and is dependent on the activation of NADPH oxidase [17]. Moreover, increased IL1 and IL6 plasma levels have been documented in rats fed a high-fructose diet [18]. Finally, in addition to the possible roles of uric acid and kidney damage

[19], the inflammatory response and oxidative stress may be key events in the development of insulin resistance in rats fed a high fructose diet. These events are likely to contribute to other aspects of the metabolic syndrome. The hypertriglyceridemic effect of fructose can be reduced by lipooxygenase inhibitors, which decrease the inflammatory response [20], and inflammation and oxidative stress are important factors in cardiovascular effects.

Inflammatory effects of magnesium deficiency

A characteristic allergy-like crisis occurs spontaneously in magnesium deficient rats. They present hyperemia and oedema of the ears and legs, and a greater spleen size due to phagocytic cell infiltration [5, 6, 21]. An inflammatory response has also been observed in other magnesium-deficient rodents. However, it was shown that in mice the response depends on the experimental conditions [22-24]. The inflammatory syndrome is accompanied by hyperalgesia, which can be prevented by an N-methyl-D-aspartate (NMDA) receptor antagonist [25]. In blood, the most prominent change is leukocytosis, which results from the increased number of PMN leukocytes, mainly neutrophils and eosinophils. An increase in the number of eosinophils is considered a typical allergic response. An important phenomenon during inflammation is the production of cytokines. Early studies reported that, in rats, experimental magnesium deficiency led to increased plasma levels of orosomucoid [26]. In the same experimental model, several positive acute phase protein plasma concentrations, and/or their liver mRNA levels, have been shown to be induced, including alpha2-macroglobulin, alpha1-acid glycoprotein, complement, fetoprotein, haptoglobulin and fibrinogen. These changes are related to increased IL6 concentrations, which stimulate the synthesis of many acute phase proteins by the liver. The decrease of several negative acute phase proteins, such as albumin, apolipoprotein (apo) E, retinol-binding protein (RBP), is a classical finding of an acute phase response. This inflammatory response and its consequences are only observed in male rats. Female animals are partially protected, suggesting that estrogens may be protective against the proinflammatory effects of magnesium deficiency [27]. It is of particular interest that females, when compared with males, do not develop the metabolic syndrome on the fructose diet. Several studies have been performed to assess the activation of inflammatory cells in magnesium deficiency [28-30]. Free radical generation from PMN

was measured *in vitro* using chemiluminescence, and via NADPH oxidase activation, both macrophages and neutrophils generated superoxide anions in response to various stimuli. There was a low basal neutrophil activity in control rats, but the basal neutrophil activity of magnesium deficient rats was significantly higher. Neutrophils from control and magnesium-deficient rats were responsive to activation by phorbol 12-myristate 13-acetate (PMA) and zymosan, and the response was higher for PMN magnesium-deficient rats. Differential gene expression analysis of stress proteins has confirmed the neutrophil activation [30] and in fact, the majority of stress proteins were upregulated in neutrophils from magnesium-deficient animals. Resident macrophages of magnesium-deficient rats present the morphological aspects of an activated cell and their chemiluminescence activity is elevated. *In vitro*, these cells are more sensitive to PMA stimulation. Magnesium-deficient rats are more sensitive to immune stress as measured by TNF response following an endotoxin challenge [31]. The specific mechanisms of the inflammatory response in magnesium deficiency have not been elucidated. However, magnesium acts as a natural calcium antagonist and the molecular basis for the inflammatory response is probably linked to the modulation of the intracellular calcium concentration [32]. Potential mechanisms include the priming of phagocytic cells, the opening calcium channels and activation of NMDA receptors [33], the release of neurotransmitters such as substance P [34, 35], the activation of NFkB [36] and the activation of the renin angiotensin systems [37].

High-fructose diet combined with magnesium deficiency

Inflammation is a key event in the initiation and development of metabolic syndrome. A high-fructose diet induces both inflammation and the metabolic syndrome in rats. If magnesium deficiency has a proinflammatory effect, one likely consequence of combining magnesium deficiency with a high-fructose diet is an amplified effect, which has been shown by recent data from rats. The fructose diet, as compared to a starch diet, induces hyperglycemia and hyperinsulinemia. Magnesium deficiency induces hyperglycemia and hyperinsulinemia when compared to a control diet. The combined effects are higher, using the high fructose low magnesium diet, and the binding of insulin to the red blood cell (RBC) insulin receptors is reduced [38]. Thus, this study clearly documents the effects of magnesium defi-

ciency in the development of insulin resistance in the rat model. We have shown that fructose feeding in magnesium deficient rats is associated with an increase in oxidative stress when compared with magnesium deficient rats fed a starch diet [39]. This observation is consistent with the hypothesis that inflammation and increased oxidative stress, induced by magnesium deficiency, contribute to the development of insulin resistance. In the same model, the proinflammatory effect of magnesium deficiency contributes to other aspects of the metabolic syndrome: hyperlipemia, elevated blood pressure, endothelial dysfunction and increased thrombosis tendency [5, 6]. Fructose has been shown to increase the hyperlipemic effect of magnesium deficiency, when compared to starch [40]. As previously discussed, magnesium deficiency is accompanied by alterations in lipid metabolism including accumulation of TG-rich lipoproteins (TGRLP), a decrease in high density lipoprotein (HDL) levels, an increase in apoB, a decrease in apoA1 and apoE, modifications in the composition of lipoproteins, and a defect in the clearance of TGRLP [41, 42]. Several factors contribute to these alterations including decreases in the activities of lipoprotein lipase, hepatic lipase [43, 44] and lecithin-cholesterol acyl transferase (LCAT) [45]. Together, these changes could contribute to decreased reverse cholesterol transport. TGRLP and tissues from magnesium-deficient rats are more susceptible to *ex vivo* oxidation than those from controls [46-48]. Thus, oxidative modification of lipoproteins could play a significant role in the pathogenesis of vascular lesions following magnesium deficiency and furthermore, magnesium affects the inflammatory-dependent events leading to atherosclerosis. The lipid metabolism changes observed in experimental magnesium deficiency have been observed in other models in inflammation [49]. On the basis of these studies, it is possible to conclude that the proatherogenic lipoprotein changes in magnesium deficient rats are the consequence of the inflammatory response. Epidemiological and experimental studies have demonstrated an inverse association between magnesium status and blood pressure [50, 51]. 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 [52, 53]. In fact, free radicals may partly inactivate NO and an increased degradation of NO by superoxide anions could contribute to hypertension during chronic magnesium deficiency. *In vitro* studies have shown that low

magnesium levels results in endothelial dysfunction [54, 55]. Serum from magnesium deficient rats stimulates the proliferation of endothelial cells, increases the adhesion of monocytes to these cells, and the endothelial cells upregulate the plasminogen activator inhibitor (PAI) factor. The presence of endothelial dysfunction and dyslipidemia triggers platelet aggregability [56]. Platelet aggregation is increased in magnesium deficiency, while magnesium supplementation inhibits platelet dependent thrombosis [44, 57]. We have investigated the thrombotic tendency in rats fed a magnesium-deficient, high fructose diet. Following epinephrine, the magnesium-deficient rats exhibited lesions in left atrium which was dilated and haemorrhagic [44]. Thus, magnesium deficiency induces predisposition to epinephrine-initiated thrombosis and inflammatory pathway promotes thrombosis which is responsible for myocardial infarction and stroke [28, 44].

Conclusion

Magnesium deficiency combined with a high-fructose diet induces insulin resistance, hypertension, dyslipidemia, endothelial activation and pro-thrombotic changes. In addition to well-known factors such as overnutrition and physical inactivity, a diet rich in fructose and deficient in magnesium results in an inflammatory response, which may represent a triggering factor in the development of the metabolic syndrome. Our data are in good agreement with clinical data [58-66] showing that low serum and dietary magnesium levels are strongly correlated with low grade systemic inflammation and metabolic syndrome [58-66]. Large epidemiological studies indicate that lower serum magnesium levels are associated with insulin resistance. Magnesium intake and systemic inflammation and the prevalence of metabolic syndrome were inversely correlated in subjects participating in the Women Health Study [62]. Furthermore, low magnesium serum levels are associated with elevated serum concentrations of both TNF-alpha [64] and C-reactive protein (CRP) [62, 63], suggesting that magnesium deficiency may also be involved in the development of the low-grade chronic inflammatory syndrome which can induce metabolic disorders. Other studies have shown the correlation between low-serum magnesium levels and TNF-alpha in obese and non-alcoholic steatohepatitis (NASH) subjects [65]. Moreover, a longitudinal association of magnesium intake with the incidence of metabolic syndrome has recently been examined and the data suggests that

young adults with higher magnesium intake have a lower risk of developing the metabolic syndrome [66]. A typical western diet is high in fructose and often magnesium deficient. Considering the well-documented detrimental effect of this eating pattern, there is urgent need to explore the details of the link between diet and the inflammatory signalling pathways.

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