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ORIGINAL CONTRIBUTION

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Implications of oxidative stress in high sucrose low magnesium diet fed rats

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Abstract *Background* Magnesium deficiency as well as excess sucrose in the diet have been shown to be associated with the generation of reactive oxygen species (ROS). *Aim of the study* In the present study we have investigated the combined effect of low magnesium high sucrose diet on the development of oxidative stress in rats. *Methods* Male Wistar rats were divided into four groups and fed control (C), low magnesium (LM), high sucrose (HS) and low magnesium high sucrose (HSLM) diet for a period of 3 months. Levels of various antioxidants, viz. ascorbic acid, vitamin E, uric acid, glutathione and non-protein thiols were determined along with malondialdehyde levels (lipid peroxidation marker). Anti-oxidant enzyme activities were determined in livers of experimental diet fed animals. *Results* Compared to controls, significantly increased lipid peroxidation was observed in plasma and liver tissue of animals in the three experimental groups, however, the combined HSLM group showed greater lipid peroxidation. Levels of various antioxidants fell significantly in plasma and tissue of LM, HS and HSLM rats. Total thiols as well as liver non-protein thiols followed a similar trend with the greatest drop in anti-oxidant potential seen in the HSLM rats. The activities of the anti-oxidant enzymes viz. SOD, GST and catalase also declined considerably in test animals w.r.t controls, with the HSLM group showing the lowest activities. *Conclusions* These findings suggest that a diet low in magnesium and high in sucrose causes oxidative stress in rats, as reflected by increased lipid peroxidation and reduced anti-oxidant potential.

Key words sucrose – low magnesium – oxidative stress – lipid peroxidation – anti- oxidant enzymes

Introduction

An overload of reactive oxygen species (ROS) that exceeds the capacity of the endogenous anti-oxidant system induces oxidative stress in cells. Reactive oxygen species are important mediators of cellular injury via damage to membranes or alterations of enzyme activity. The poly-unsaturated fatty acids of membranes and of lipoprotein particles are particularly susceptible to free radical attack, ultimately forming lipid hydroperoxides, lipid peroxides, hydrocarbons and aldehydes as their stable degrada-

tive products; which are implicated in many pathologies such as atherosclerosis, ageing, cancer, diabetes etc. [44]. Several dietary factors may contribute towards the generation of ROS. In recent years there has been a growing interest in magnesium as well as sucrose content in the diet and there is a large volume of literature suggesting that manipulation of these two dietary components individually contributes towards generation of ROS. Rayssiguier and coworkers [29] reviewed the evidence that magnesium deficient animal tissues show increased susceptibility to lipid peroxidation, which is alleviated by co-incubation with antioxidants, suggesting that free radicals are involved. It has been proposed that natural antioxidant defenses present in mammalian tissues against oxidative stress may be compromised during magnesium deficiency [12]. Studies carried out in our own laboratory [19] have reported a reduction in some anti-oxidants plasma of rats consuming low magnesium diet. Therefore, the possibility exists that dietary magnesium deficiency results in the depletion of anti-oxidant capacity thereby predisposing to oxidative stress.

High sucrose intake is considered to be another important factor contributing towards oxidative stress. Busserolles and co-workers [9] have demonstrated that short-term consumption of sucrose rich diet has a pro-oxidant effect in rats. Similar findings were reported by Faure et al. [15], who reported that fructose component of sucrose diet has a deleterious effect on the anti-oxidant defense system, which was supported by reduced anti-oxidant defenses [30], and increased antioxidant production [31] after sucrose feeding. However, the possibility exists that sucrose feeding facilitated oxidative damage [27] contributes to the pathogenesis of disorders associated with high sucrose intake.

Since both high sucrose feeding and low dietary magnesium have been independently associated with increased oxidative stress in various studies, the present study was designed to elucidate the combined effect of a high sucrose and low magnesium diet on anti-oxidant potential and thus on the development of oxidative stress in male rats.

Materials and methods

Chemicals

Methyl thymol blue (MTB), poly vinyl pyrollidine (PVP), ethylene glycol tetra acetic acid (EGTA), α-tocopherol and 2,4,6-tripyridyl-s-triazine (TPTZ) were from Sigma Chemical Company, St. Louis, Mo. USA and were kindly provided by Prof. Ronal R. MacGregor, Department of Anatomy and Cell Biology, University of Kansas Medical Centre, Kansas City, Kansas, USA. All other chemical used were of analytical reagent grade.

Animals and diet

Male Wistar rats each weighing approximately 130 g were procured from Central Animal House, Panjab University, Chandigarh. The animals were kept in polypropylene cages under controlled conditions of temperature and light. The institution's guidelines for the care and use of laboratory animals were followed. Rats were randomly divided into four groups of six animals each and fed the respective diets for a period of 3 months. Group C animals were fed a control diet, LM group was fed a low magnesium diet, HS group was fed a sucrose rich diet and HSLM group rats were kept on a low magnesium high sucrose diet. Experimental diets were prepared in laboratory on a weekly basis and diet compositions are shown in Table 1. Rats were given feed in form of loose pellets in small metal dishes just before the beginning of dark cycle. Any spillage was collected in the morning and its weight equivalent was added to the following day's feed. Diets were freshly made every 3–4 days and stored at 4°C. The rats were allowed free assess to water.

Sample preparation

Rats were fasted overnight and blood samples were drawn every month from the orbital sinus of the eye under ether anesthesia. Plasma was collected immediately and RBCs were processed for hemolysate preparation. For the estimation of RBC magnesium red cells were washed thrice with normal saline in cold centrifuge and finally packed. An aliquot of RBC was digested using digestion mixture (HNO_3:$HClO_4$; 3:1) and dried to ash. Magnesium was estimated after appropriate digestion. At the end of 3 months of feeding experimental diets, the animals were killed by exsanguinations from heart under diethyl ether anesthesia. Liver was removed immediately and washed thrice with 0.9% NaCl followed by 50 mM Tris–0.1 mM EDTA buffer. Tissue was blotted, weighed, minced and homogenized with ice-cold 50 mM Tris–0.1 mM EDTA buffer (10 ml/g of tissue), using a motor driven glass Teflon homogenizer. The homogenate was centrifuged at 1000*g* at 4°C for 10 min to obtain nuclear pellet and supernatant followed by re-centrifugation at 10000*g* for 15 min. The pellet thus obtained was suspended in Tris–EDTA buffer and was used to estimate MDA in mitochondrial fraction. The supernatant was used for enzyme activity assays (SOD, GST and catalase).

Table 1 Composition of the experimental diets

Ingredients (g/kg diet)	Control	High sucrose (HS)	Low magnesium (LM)	High sucrose low magnesium (HSLM)
Starch	650	–	650	–
Sucrose	–	650	–	650
Casein	200	200	200	200
Corn oil	50	50	50	50
Cellulose	50	50	50	50
Salt mixture[a,b]	35	35	35	35
Vitamin mixture[c]	10	10	10	10
DL-methionine	3	3	3	3
Choline chloride	2	2	2	2

By calories, the diets were 68% carbohydrate, 19.8% protein and 11.5% fat

[a] Salt mixture expressed in g/kg: $CaHPO_4$, 60 g; KCl, 200 g; NaCl, 120 g; MgO, 21.0 g; $MgSO_4 \cdot 2H_2O$, 100 g; Fe_2O_3, 6 g; $FeSO_4 \cdot 7H_2O$, 10 g; trace elements 10 g/kg including Mn, 0.8 g; CuO, 125 g; Co, 0.0009 g; Zn, 0.450 g; I, 0.0049 g

[b] A similar composition of salt mixture was used in all the experimental groups, except for the addition of MgO and $MgSO_4 \cdot 2H_2O$ to provide (per kg) 507.0 mg of Mg in the control and high sucrose diets and 90.0 mg of Mg in the low magnesium and the high sucrose low magnesium diets

[c] Expressed per kg of the vitamin mixture: retinol, 539 g; cholecalciferol, 6.250 mg; thiamine, 2000 mg; riboflavin, 1500 mg; niacin, 7000 mg; pyridoxine, 1000 mg; cyanocobalamine, 5 mg; ascorbic acid; 80.000 mg; D,L-α-tocophenyl acetate, 17,000 mg; menadione, 1000 mg/kg; nicotinic acid, 10,000 mg; folic acid, 500 mg; *para*-amino benzoic acid, 5000 mg; biotin, 30 mg/kg

Biochemical analysis

The lipid peroxidation was estimated by the method of Buege and Aust [7]. The levels of plasma ascorbic acid, Vitamin E and total thiols were measured by the methods of Roe and Kuther [34], Martinek [26] and Koster et al. [23], respectively. RBC GSH was measured by the method of Beutlar et al. [2]. The activities of superoxide dismutase (SOD), glutathione-*S*-transferase (GST) and catalase were measured by the method of Kono [22], Habig et al. [17] and Luck [25], respectively. Plasma uric acid was assayed by enzymatic uricase method [39]. Plasma as well RBC magnesium was estimated colorimetrically by the modified method of Thuvasethakul and Wajjwalku [41].

Statistical analysis

Statistical analysis was performed using GraphPad InStat (GraphPad Inc., San Diego, CA, USA) software package. Results were expressed as mean and SD of six observations in each group. Further, the statistical significance of the differences among the various dietary groups was determined by subjecting the data to one way ANOVA with diet as the main effect, followed by inspection of all differences between pairs of means by Tukey's test. Differences were considered statistically significant at $P < 0.05$.

Results

The data in Table 2 represents the values of body weight, lipid peroxidation, plasma ascorbic acid, vitamin E, uric acid, total thiols, RBC GSH, non-protein thiols and plasma as well as RBC magnesium levels at the end of 1 month of feeding experimental diet, whereas Tables 3 and 4 depict the values of these parameters at the end of 2 and 3 months of feeding, respectively. Animals in LM and HSLM groups showed lesser weight gain compared to control rats. As is evident from these tables, the level of peroxidation marker (TBARS) in LM, HS and HSLM groups started increasing from the first month of feeding experimental diet and this effect was maximally observed in HSLM fed rats. At the end of the study period, plasma MDA levels were significantly increased in all three experimental groups as compared to controls as seen in Fig. 1. However, the maximum elevation was observed in HSLM group rats. Plasma ascorbic acid and vitamin E values of control animals remained almost constant throughout the experimental period. However, animals in LM, HS and HSLM groups showed a continuous decline in these parameters with time. Ascorbic acid levels reduced drastically (64.86%) in HSLM group animals by the end of the study period, whereas vitamin E decreased by 46.67% with respect to their original values. An almost similar trend was seen in case of uric acid. Animals in LM, HS and HSLM groups showed a reduction in total thiols by first month of feeding but the magnitude of the decrease was more pronounced in the rats fed the combined high sucrose low magnesium diet. Significant reduction was observed in RBC glutathione levels of animals in LM, HS and HSLM groups with the maximum decline seen in the HSLM rats. Plasma as well as RBC magnesium was decreased significantly in group LM and HSLM diet fed rats, though

Table 2 Body weights, levels of plasma ascorbic acid, vitamin E, uric acid, total thiols and magnesium at the end of 1 month of feeding

Parameters	C	LM	HS	HSLM
Body weight	180.34 ± 4.08	148.67 ± 2.87***	180.83 ± 2.25†††	185.34 ± 3.26†††
Ascorbic acid (μmol/l)	47.49 ± 1.17	27.34 ± 3.65***	31.47 ± 4.40***	29.11 ± 2.63***
Vitamin E (μmol/l)	19.98 ± 0.99	15.46 ± 1.22***	16.11 ± 1.57***	15.46 ± 1.22***
Uric acid (μmol/l)	104.66 ± 2.18	80.72 ± 2.75***	91.10 ± 2.99***,†	76.04 ± 1.58***,†,‡‡
Total thiols (μmol/l)	332.67 ± 7.65	272.66 ± 6.15***	288.34 ± 6.62***,††	263.66 ± 3.88***,‡‡
RBC GSH (μmol/l)	10.62 ± 0.20	8.875 ± 0.18***	9.75 ± 0.26***,†	8.46 ± 0.16***,†,‡‡
Plasma magnesium (mmol/l)	0.934 ± 0.08	0.54 ± 0.08***	0.984 ± 0.05†††	0.602 ± 0.08***,‡‡‡
RBC magnesium (mmol/l)	1.70 ± 0.05	1.33 ± 0.05***	1.70 ± 0.05†††	1.35 ± 0.05***,‡‡‡

Mean values with their standard deviations, $n = 6$. LM, low magnesium; HS, high sucrose; HSLM, high sucrose low magnesium
* Mean values were significantly different from control group, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$
† Mean values were significantly different from LM group, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.005$
‡ Mean values were significantly different from HS group, $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$, $^{\ddagger\ddagger\ddagger}P < 0.005$

Table 3 Body weights, levels of plasma ascorbic acid, vitamin E, uric acid, total thiols and magnesium at the end of 2 months of feeding

Parameters	C	LM	HS	HSLM
Body weight	244.16 ± 5.84	185.34 ± 4.54***	239.16 ± 3.79***,†††	220.16 ± 3.54***,†††,‡‡
Ascorbic acid (μmol/l)	46.50 ± 2.05	24.39 ± 3.51***	26.18 ± 3.69***	22.60 ± 1.83***
Vitamin E (μmol/l)	20.62 ± 0.99	14.50 ± 1.06***	13.53 ± 2.11***	14.82 ± 1.00***
Uric acid (μmol/l)	104.71 ± 2.86	71.95 ± 2.80***	82.33 ± 3.08***,†	67.30 ± 2.21***,†,‡
Total thiols (μmol/l)	332.34 ± 6.50	214.00 ± 7.89***	252.33 ± 7.08***,††	201.33 ± 12.37***,‡‡
RBC GSH (μmol/l)	10.69 ± 0.13	8.52 ± 0.2***	9.04 ± 0.30***	8.18 ± 0.09***,†,‡‡
Plasma magnesium (mmol/l)	0.836 ± 0.04	0.484 ± 0.01***	0.848 ± 0.03†††	0.545 ± 0.06***,‡‡‡
RBC magnesium (mmol/l)	1.70 ± 0.05	1.25 ± 0.04***	1.70 ± 0.05†††	1.358 ± 0.05***,‡‡‡

Mean values with their standard deviations, $n = 6$. LM, low magnesium; HS, high sucrose; HSLM, high sucrose low magnesium
* Mean values were significantly different from control group, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$
† Mean values were significantly different from LM group, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.005$
‡ Mean values were significantly different from HS group, $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$, $^{\ddagger\ddagger\ddagger}P < 0.005$

Table 4 Body weights, levels of plasma ascorbic acid, vitamin E, uric acid, total thiols and magnesium at the end of 3 months of feeding

Parameters	C	LM	HS	HSLM
Body weight	285 ± 4.47	198 ± 5.09***	283.34 ± 4.08†††	236.67 ± 4.08***,†††,‡‡‡
Ascorbic acid (μmol/l)	46.84 ± 2.19	19.63 ± 1.95***	20.82 ± 1.45***	16.06 ± 1.95***,†,‡
Vitamin E (μmol/l)	20.30 ± 1.05	12.56 ± 1.05***	12.87 ± 1.57***	10.63 ± 1.61***
Uric acid (μmol/l)	105.24 ± 3.03	71.23 ± 2.11***	80.01 ± 2.51***,†	66.20 ± 1.30***,††,‡‡
Total thiols (μmol/l)	333.33 ± 7.11	199.66 ± 3.44***	245.66 ± 4.96***,††	191.00 ± 4.33***,††,‡‡
RBC GSH (μmol/l)	10.50 ± 0.25	8.34 ± 0.13***	8.54 ± 0.21***	8.00 ± 0.15***,†,‡
Plasma magnesium (mmol/l)	0.885 ± 0.05	0.549 ± 0.11***	0.995 ± 0.08†††	0.549 ± 0.14***,‡‡‡
RBC magnesium (mmol/l)	1.696 ± 0.05	1.098 ± 0.07***	1.879 ± 0.09†††	1.17 ± 0.07***,‡‡‡

Mean values with their standard deviations, $n = 6$. LM, low magnesium; HS, high sucrose; HSLM, high sucrose low magnesium
* Mean values were significantly different from control group, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$
† Mean values were significantly different from LM group, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.005$
‡ Mean values were significantly different from HS group, $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$, $^{\ddagger\ddagger\ddagger}P < 0.005$

no significant change was observed in case of high sucrose diet fed animals. Figure 2A and B depict hepatic MDA levels and levels of non-protein thiols, respectively, in the four different groups at the end of study period. MDA levels increased by 113% while NPSH showed ~45% decline in livers of HSLM rats as compared to controls. Assessment of antioxidant enzymes in liver of animals demonstrated that SOD, GST and catalase activities fell significantly (Fig. 3) in LM, HS and HSLM group rats as compared to controls.

Discussion

Within first week of feeding the experimental diet, classical signs of magnesium deficiency (including hyperemia of the ears, growth retardation, hair loss

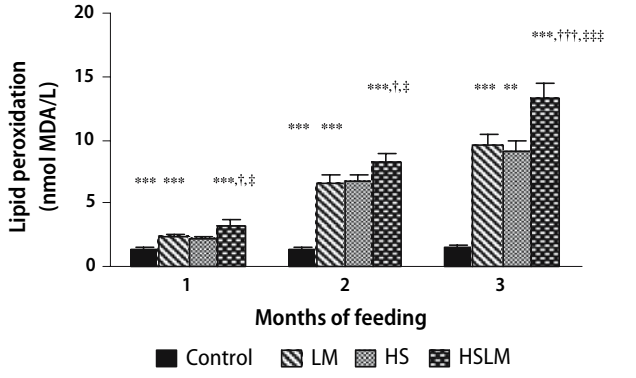

Fig. 1 Lipid peroxidation in plasma of four different groups of rats. Mean values with their standard deviations, $n = 6$. LM, low magnesium; HS, high sucrose; HSLM, high sucrose low magnesium. *Mean values were significantly different from control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$. †Mean values were significantly different from LM group, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.005$. ‡Mean values were significantly different from HS group, $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$, ‡‡‡P < 0.005

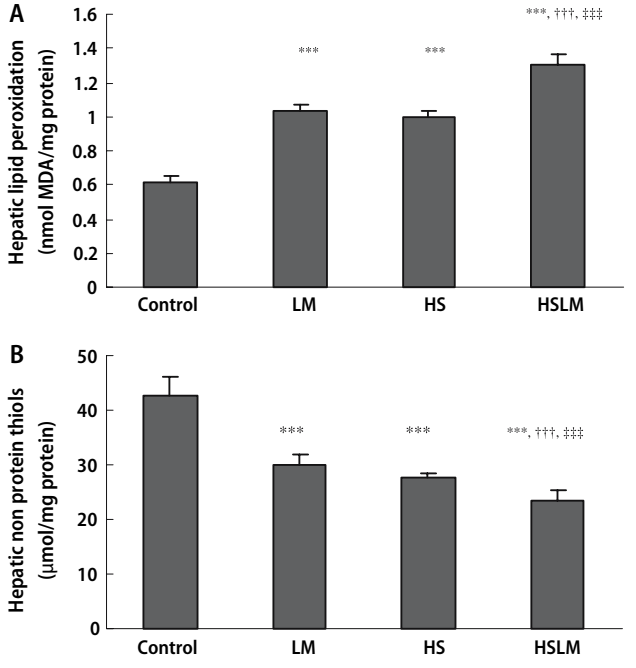

Fig. 2 Lipid peroxidation (**A**) and non-protein thiol (NPSH) levels, (**B**) in livers of four different groups of rats Mean values with their standard deviations, $n = 6$. LM, low magnesium; HS, high sucrose; HSLM, high sucrose low magnesium. *Mean values were significantly different from control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$. †Mean values were significantly different from LM group, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.005$. ‡Mean values were significantly different from HS group, $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$, $^{\ddagger\ddagger\ddagger}P < 0.005$

and edema of paws) were observed in the group II and group IV animals. The present study clearly indicates that the body weights of animals fed the combined high sucrose low magnesium diet remained below those of the control animals ($P < 0.05$). Previous studies have shown that magnesium deficiency leads

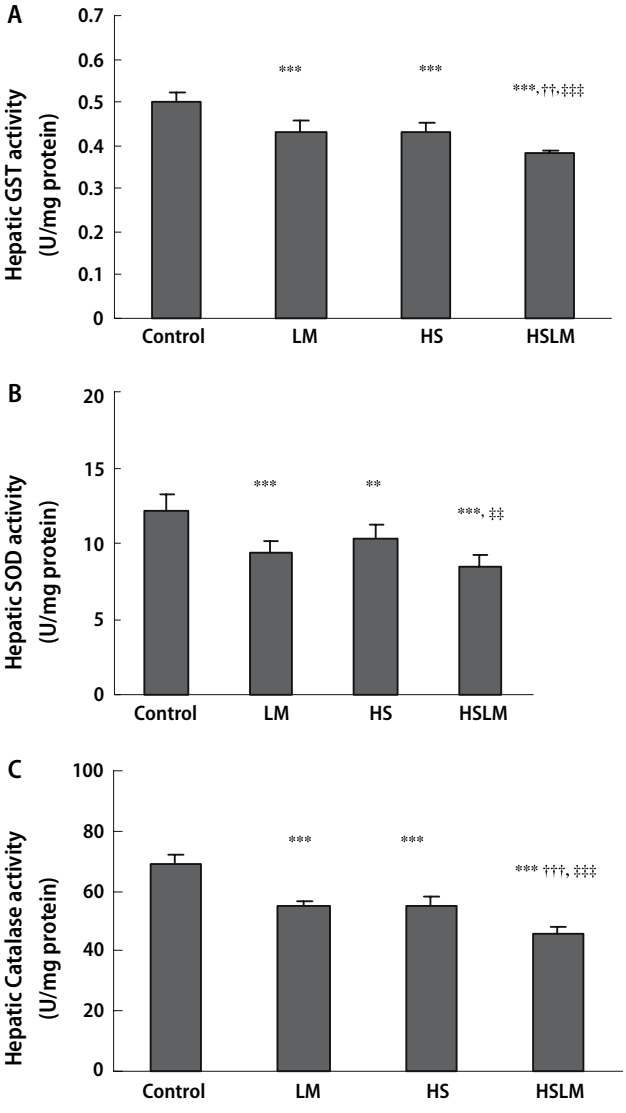

Fig. 3 Anti-oxidant enzyme activities in hepatic tissue of experimental diet fed rats. (**A**) Glutathione-*S*-transferase activity. (**B**) Superoxide dismutase activity and (**C**) Catalase activity. Enzyme activities are expressed in Units/mg protein where one unit corresponds to one mole of product formed or decomposed per minute. Mean values with their standard deviations, $n = 6$. LM, low magnesium; HS, high sucrose; HSLM, high sucrose low magnesium. *Mean values were significantly different from control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$. †Mean values were significantly different from HS group, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.005$. ‡Mean values were significantly different from LM group, $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$, $^{\ddagger\ddagger\ddagger}P < 0.005$

to a decrease in body weight [8, 13] whereas sucrose has been shown to either cause an increase in body weight or to not affect body weight [6, 32]. Since the present work has been carried out to study the combined effect of low magnesium high sucrose diet it appears that the net effect is a lesser increase in body weight associated with a low magnesium diet which is somewhat compensated by increased weight gain due to high sucrose feeding. It seems that inadequate

magnesium in the diet exerts a growth retarding effect as observed by lesser gain in body weight in animals fed low magnesium and high sucrose low magnesium diets which might be attributable to a general decrease in feeding efficiency.

This study clearly demonstrates that rats fed a high sucrose low magnesium diet had increased lipid peroxidation and decreased anti-oxidant potential. Measurement of TBARS concentration, although non-specific [33], is widely used as an indicator of lipid peroxidation process and indirectly of oxidative stress. The levels of peroxidation marker (TBARS) increased significantly in plasma of HSLM rats. Malondialdehyde (MDA), the main component of plasma TBARS, originates from several sources (i) peroxidation of plasma lipids, (ii) blood platelets, (iii) peroxidation of lipids in endothelial and other cells [16]. Therefore the mechanism of increased TBARS in high sucrose low magnesium diet fed rats may be multifactorial. First, high calorie diet may stimulate mitochondrial oxidation metabolism and increased leakage of electrons from mitochondrial respiratory chain [1]. The opposite i.e. reduced mitochondrial free radical formation and improvement of antioxidant defense was observed following caloric restriction [36]. Second, free radical cascade may be triggered by activated phagocytes, which generate oxygen free radicals during respiratory burst [35]. Besides, we also found significant decrease in vitamin E, vitamin C and uric acid levels in plasma. Since vitamins E and C and uric acid normally act as antioxidants; low levels of these parameters may probably be the result of their increased utilization. Studies conducted in our own laboratory [10] and elsewhere [20, 11] have reported that sucrose given at abnormally high amounts in the diet leads to increased plasma triacylglycerol concentrations. Hyperacylglycerolemia observed in our model of high sucrose low magnesium diet fed rats could contribute significantly towards oxidant-antioxidant imbalance. It may increase lipid peroxidation simply by an excessive availability of substrate fatty acids contained in plasma triacylglycerols. It has been reported that plasma lipid peroxide concentration is significantly higher in patients with hypertriacylglycerolemia [38]. Unlike other fat soluble vitamins, vitamin E has no specific transport proteins, but rather is transported in plasma lipoproteins and tocopherol is secreted in the liver in VLDL and protects lipoproteins by preventing oxidation. When lipoproteins are depleted of antioxidants, unsaturated fatty acids are rapidly oxidized and the vitamin E depletion in sucrose fed rats may predispose lipoproteins to subsequent oxidative stress [14]. Alternatively, there is considerable evidence that hyperglycemia results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in a variety of tissues. We have already reported [10] that rats kept on a high sucrose low magnesium diet develop considerable hyperglycemia. Elevated glucose causes oxidative stress due to increased production of mitochondrial ROS [4], non-enzymatic glycation of proteins [3] and glucose auto-oxidation [43].

Thus there are several possible pathways by which a diet rich in sucrose may alter cellular metabolism, which in turn may accelerate oxidative stress. The increased oxidative stress could be due to oxygen free radical production and/or decreased protection by non-enzymatic or enzymatic anti-oxidants [18]. Moreover, the possibility exists that sucrose feeding leads to accumulation of advanced glycation end products and that oxidative degradation of glucose or sucrose adducts leads to the production of free radicals [24].

It was reported that increase in oxidative products provides indirect evidence that endogenous anti-oxidants may be compromised during magnesium deficiency [5]. This finding may result from either oxidative loss of endogenous anti-oxidants during magnesium deficiency or may indicate that anti-oxidants are insufficient to cope up with increased oxidative stress during magnesium deficiency. Magnesium deficiency has been implicated in causing increased oxidative stress that renders the cell more susceptible to oxidative damage [37, 42]. Further, magnesium itself has been reported to have anti-oxidant potential, scavenging oxygen radicals possibly by the spontaneous dismutation of superoxide ions and also as an essential requirement for the synthesis of some important natural antioxidants [21]. The decrease in ascorbic acid may be an indication of magnesium requirement in the biosynthesis of ascorbic acid in vivo. This has been explained by Hsu et al. [21] who observed a decrease in ascorbic acid in liver of magnesium depleted rats. They suggested that synthesis of L-ascorbic acid from D-glucouronolactone in the liver of magnesium depleted rats was significantly suppressed as compared to control rats. Ascorbate is known to regenerate reduced vitamin E from oxidized vitamin E [40] and loss of ascorbate during magnesium deficiency suggests a possible subsequent loss of reduced form of vitamin E. Decrease in RBC GSH levels suggest that magnesium is essential in the maintenance of GSH to protect against oxidative damage. Minnich et al. [28] 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 reduced 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. Decreased activities of antioxidant enzymes

seen in our study may be secondary to enzyme inactivation by oxygen free radicals.

Conclusion

This study clearly indicates that a high sucrose low magnesium diet induces lipid peroxidation and depletes various anti-oxidants viz., ascorbic acid, vitamin E, uric acid, total thiols, RBC GSH, NPSH and a causes a reduction in the activities of the anti-oxidant enzymes, SOD, GST and catalase. Therefore it is postulated that a high sucrose low magnesium diet leads to reduction of threshold anti-oxidant capacity and enhanced susceptibility to free radical damage.

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