Magnesium deficiency increases oxidative stress in rats

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Magnesium deficiency has been implicated in the development of atherosclerosis and late diabetic complications, diseases often associated with increased oxidative stress. Present study was carried out to examine the effect of magnesium deficiency on oxidative stress and total radical trapping antioxidant parameter (calculated) in rats and correlate it with the development of free radical mediated diseases. Male Wistar rats were divided into two groups and pair fed for six weeks with low magnesium diet (70 mg/kg) and control diet (990 mg/kg) prepared synthetically. Deionized water was given ad libitum. Low magnesium diet caused a significant decrease in plasma and red blood cell magnesium levels. A marked increase in plasma malondialdehyde and corresponding decrease in total radical trapping antioxidant parameters (calculated) were observed in the low magnesium diet group than control group. The level of plasma glucose increased moderately in the low magnesium diet group. Hypertriglyceridemia and significantly decreased plasma HDL (high density lipoprotein)-cholesterol levels were observed in the low magnesium diet group. The results clearly demonstrate that magnesium deficiency is associated with increased oxidative stress through reduction in plasma antioxidants and increased lipid peroxidation suggesting that the increased oxidative stress may be due to increased susceptibility of body organs to free radical injury.

Magnesium is a key intracellular cation and a critical cofactor for more than 320 enzymatic reactions many of which involve energy metabolism. All the enzymatic reactions that hydrolyze and transfer phosphate group including those associated with reactions involving adenosine triphosphate (ATP) show an absolute requirement for magnesium. Since ATP is essential for the utilization of carbohydrate, fat and protein, synthesis of nucleic acids and coenzymes, muscle contraction, signal transduction, protecting biological membranes and transport of K+ and Ca2+ ions, alterations in magnesium homeostasis can potentially have profound effect on multiple cellular functions.

Primary magnesium deficit occurs from two aetiological mechanisms: deficiency and depletion. Primary magnesium deficiency is due to insufficient magnesium intake and primary magnesium depletion is due to dysregulation of factors controlling magnesium status such as intestinal hypo-absorption, reduced bone uptake and mobilization and urinary loss.

Physicians are now recognizing magnesium deficiency frequently due to increased clinical awareness and greater frequency of assessment of magnesium status. In recent years there has been a growing interest in magnesium and its correlation with the development of various age related diseases viz: hypertension, diabetes mellitus, cardiovascular diseases, atherosclerosis, myocardial damage and cardiac arrhythmias through free radical mediated oxidation of cellular components. Dickens et al. have proposed that during magnesium deficiency, natural antioxidant defenses present in mammalian tissues against oxidative stress may be compromised. Free radical mediated oxidation of cellular components is a well established mechanism of cellular injury in many of the above-mentioned age related diseases as already demonstrated by recent studies.

The present study has therefore been designed to elucidate the effect of magnesium deficiency on antioxidant potential and oxidative stress in rats. The effect of magnesium deficiency on plasma glucose and lipid profile was also included in the study to assess the role of magnesium deficiency in the development of diabetes and cardiovascular diseases. Attempts have also been made to correlate magnesium deficiency and total radical trapping antioxidant parameter (TRAPc), which has been proposed to explore the antioxidant property of plasma sample taking into account the plasma levels of four natural antioxidants: thiol groups (-SH), uric acid, vitamin E and vitamin C.

Chemicals—Methyl thymol blue (MTB), poly vinyl pyrrolidone (PVP), ethylene glycol tetra acetic acid (EGTA), α-tocopherol and 2,4,6-tripyridyl-S-triazine

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(TPTZ) were from Sigma Chemical Company, St. Louis, Mo, USA and were kindly provided by Prof. Ronald R. MacGregor, Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas, USA. 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB), thio-barbituric acid (TBA) and reduced glutathione were procured from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. All other chemicals used were of analytical grade and obtained locally.

**Animals and diets**—Male Wistar rats (16) weighing approximately 100 g were obtained from Central Animal House, Panjab University, Chandigarh. The animals were kept in polypropylene cages under controlled environment of temperature, relative humidity and light: dark cycle and were given commercial standard pellet diet and water *ad libitum*. After acclimatization for one week, the rats were randomly divided into two groups of 8 rats each: control group and low magnesium diet group, and pair fed with the appropriate synthetic diet for 6 weeks. The synthetic diet contained (g/kg) casein 200, sucrose 700, corn oil 50, mineral mixture 35 and vitamin mixture 10 as described by Rayssiguier *et al*.

The low magnesium diet was of the same composition as the control diet except for the magnesium content (70 mg/kg in low magnesium diet and 990 mg/kg in the control diet as determined by analysis). The rats were allowed free access to synthetic diet and deionized water.

**Biochemical analysis**—Six weeks after feeding the appropriate diet, rats were fasted overnight and blood was drawn from the orbital sinus under light ether anesthesia and collected into heparinised tubes. Blood was immediately centrifuged at 2000 g for 15 min at 4°C. The levels of plasma glucose (Accurex Biomedical Pvt Ltd, Thane, India), uric acid (Tranasia Bio-Medicals Ltd, Mumbai), triglycerides (Boehringer Mannheim), total cholesterol and HDL-cholesterol (Boehringer Mannheim) were measured by enzymatic assays by using standard commercial kits. The plasma magnesium concentration was estimated spectrophotometrically by dye method using methyl thymol blue. For the estimation of RBC magnesium and GSH (glutathione), packed red cells were washed with normal saline thrice in cold centrifuge and finally packed. For magnesium estimation, a portion was digested with digestion mixture (HNO₃: HClO₄: 3: 1) and dried to ash. After appropriate dilution, magnesium was analyzed as previously explained. RBC GSH was analyzed by method of Beutler *et al*. Plasma MDA (malondialdehyde), vitamin C, vitamin E and thiol group (-SH) were analyzed by the methods of Beu ge and Aust, Roe and Kuether, Martinek and Koster *et al* respectively. TRAPc was calculated according to the formula [2.0 (vitamin E) + 1.7 (uric acid) + 1.3 (vitamin C) + 0.66( SH)] as proposed by Wayner *et al*.

**Statistical analysis**—All values are expressed as mean with their standard errors. The significance of the difference between the means of two groups of values was determined by Student’s *t*-test. A *P* value < 0.05 was considered statistically significant.

**Results and Discussion**

Results are presented in Table 1.

Within first week of feeding the diet, classical signs of magnesium deficiency (including hyperemia of the ears, growth retardation, hair loss and edema of paws) were observed in the low magnesium diet group.

Low magnesium diet had marked effect on weight gain, plasma and RBC magnesium levels, plasma MDA levels, RBC GSH levels, TRAPc and lipid profile. However, plasma glucose levels were altered moderately.

Weight gain in the low magnesium diet group after 6 weeks of feeding the diet was significantly less (*P* < 0.005) as compared to control rats. Rats fed with low magnesium diet gained only 46% weight in comparison to control rats that gained 92% of their original weight.

A significant impairment in the ability of rats to gain weight on low magnesium diet occurred in the present study can be attributed to the fact that magnesium has an indispensable role in normal growth. Reduced protein synthesis and protein utilization, which are common findings in magnesium deficiency may also be responsible for growth retardation.

These data clearly demonstrate that magnesium deficiency is associated with increased lipid peroxidation and decreased antioxidant potential in plasma. The increase in oxidative products provides indirect evidence that endogenous antioxidants may be compromised during magnesium deficiency. This finding may result from either oxidative loss of endogenous antioxidants during magnesium deficiency or may indicate that antioxidants are insufficient to cope up with increased oxidative stress during magnesium deficiency. After correction for changes in the triglyceride and cholesterol levels the MDA levels were still significantly higher in magnesium deficient rats suggesting that the levels of TBARs depend not only on...
the availability of lipids, the substrate for lipid peroxidation and other factors may also be responsible for increased oxidative stress. Magnesium deficiency has been implicated in causing increased oxidative stress that renders the cells more susceptible to oxidative damage. Further, magnesium itself has been reported to have antioxidant potential, scavenging oxygen radicals, possibly by affecting the rate of spontaneous dismutation of superoxide ions and also as an essential requirement for the synthesis of some important natural antioxidants.

Lipid peroxidation and derived oxidized products are being intensively investigated because of their potential to cause injury and their pathogenic role in several clinically significant diseases. The decrease in vitamin C may be an indication of magnesium requirement in the biosynthesis of vitamin C in vivo. Ascorbate is known to regenerate reduced vitamin E from oxidized vitamin E and loss of ascorbate during magnesium deficiency suggests a possible subsequent loss of reduced form of vitamin E. The plasma vitamin E levels remained significantly lower even after correcting for triglyceride and cholesterol. Decrease in RBC GSH levels suggests that magnesium is essential in the maintenance of GSH to protect against oxidative damage. Minnich et al. have reported that magnesium is an essential cofactor for the enzymatic synthesis of glutathione in the red blood cell cytosol. Therefore, it may be suggested that this ion is directly responsible for the decreased glutathione content. Alternatively, it may also be suggested that decrease in RBC GSH could be due to its increased consumption because of increased free radical activity.

TRAPc has been proposed by Ceriello et al. to explore the antioxidant potential of plasma taking into account the four main natural antioxidants present in the plasma (thiol group, uric acid, vitamins E and C). The present results clearly show that TRAPc is significantly decreased in the magnesium deficient rats suggesting the existence of oxidative stress in magnesium deficiency.

With regard to glucose metabolism, although it will be premature to conclude that magnesium deficiency is a risk factor for the development of diabetes, the elevated levels of glucose in magnesium deficient rats suggest that diabetics with hypomagnesemia are at increased risk of late diabetic complications. Contradictions exist with regard to primary role involvement of magnesium in glucose homeostasis. Though some studies have reported hyperglycemia in the magnesium deficient rats, improvement in the glucose homeostasis has also been observed by some researchers. Acute magnesium depletion leads to insulin resistance and hyperglycemia, while chronic cellular magnesium depletion leads to accelerated glucose uptake in muscles. Elevated free radical
activity has also been reported to induce a defect in insulin-mediated glucose uptake. Glutathione has also been reported to improve glucose metabolism by enhancing glucose-induced insulin secretion in aged patients with impaired glucose tolerance and increasing insulin action in non-insulin dependent diabetic patients. Thus it may be concluded that magnesium is one of the potential factors mediating the antioxidant-sensitizing effects of GSH.

The present study has also demonstrated that magnesium deficiency caused elevation of triglycerides and decreased levels of HDL-cholesterol indicating abnormalities in lipoprotein metabolism. The hypertriglyceridemia may be due to reduced clearance of triglycerides from the blood. The present findings are in agreement with other studies, which reported slight variation in total cholesterol in severe magnesium deficiency of short duration and significant increase in total cholesterol during moderate magnesium deficiency for long durations. These observations combined with present knowledge that dyslipidemia is one of the major factors for the development of cardiovascular disease suggest that magnesium deficiency may be responsible for the development of cardiovascular disease. The mechanism responsible for the atherogenicity of the hypertriglyceridemic state may be related to increased susceptibility of triglyceride-rich lipoprotein against oxidative modification. Epidemiological surveys have also concluded that populations living in areas having hard water or taking magnesium rich diet are less prone to cardiovascular diseases. It has also been suggested that magnesium is beneficial in the treatment of myocardial infarction because it dilates the coronary arteries, which results in improved delivery of oxygen to the heart. Deficiency of magnesium may also increase the risk of heart disease by weakening the heart’s defense against free radical damage.

In conclusion, the experimental data presented here suggests that magnesium deficiency may be represented as an independent risk factor for increased oxidative stress and decreased antioxidant potential. The increased oxidative stress has been implicated in many free radical mediated diseases including hypertension, abnormal glucose metabolism and accelerated atherosclerosis through oxidative modification of lipoproteins and other biochemical abnormalities. Therefore it is postulated that magnesium deficiency leads to reduction of threshold antioxidant capacity and enhanced susceptibility to free radicals, which may eventually culminate in above said disorders.

References
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