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Insinuation of exacerbated oxidative stress in sucrose-fed rats with a low dietary intake of magnesium: Evidence of oxidative damage to proteins

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Abstract
High sucrose diets and low magnesium intake have been independently implicated in induction of oxidative stress in animal models. The aim of this study was to investigate whether low dietary magnesium intake exacerbates the prooxidant effects of high sucrose feeding. Rats were fed control (C), high sucrose (HS); low magnesium (LM) and high sucrose low magnesium (HSLM) diets for 90 days and oxidative stress evaluated in terms of formation of TBARS, advanced oxidation protein products and protein carbonyls. HS and LM rats showed evidence of lipid peroxidation and protein oxidation in plasma and liver. Enhanced oxidative injury to lipids and proteins after HSLM feeding was indicated by increased carbonyl content ($p < 0.01$) and significantly ($p < 0.005$) higher levels of TBARS in plasma and hepatic tissue relative to both HS and LM groups. Altogether, these results illustrate the potential detrimental and cumulative effects of low magnesium intake combined with high sucrose consumption on oxidative stress variables.

Keywords: *Sucrose, low dietary magnesium, lipid peroxidation, protein carbonyls, advanced oxidation protein products*

Introduction

The combination of the modern diet and sedentary lifestyle has resulted in an explosive increase in the incidence of type 2 diabetes worldwide. A cardinal feature of type 2 diabetes is insulin resistance, which is also associated with a wide range of other clinical and experimental settings. Since both genetic as well as environmental factors predispose to insulin resistance, it would appear likely that diet composition plays a significant role in inducing adaptations that are characteristic of pre-diabetic states and insulin resistance [1]. Studies in humans and animals have suggested that type 2 diabetes and impaired glucose tolerance are accompanied by the presence of an oxidative stress with post-prandial hyperglycemia contributing to oxidative stress substantially [2].

Current dietary guidelines for reduced fat intake have led to macronutrient substitution in favour of carbohydrates. Surplus amounts of ingested sugars, especially sucrose and fructose, have been associated with numerous metabolic abnormalities in humans and experimental animals, including hypertriacylglycerolemia, hyperglycemia and defects in insulin signalling. Although most of the adverse effects of dietary sucrose occur only at high intakes [3,4], the UK National Dietary and Nutrition Survey [5], among others [6], showed that a considerable section of the population derived a significantly high proportion of food energy from non-milk extrinsic sugars (predominantly sucrose), which was well above the current dietary recommendations. High sucrose and high fructose diets have been widely used in animal

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models to induce the metabolic changes observed in the insulin resistance syndrome [1,7] Although the underlying mechanism for the detrimental consequences of high sucrose diet in animal models is not clear, there is evidence to suggest that sucrose feeding may facilitate oxidative damage [8,9].

Clearly, dietary macronutrients play a key role in the development of insulin resistance as well as type 2 diabetes; however, the role of micronutrients in this process is not very clear. A strong relationship between magnesium and insulin action has been reported [10,11]. Low serum and intracellular magnesium concentrations are associated with insulin resistance, impaired glucose tolerance and defects in insulin secretion [11–13]. Over-processing of foods has contributed to the substantially reduced intake of magnesium in the diet over the last few decades [14]. Dietary intake of Mg may not be high enough in many people to promote an optimal magnesium status that imparts protection against various disorders [11,15,16]. Work done in our laboratory [17] is consistent with other observations that support a link between low Mg intake and increased oxidative stress. Hypomagnesaemia may induce an impairment of redox status by facilitating an increased generation of reactive oxygen species [18–20] as well as compromised anti-oxidant defences [21,22].

There are some short-term metabolic studies that have examined the combined effect of high fructose/sucrose feeding in magnesium deficient rats [23,24]. However, no studies to our knowledge have examined the wide-ranging effects of dietary magnesium deficiency combined with high sucrose feeding in the context of oxidative injury to lipids, proteins and DNA. Since high sucrose consumption and low magnesium intake have been independently implicated as causes of increased free radical generation with subsequent damage to cellular processes; in the present study we endeavoured to assess the evidence of exacerbated oxidative injury to lipids, protein and DNA in normal rats.

Methods and materials

Experimental animals and feeding protocol

Male Wistar rats weighing ~150 g were obtained from an institutional breeding stock. Rats were housed in polypropylene cages in a temperature-controlled room with a 12:12-h light–dark cycle and free access to water. The institution's guidelines for the care and use of laboratory animals were followed. On initiation of the study, all animals were provided free access to a semi-purified high-starch diet for a 2-week baseline period. Food intake was measured daily and body weight was recorded at weekly time intervals. After the 2-week baseline period, rats were divided into four groups of 6–8 animals each: control (C), high sucrose (HS), low magnesium (LM) and high sucrose low magnesium (HSLM). Animals in the control group remained on the high starch diet for 12 weeks while animals in the other three groups were switched to the respective experimental diets. Rats in the high sucrose group were fed a high sucrose diet, low magnesium group animals were maintained on the low magnesium diet whereas animals in the high sucrose low magnesium group were fed the high sucrose low magnesium diet. These diets were isocaloric and their compositions are given in Table I.

During this period, rats were fed 95% of the average food intake recorded during the second week of baseline feeding. Such a feeding protocol was followed during the experimental feeding period to ensure similar rates of weight gain and body composition across the groups [25].

Table I. 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*, †	35	35	35	35
Vitamin mixture‡	10	10	10	10
DL-methionine	3	3	3	3
Choline chloride	2	2	2	2

* Salt mixture expressed in g/kg: $CaHPO_4$, 60 g; KCl, 200 g; NaCl, 120 g; MgO, 21.0 g; $MgSO_4$. $2H_2O$, 100 g; Fe_2O_3, 6 g; $FeSO_4$. $7H_2O$, 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.

† A similar composition was used in all the experimental groups, except for the addition of MgO and $MgSO_4.2H_2O$ to provide (per kg) 50 7.0 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.

‡ Expressed per kg of the vitamin mixture: retinol, 539 mg; 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.

Sample collection

Blood was collected from the supra orbital sinus into heparinized tubes at monthly intervals. Plasma samples were obtained by low speed centrifugation (3000 *g* for 15 min at 4°C) and were stored at −80°C for biochemical analysis. After 12 weeks on the diets, non-fasted rats were weighed and killed under diethyl ether anaesthesia. Hepatic tissue was rapidly excised, washed in ice cold 0.9% NaCl and stored at −80°C.

Plasma and liver TBARS

The levels of TBARS were measured in plasma of experimental rats using the following method [19]. The susceptibility of hepatic tissue to peroxidation was determined in liver homogenate after iron-ascorbate induced lipid peroxidation, as described by Ohkawa et al. [26].

Biochemical analysis

Plasma magnesium levels were measured by the method of Thuvasethakul and Wajjwalku [27]. Briefly, a stock dye was prepared containing per 100 ml methylthymol blue (Na salt), 18 mg, polyvinylpyrrolidine, 0.6 g, 1 M HCl, 10 ml. The stock base contained per 100 ml Na sulphite, 2.4 g; Na azide 0.1 g; glycine, 750.5 mg; EGTA, 95.1 mg; NaOH 1 M, 23.5 ml. Working dye was freshly prepared before use by mixing equal volumes of stock dye and base. Analysis was performed by mixing 50 µl serum or standard or deionized water (as blank) with 2 ml working dye solution and absorbance was measured at 582 nm. Soluble protein was quantified by the refined Coomassie blue method [28].

Oxidized proteins

Oxidative damage to proteins was assessed in terms of formation of protein carbonyls and advanced oxidation protein products. Levels of protein carbonyls in plasma as well as liver were detected and quantitated after reaction with dinitrophenylhydrazine to form protein hydrazones [29]. Carbonyl content was calculated by obtaining the spectra of DNPH-treated samples at 355–390 nm. Advanced Oxidation Protein Products (AOPP) levels in plasma were measured by a spectrophotometric method [30]. Briefly, 100 µl acetic acid was added to 100 µl of plasma, diluted 1:10 in phosphate-buffered saline or chloramine-T standard solutions (0–100 µµ). Then, 50 µl of potassium iodide (1.16 M) was added, followed by 100 µl acetic acid. Absorbance of the reaction mixture was immediately read at 340 nm on a spectrophotometer. Chloramine-T absorbance at 340 nm was linear within the range of 0–100 µM. AOPP concentrations were expressed in µmol/l of chloramine-T equivalents.

DNA fragmentation

For the determination of oxidative injury to DNA, DNA was extracted from liver tissue by the phenol-chloroform method and fragmentation of chromatin was evaluated after electrophoresis of DNA on a 1% agarose gel.

Statistical analysis

Statistical analysis was performed using the GraphPad InStat (GraphPad Inc., San Diego, CA, USA) software package. Results were expressed as mean and SD of 6–8 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. Correlation coefficients were calculated using Spearman's procedure. Differences were considered statistically significant at $p < 0.05$.

Results

Throughout the experiment, no difference was observed in the food intake of animals in the four groups (data not shown). After 12 weeks on the experimental diets, body weights of animals in the control and sucrose fed groups did not differ while dietary magnesium deficiency resulted in lesser gain in body weight in the LM and the HSLM groups (data not shown).

Compared to controls, higher levels of the peroxidation marker TBARS ($p < 0.001$) were found in the plasma of HS, LM as well as the HSLM group animals (Table II). The level of plasma TBARS in the high sucrose low magnesium diet fed rats was significantly ($p < 0.01$) higher than those seen in the low magnesium and high sucrose groups, separately. After exposure of liver homogenate to Fe-induced lipid peroxidation, TBARS levels were raised by 72% in the HS, 67% in the LM and by 103% in the combined high sucrose low magnesium group, showing an increase significantly different from both the high sucrose or the low magnesium groups ($p < 0.01$).

Specific markers used to determine protein oxidation in high sucrose low magnesium diet fed rats are given in Table III. When compared with the control animals, significantly higher protein carbonyl (PCO) levels were seen in plasma of LM group ($p < 0.01$), HS group ($p < 0.01$) and HSLM group ($p < 0.001$). PCO content in plasma of HSLM diet fed rats was significantly higher than values obtained for

Table II. Effect of combined high sucrose low magnesium diet on extent of TBARS formation in plasma and liver.

	TBARS (nmol MDA/mg protein)	
	Plasma TBARS	Liver TBARS
Control	0.108 ± 0.012	2.04 ± 0.06
HS	0.383 ± 0.023***	3.36 ± 0.076***
LM	0.427 ± 0.021***, †	3.83 ± 0.067***, †
HSLM	0.540 ± 0.024***, †††, ‡‡‡	4.92 ± 0.073***, †††, ‡‡‡

Mean values with their standard deviations, $n = 6–8$.
TBARS, Thiobarbituric acid reactive substances; HS, High Sucrose; LM, Low Magnesium; 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, † $p < 0.05$, ††† $p < 0.005$.
‡ Mean values were significantly different from LM group, ‡ $p < 0.05$, ‡‡ $p < 0.01$, ‡‡‡ $p < 0.01$.

LM ($p < 0.01$) and HS ($p < 0.01$) groups. We found significant ($p < 0.001$) increases in protein carbonyl content in hepatic tissue of low Mg, high sucrose and the combined high sucrose low Mg groups, relative to the values obtained for control group. A trend similar to that seen in the case of plasma carbonyls was observed in the liver tissue of HSLM group animals which showed significant increases ($p < 0.01$) relative to both the HS and the LM groups.

Figure 1 depicts the levels of a novel oxidative stress marker, AOPP, in plasma of experimental diet fed rats at monthly intervals. Evidently, AOPP levels started to rise as early as after 1 month of feeding (Figure 1A) and the trend continued over the entire period of study. Upon the completion of the stipulated period (Figure 1C), relative to controls, AOPP levels were seen to be increased significantly in the other three groups with the greatest increase in AOPP levels witnessed in the HSLM group (~ 112%), while the HS group showed a rise of 96% and an 87%

Table III. Protein carbonyl content in plasma and liver of high sucrose low magnesium diet fed animals.

	PCO (nmol/mg protein)	
	Plasma PCO	Liver PCO
Control	0.575 ± 0.077	2.090 ± 0.070
HS	1.474 ± 0.101***	2.920 ± 0.091***
LM	1.650 ± 0.086***, ††	3.024 ± 0.105***
HSLM	2.250 ± 0.100***, †††, ‡‡	3.409 ± 0.217***, ††, ‡‡

Mean values with their standard deviations, $n = 6–8$.
PCO, Protein Carbonyls; HS, High Sucrose; LM, Low Magnesium; 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, † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.005$.
‡ Mean values were significantly different from LM group, ‡ $p < 0.05$, ‡‡ $p < 0.01$.

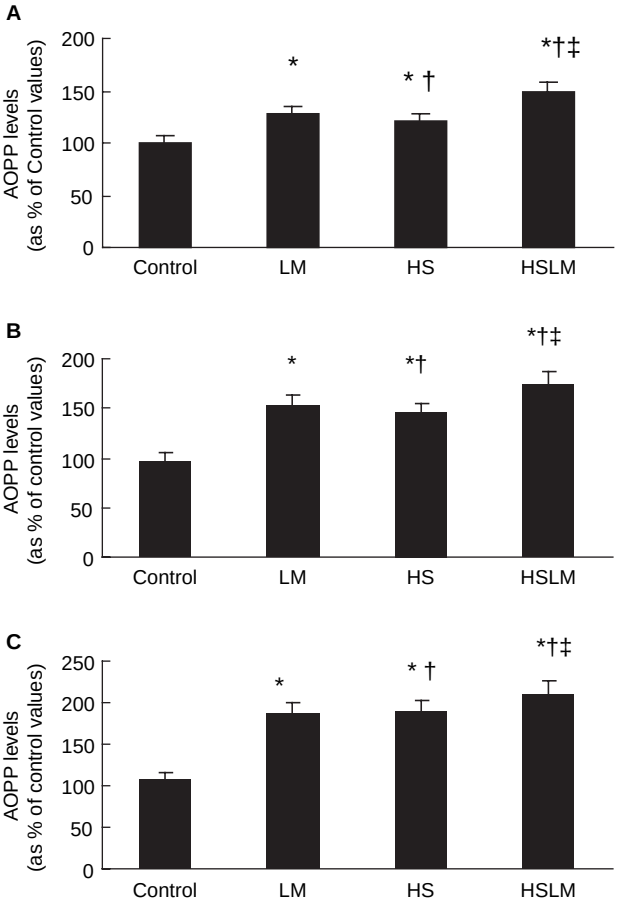

Figure 1. Advanced oxidation protein products (AOPP) in plasma of high sucrose low magnesium diet fed rats. (A) After 4 weeks of feeding, (B) After 8 weeks of feeding, (C) After 12 weeks of feeding. HS, High Sucrose; LM, Low Magnesium; HSLM, High Sucrose Low Magnesium. * Mean values were significantly different from Control group, $p < 0.05$; † Mean values were significantly different from HS group, $p < 0.05$; ‡ Mean values were significantly different from LM group, $p < 0.05$.

elevation observed in the plasma of LM group animals.

Fragmentation of chromatin into units of single or multiple nucleosomes is specific for apoptosis. The appearance of the nucleosomal DNA ladder after electrophoresis on agarose gels is considered a hallmark of programmed cell death. Figure 2 presents the results of DNA separation on a 1% agarose gel in livers of control and treated animals.

Discussion

Sucrose enriched diets and hypomagnesemia have independently been implicated in the induction of insulin resistance in studies on rodents. The current work provides new information on the combined effect of high sucrose feeding and low dietary magnesium intake on certain parameters of oxidative stress measured in plasma and liver of normal rats, including unsaturated lipids in cell membranes, amino acids in

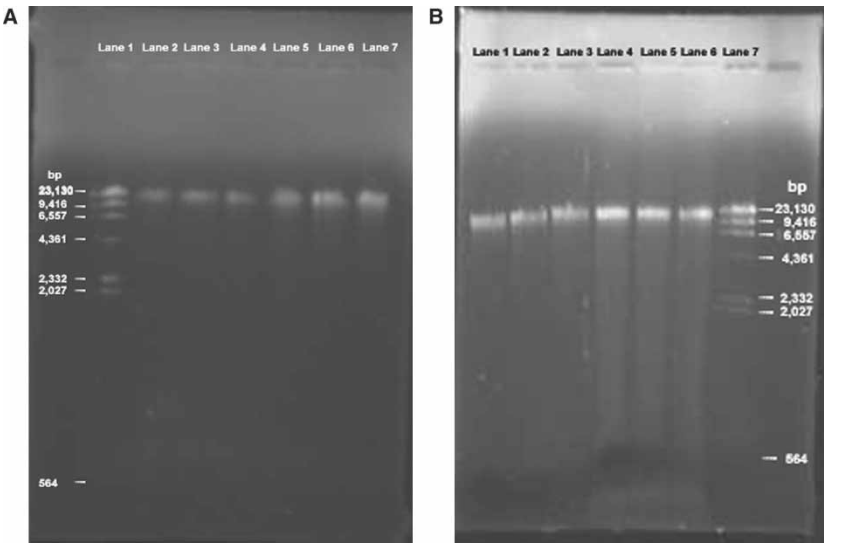

Figure 2. Agarose gel electrophoresis of DNA samples from livers of experimental diet fed animals. (A) Electrophoretogram of DNA from HS and HSLM group rats. (B) Electrophoretogram of DNA from livers of Control and LM group rats.

proteins and nucleotides in DNA, since these are the prime targets for the more reactive free radicals [19]. We have previously reported that relatively long-term feeding of high sucrose low magnesium diet induces insulin resistance in rodents [31].

Insulin resistance is associated with increased lipid peroxidation and free radical formation. The level of the peroxidation marker, TBARS, was found to be significantly increased in the plasma of treated animals in our study. An increased susceptibility to peroxidation was noted in liver homogenates of HS, LM and HSLM group rats. Our results are in accordance with previous publications which have reported enhanced lipid peroxidation in studies on livers from sucrose fed [8,9] and magnesium deficient rats [17,19,32,33] as well as increases in plasma TBARS in magnesium deficient animals.

There are several possible pathways by which diet-induced alterations in cellular metabolism may accelerate oxidative stress. Enhanced susceptibility to peroxidation could be due to increased oxygen free radical production and/or reduced protection by enzymatic and non-enzymatic antioxidants [34]. There is the likelihood that, due to the diet-induced hyperglycemia, glucose oxidation through the pentose phosphate pathway leads to excessive formation of NADPH, which in turn can promote lipid peroxidation in the presence of the cytochrome P-450 system. Increased hepatic activity of the NADPH generating enzyme, glucose-6-phosphate dehydrogenase, seen in HSLM diet fed animals in our study (data not shown) lends support to this hypothesis. Alternatively, inhibition of antioxidant enzymes by glycosylation may give rise to increased lipid peroxidation [35].

Moreover, tissue susceptibility to oxidative stress can be dependent on alterations in lipid composition. We have observed increased levels of triacylglycerols and free fatty acids (FFA) in rats fed a high sucrose low magnesium diet (unpublished observations). Although several studies have reported that fatty acids stimulate ROS production [36,37], only recently Du et al. [38] were able to demonstrate that increased levels of free fatty acids seen in people with insulin resistance caused increased superoxide production by providing increased electron donors (NADH and $FADH_2$) to the mitochondrial electron transport chain.

In our study, a positive correlation between lipid peroxidation in plasma, as measured by TBARS levels, and diet-induced hypertriacylglycerolemia ($r = 0.60$, $p < 0.05$) was noted. We hypothesize that lipid peroxidation possibly accompanies the oxidation of LDL protein thereby implicating reactive lipid intermediates in the reaction pathway. In addition, due to the greater oxidizability of lipids compared to glucose and reactive carbonyls, reactions driven by unsaturated fatty acids could be an important source of oxidizing intermediates in the tissues of magnesium deprived animals fed a high sucrose diet.

Reactive Oxygen Species (ROS) has previously been suggested to be involved in insulin resistance. Work done by Houstis et al. [39] has shown a causal role for ROS based on the observation that several pharmacological and genetic interventions designed to decrease ROS levels substantially prevented the development of insulin resistance. There is evidence to suggest that formation of ROS such as superoxide and H_2O_2 may be a direct consequence of hyperglycemia [40]. Experimental induction of hyperglycemia has been shown to result in elevated markers of oxidative stress. Furthermore, several studies have reported the inhibitory effects of ROS on insulin secretion and signalling [41,42]. Our study found that plasma glucose values correlated well with

indices of lipid peroxidation as well as protein oxidation, glucose vs TBARS ($p < 0.01$) and glucose vs PCO ($p < 0.05$), respectively. The correlation between glucose and TBARS as well as PCO is an indicator of the close relationship between the hyperglycaemic state and oxidant damage to lipids and proteins. Another possibility exists, as suggested by Ceriello and Motz [43] that insulin resistance may be a compensatory mechanism that imparts protection against further insulin-stimulated nutrient uptake and, hence, subsequent oxidative stress. The generation of excessive NADH, which leads to the formation of ROS, may be prevented by inhibiting oxidation of free fatty acids. Increased intracellular free fatty acids may in turn lead to reduced translocation of glucose transporters and consequently resistance to insulin-stimulated glucose uptake.

Lipoxidation end products and/or advanced glycation end products (AGEs) together with dysregulated glucose and lipid metabolism are believed to be important contributors to oxidant or carbonyl stress. Oxidative modification of proteins may cause alterations in protein function and may lead to their degradation [44,45]. Protein oxidation, measured as an increase in dinitrophenylhydrazine (DNP)-reactive carbonyl groups, has been shown to be an early event in oxidative stress *in vitro* [46]. Protein oxidation may be a more significant pathological event than damage to lipids because enzyme inactivation can have fast and suprastoichiometric effects by virtue of the nature of the catalytic functions of enzymes [47]. In our study, levels of protein carbonyls (PCO) were found to be considerably elevated in both the plasma and hepatic tissue of the three treated groups, with the combined HSLM diet fed rats showing the greatest increase (Table III). Our findings lend support to limited reports in the literature on carbonyl formation in association with sucrose feeding [48,49]. Increased protein carbonyl content has also been demonstrated in the kidney and brain of magnesium deficient rats [50]. Correlation analysis showed a positive correlation between TBARS and PCO content in hepatic tissue in our study ($r = 0.72$, $p < 0.05$). As seen in Figure 3, a significant positive correlation was also observed between lipid peroxidation and carbonyl formation in plasma of experimental diet fed animals ($r = 0.68$, $p < 0.01$). The coexistence of elevated MDA levels and increased protein carbonyl content in high sucrose low magnesium diet fed rats in our study seems to suggest that an increase in lipid peroxidation might play an important role in enhanced protein oxidation.

The presence of advanced oxidation protein products may be a good marker of oxidative stress. These are defined as dityrosine containing cross-linked protein products, thereby excluding protein aggregates that form as a result of disulphide links [51].

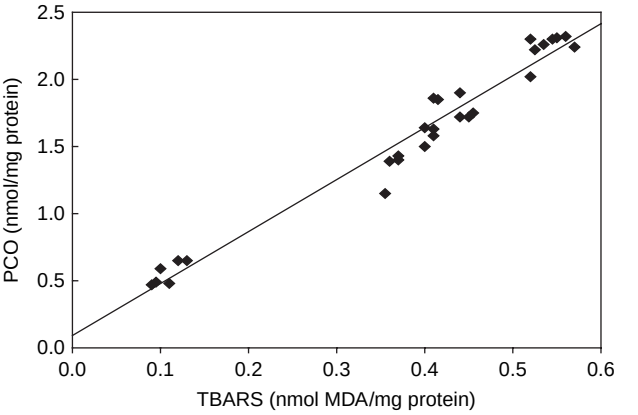

Figure 3. Correlation between lipid peroxidation and protein carbonyl levels in plasma of experimental diet fed rats ($r = 0.68$, $p < 0.01$).

AOPP are formed during oxidative stress by the action of chlorinated oxidants, mainly hypochlorous acid and chloramines. They are supposed to be structurally similar to advanced glycation end products (AGE) and to exert similar biological activities as AGEs, i.e. induction of proinflammatory cytokines and adhesive molecules. Albumin is the most abundant plasma protein and is a powerful extracellular antioxidant [52]. AOPP is considered to be an oxidation index for albumin [30,53]. Both oxidative and carbonyl stress have been shown to induce AOPP formation [53].

Figure 1 depicts the results obtained upon the measurement of advanced oxidation protein products (AOPP) levels in plasma of experimental diet fed rats. AOPP measurements were made at monthly intervals in plasma of experimental animals and elevated AOPP concentrations were found in animals on the LM, HS and HSLM diets. The increase in advanced oxidation protein products was evident after 1 month of feeding the respective diets to experimental animals. The same trend continued through the entire study period across all the groups and by the end of the feeding schedule plasma AOPP levels had risen

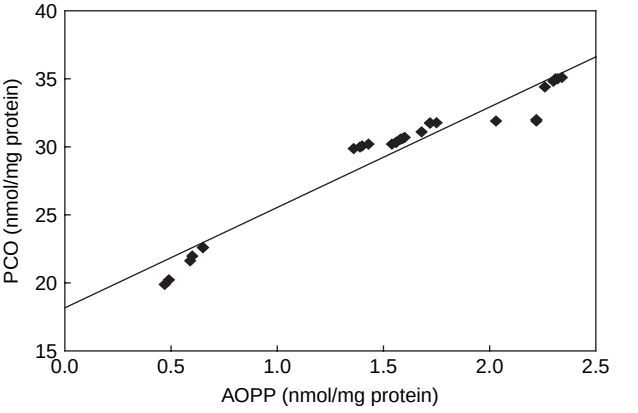

Figure 4. Correlation between protein carbonyl levels and AOPP in plasma of experimental diet fed rats ($r = 0.88$, $p < 0.005$).

significantly in all the treated groups with the HSLM group rats showing the greatest increase. The high AOPP values seen in the experimental diet fed animals relative to controls reflect excessive free radical generation and oxidative damage to proteins as a result of Mg deprivation and/or high sucrose feeding. It is important to note that, in our study, AOPP values correlated tightly with carbonyl content in both plasma (Figure 4) and liver of normal rats ($r = 0.88$, $p < 0.005$; $r = 0.52$, $p < 0.05$, respectively). This indicates a close association between these two indices of protein oxidation. In addition, plasma glucose values in our study correlated well (Table IV) with markers of both protein oxidation (AOPP & PCO) and lipid peroxidation in plasma as well as hepatic tissue, suggesting interplay among lipid peroxidation, protein oxidation and diet-induced hyperglycemia.

In most industrialized countries, the daily magnesium intake compares poorly with the recommended daily dietary allowances (RDA) and thus marginal magnesium deficiencies are very common. Magnesium is highly required to maintain genomic stability, besides its requirement as an essential cofactor in almost all enzymatic systems involved in DNA processing [54,55]. In the present study, we examined whether the oxidative stress induced by low dietary magnesium exerted any genotoxic effects or induced apoptosis in sucrose fed rats. ROS may inflict damage on DNA leading to DNA strand breaks, DNA-protein cross-links and a broad spectrum of oxidative DNA base modifications [56]. The appearance of the nucleosomal DNA ladder on agarose gels has become a hallmark of programmed cell death. The electrophoretic pattern of DNA extracted from hepatic tissue of high sucrose low magnesium diet fed rats was examined on an agarose gel for any evidence of DNA strand breaks/nicks. Agarose gel electrophoresis of DNA extracted from hepatic tissue of experimental animals in our study did not appear to show any fragmentation as evidenced by a single band of genomic DNA suggesting that oxidative damage does not affect integrity of DNA as a result of these dietary regimens. As discussed earlier, markedly elevated levels of various biomarkers of oxidative stress to lipids and proteins have been found in LM, HS and HSLM rats, suggesting that these experimental diets induced oxidative stress. The apparently intact DNA bands seen in the HS group suggest that oxidative damage is limited to lipid peroxidation and protein oxidation and the stress is not acute enough to threaten genomic stability as assessed by DNA electrophoresis. Interestingly, the HSLM and LM group DNA lacked evidence of DNA fragmentation as well, despite the less than adequate intake of magnesium that is crucial for DNA helix stability [57] and maintenance of chromatin structure and compactness of heterochromatin [58]. The possibility exists that the reduced availability of free magnesium due to low Mg in the diet is made up for by mobilization of bound magnesium from intracellular compartments such as mitochondria and endoplasmic reticulum.

Our results showed that low dietary magnesium intake in sucrose-fed rats was associated with significantly increased levels of several of the biomarkers routinely used to assess oxidative stress in animal models. Although increases were noticed in rats fed high sucrose and low magnesium diets separately; however, the greater increases seen in the combined HSLM group would prompt us to construe that the combined high sucrose low Mg diet exerts a cumulative effect and worsens the oxidant stress. We propose that the diet-induced insulin resistance observed in high sucrose low magnesium feeding invokes a mechanism that involves oxygen free radicals. This is corroborated by the observation that lipid and protein oxidation markers were affected in plasma and livers of high sucrose low magnesium diet fed rats and these changes correlate significantly with the hyperglycemia and hypertriacylglycerolemia seen in these animals. In addition to the ability of ROS to directly inflict macromolecular damage, it is believed that ROS can function as signalling molecules to activate a number of cellular stress-sensitive pathways that cause cellular damage [59]. Further, these same pathways are believed to be linked to insulin resistance and reduced insulin secretion. Collectively, these observations suggest that a potential oxidative pathway requiring both glucose and unsaturated fatty acids could contribute to diet induced insulin resistance seen in our study.

Table IV. Spearman's correlation coefficients between plasma glucose, triacylglycerols and oxidative stress markers in plasma and livers of high sucrose low magnesium diet fed rats.

	Plasma TBARS	Plasma PCO	Plasma AOPP	Liver TBARS	Liver PCO
Plasma Glc	0.78*	0.52*	0.64*	0.57*	0.45*
Plasma TAG	0.84**	0.56*	0.41	0.37	0.25
Plasma TBARS	—	0.68**	0.57**	0.44**	0.13
Plasma PCO	—	—	0.88***	0.28	0.61*
Plasma AOPP	—	—	—	0.17	0.52*

HS, High Sucrose; LM, Low Magnesium; HSLM, High Sucrose Low Magnesium; Glc, Glucose; TAG, Triacylglycerol; TBARS, Thiobarbituric acid reactive substances; PCO, Protein carbonyls; AOPP, Advanced oxidation protein products

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.

In summary, we showed that low magnesium intake might exaggerate the oxidative stress in sucrose-fed rats and amplify the oxidative damage to unsaturated lipids in membranes and amino acids in proteins. Magnesium-deficient animals fed a high sucrose diet showed an increased susceptibility to *in vivo* oxidative stress and their tissues were more susceptible to *in vitro* peroxidation. Extrapolating to the human condition, we conclude that excessive sucrose consumption, coupled with low Mg intake in the current dietary/social structure, predisposes to inappropriate oxidant stress responses, which may promote the premature manifestation of multifaceted diseases such as diabetes and cardiovascular disease. Overall, the current investigation illuminates the concept that oxidative stress may indeed be involved in the pathogenesis of certain types of diet-induced insulin resistance.

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