Effect of a low magnesium diet on in vitro glucose uptake in sucrose fed rats

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Abstract. Dietary magnesium deficiency and excess sucrose in the diet have been shown to play an important role in the development of insulin resistance. This study is an extension of a previously published experiment (Magnes Res 2004; 17: 293-300) and is focused on the effect of a low magnesium diet on in vitro glucose uptake in sucrose fed rats. For this purpose male Wistar rats were divided into four groups and fed control, high sucrose, low magnesium and high sucrose low magnesium diets for a period of three months. Serum and erythrocyte magnesium values demonstrated a significant drop in the low magnesium and high sucrose low magnesium groups. A significant increase was observed in the body weight of the high sucrose group, whereas the weights of animals in the high sucrose low magnesium group remained unchanged from controls. The biochemical analysis showed a significant decrease in in vitro glucose uptake in liver, muscle and diaphragm of rats consuming high sucrose, low magnesium and high sucrose low magnesium diets. The maximum reduction, however, was observed in the combined high sucrose low magnesium group. These findings seem to suggest the potential of a high sucrose low magnesium diet to cause insulin resistance by reducing glucose uptake in target tissues of rats.

Key words: low magnesium, sucrose, insulin resistance, glucose uptake

Magnesium is the second most abundant intracellular cation. It serves as a co-factor in a number of enzymatically catalyzed steps regulating glucose metabolism [1]. All of the enzymatic reactions that hydrolyze and transfer phosphate groups, including those associated with the reactions involving adenosine triphosphate (ATP), show an absolute requirement for magnesium [2]. Magnesium deficiency is common and multifactorial and is associated with a number of diseases as hypertension, diabetes mellitus, atherosclerosis, cardiovascular disease and myocardial infarction, etc. It is believed that an estimated 50%-85% population of the United States is receiving an inadequate magnesium intake. There is a great volume of literature that suggests an association between reduced magnesium intake and insulin resistance, central to type 2 diabetes. Hall et al. [3] have reported that magnesium deficiency produces insulin resistance in isolated skeletal muscles preparations. In the recent past, many researchers have studied and elaborated the link between magnesium deficiency and insulin resistance [4-6].

Serum magnesium concentrations have been shown to correlate inversely with glucose disposal in diabetic patients and magnesium administration has been found to increase the utilization of carbohydrates [5]. The link between magnesium deficiency and the development of diabetes is strengthened by the observation that several treatments for type 2 diabetes appear to promote an increase in magnesium levels. Metformin, for example, has been shown to raise magnesium levels in the liver [7]. Pioglitazone, a thiazolidinedione anti-diabetic agent that increases insulin sensitivity, is reported to increase free magnesium concentration in adipocytes [8].
Sucrose is considered to be another important factor associated with the development of insulin resistance. It has been reported that the inclusion of refined sucrose in the diet for 2-8 weeks could increase fasting glucose, insulin, total triglycerides, and very low-density lipoprotein triglycerides in non-insulin diabetes mellitus, non-diabetic hyperinsulinemia and normal humans [9-11]. In particular, Storlein et al. [12] demonstrated that sucrose feeding for a 4-week period produced a major impairment in insulin action that was predominantly accounted for by impaired suppression of hepatic glucose production and to a lesser extent by reduced insulin mediated glucose utilization. However, the effect of sucrose remains quite controversial. A range of in vitro and in vivo studies have shown impairment [10, 13-16], no change [17-21], and even improvement [22, 23] in various aspects of glycemic control after increases in dietary intake of sucrose. The controversies regarding the ability of sucrose to produce insulin resistance is probably due to the various populations studied, the dose of sucrose used and/or the duration of sucrose exposure. However, the fructose component of sucrose is non-controversial regarding its ability to produce insulin resistance. Thornburn et al. [24] have reported that feeding rats 35% of their calories as fructose for 4 week severely reduced insulin sensitivity with major impairments in both liver and peripheral tissues. Fructose has been labelled as more deleterious than sucrose in regard to insulin resistance [25, 26]. Nevertheless, it is becoming increasingly clear that dietary nutrients can modify insulin action in a number of target tissues. Therefore, it seems that sucrose consumption as well as low magnesium intake are two dietary patterns that independently appear to play increasingly important roles in the development of insulin resistance.

We have already reported that the feeding of a high sucrose low magnesium diet to male Wistar rats for a period of three months produces substantial hyperglycemia, hyperinsulinemia and hypertriglyceridemia [27]. The present study is an extension of our previously published work and investigates the effect of a low magnesium diet on in vitro 14C-glucose uptake in liver, diaphragm and thigh muscle of sucrose fed rats.

Materials and methods

Chemicals

2-[14C]-deoxy-D-glucose was procured from Bhabha Atomic Research Centre (Mumbai, India). Methyl thymol blue (MTB), poly vinyl pyrrolidinone (PVP) and ethylene glycol tetra acetic acid (EGTA) 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). All other chemicals used were of analytical grade.

Animals and diet

Male Wistar rats each weighing approximately 130 g were obtained from the Central Animal House (Panjab University, Chandigarh, India). The animals were kept in polypropylene cages under controlled conditions of temperature and light. The rats were randomly divided into four groups of six animals each. Group I was fed a synthetic control diet (C), group II was fed a low magnesium diet (LM), group III was fed a high sucrose diet (HS) while group IV was fed a diet low in magnesium and high in sucrose (HSLM). The animals were fed these experimental diets for a period of three months. Composition of the diets is shown in Table 1. The rats were given feed in small metal dishes just before the beginning of dark cycle. Any spillage was collected in the morning and its weight equivalent added to following day’s feed. Diets were freshly made every 3-4 days and stored at 4°C. The rats were allowed free access to deionized water to avoid consumption of magnesium from normal drinking water.

Sample preparation and biochemical analysis

Blood samples were drawn from the orbital sinus of light ether anaesthetized, overnight fasted rats and immediately centrifuged at 2000 g for 15 minutes at 4°C. Serum was separated immediately and the red cells were washed thrice with normal saline in cold centrifuge and finally packed; an aliquot of RBCs was digested using digestion mixture (HNO3:HClO4: 3:1) and dried to ash. After appropriate dilution magnesium was analyzed by the method of Thuvasethakul and Wajiwalku [28].

At the end of three months of feeding synthetic diets, glucose uptake was determined using the uptake of the radioactive glucose analogue, 2-[14C]-deoxy-D-glucose (2-DG) as described previously by Chang et al. [29]. The target tissues were quickly excised after sacrificing the animals, dissected free of any adjoining connective tissue, blotted and divided into long longitudinal strips (25-30 mg each). Tissues were placed in 3 mL of Krebs-Ringer bicarbonate buffer (KRB) (37°C, pH 7.4) containing 1 mmol/L glucose, 1% fatty acids, free bovine serum albumin (BSA) under aeration of 5% CO2 in O2.
After preincubation for 30 min, tissues were incubated with 1.0 nM bovine insulin for 30 min and then with 50 lL Krebs’ Ringer Bicarbonate buffer containing 2-DG (1 lCi/mL) for 5 min at 37°C in the shaking water bath under aeration. Reactions were terminated by quickly blotting the tissues and dissolving them in 0.5 mL of 0.5 N NaOH for 45 min before neutralization with 0.5 mL of 0.5 N HCl. After centrifugation, 800 lL of the supernatant was added to 1 mL of aqueous counting scintillant and the radioactivity was determined using a β-counter.

Statistical analysis

Results were expressed as means with their standard deviation (S.D.). Further, the statistical significance of the differences among the various dietary groups was determined by subjecting the data to two way ANOVA with carbohydrate (starch/sucrose) and magnesium (adequate/low) as the two factors, followed by inspection of all differences between pairs of means by Tukey’s test. Differences were considered statistically significant at p < 0.05.

Results

This paper shows additional results on the effect of low magnesium diet on in vitro glucose uptake in sucrose fed rats and is an extension to our previously published results [27] on the combined effect of a high sucrose low magnesium diet in rats. Figure 1 represents weight gain in rats after feeding the respective diets for 3 months in different experimental groups. Animals of LM and HSLM groups exhibited slower body weight gain compared to control and high sucrose fed rats (p < 0.05). Compared to initial values, body weights of control and sucrose fed rats increased by ≈ 117%, whereas rats in LM and HSLM groups registered a lesser increase in body weight of 46.15% and 76.92% respectively. Two way analyses of variance revealed that the magnesium content of the diet had a significant effect on changes in the body weight of experimental animals while the carbohydrate constituent was not a significant factor.

Figures 2 and 3 represent time course changes in serum as well as RBC magnesium levels, respectively, during the experimental period. The magnesium status of rats fed the magnesium adequate diets (control and HS groups) remained constant throughout the study period. However, serum as well as RBC magnesium levels in rats fed low magnesium diets showed a significant (p < 0.005) fall in low magnesium and high sucrose low magnesium fed animals. When the data was subjected to two way ANOVA, out of the two factors on which comparisons were based, it was observed that it was the Mg content of the diet which exerted a significant effect.

Table 1. Composition of the experimental diets.

<table>
<thead>
<tr>
<th>Ingredients (g/kg diet)</th>
<th>Control (C)</th>
<th>High sucrose (HS)</th>
<th>Low magnesium (LM)</th>
<th>High sucrose low magnesium (HSLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>650</td>
<td>-</td>
<td>650</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>650</td>
<td>-</td>
<td>650</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Salt mixture&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Salt mixture expressed in g/kg: CaHPO₄, 60 g; KCl, 200 g; NaCl, 120 g; MgO, 21.0 g; MgSO₄., 2H₂O, 100 g; Fe₂O₃, 6 g; FeSO₄., 7H₂O, 10 g; trace elements 10 g/kg including Mn, 0.8 g; CuO, 125 g; Co, 0.0009 g; Zn, 0.450 g; 1.0, 0.0049 g.<br>

<sup>b</sup> A similar composition was used in all the experimental groups, except for the addition of MgO and MgSO₄.2H₂O 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.<br>

<sup>c</sup> Expressed per kg of the vitamin mixture: retinol, 539 mg; cholecalciferol, 6250 mg; thiamine, 2000 mg; riboflavin, 1500 mg; niacin, 7000 mg; pyridoxine, 1000 mg; cyanocobalamin, 5 mg; ascorbic acid; 80,000 mg; DL-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.
Figure 4 depicts the in vitro glucose uptake in liver, thigh muscle and diaphragm of rats after feeding the respective experimental diets for three months. As is clear from this figure, compared to control animals, insulin-mediated glucose uptake was reduced significantly (p < 0.005) in all the above mentioned tissues of rats consuming low magnesium, high sucrose and high sucrose low magnesium diets. Glucose uptake values in the combined high sucrose low magnesium group rats were found to be lower than those in the low magnesium (p < 0.01) and high sucrose groups (p < 0.01). 2 X 2 analyses of variance of data obtained on glucose uptake in liver, thigh muscle and diaphragm showed that both carbohydrate constituent and magnesium content of diet had significant effects on glucose uptake, while the interaction of these two factors also had a significant effect, as seen in the combined HSLM group. The greater magnitude of reduction in HSLM rats would appear to suggest that the combined high sucrose low magnesium diet has an additive effect in lowering the insulin-mediated glucose uptake in these target tissues.

Discussion

Within the first week of feeding the experimental diet, classical signs of magnesium deficiency (including hyperemia of the ears, growth retardation, hair loss and edema of paws) were observed in the LM and HSLM group 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 to a decrease in body weight [30-32] whereas sucrose has been shown to either cause an increase in body weight or to not affect body weight [33-37]. Since the present work has been carried out to study the combined effect of a 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 after statistical analysis using 2 way ANOVA.
The deficiency in dietary intake of magnesium was reflected by a significant reduction in serum and RBC magnesium levels in the LM and HSLM groups. Serum magnesium levels are considered to be the first indicator of magnesium deficiency. Shills et al. [38] reported that during experimentally induced magnesium deficiency, the first change appears to be a fall in serum magnesium concentration. Some previous studies have reported similar findings and suggested that serum magnesium falls rapidly during magnesium depletion in humans [39, 40] and animals [41, 42]. Alfrey et al. [43] have correlated serum magnesium with bone magnesium status during both hyper- and hypomagnesaemia and suggested that for clinical purposes serum magnesium is a suitable indicator of total body magnesium. Similarly, erythrocyte magnesium content is reported to be another reliable indicator of total body stores of magnesium. Ellin et al. [44] have found a marked reduction in plasma and erythrocyte magnesium contents in magnesium deficient animals. Therefore our findings of significantly (p < 0.005) reduced serum as well as RBC magnesium content in the LM and HSLM groups is basically an indication of reduced body magnesium stores.

The present study demonstrates that a high sucrose low magnesium diet significantly suppresses insulin-mediated glucose uptake in liver, thigh muscle and diaphragm of rats. Statistical analysis revealed that both the carbohydrate composition as well as the magnesium content of the diet were independent factors that have significant effects on glucose uptake in various tissues, while the interaction of these factors also affected glucose uptake significantly. Our results are consistent with reports published previously. Tobey et al. [45] have reported that liver is the most likely site responsible for the decline in insulin mediated glucose uptake in sucrose fed rats. In a previous study, Vrana et al. [46] observed that insulin stimulated glucose uptake was reduced in adipose tissue from sucrose fed rats. However, adipose tissue accounts for only a small fraction of total glucose disposal in the intact organism [47]; a defect at this level cannot account for the glucose intolerance and insulin resistance seen in sucrose fed animals. In another study Maegawa et al. [48]...
compared a high sucrose diet with a high fat diet and found that insulin-stimulated glucose uptake into soleus muscle was decreased with both the experimental diets. In vivo insulin-stimulated glucose uptake was reportedly decreased in rats fed a diet high in sucrose for a period of ten months as compared with animals given a diet high in complex carbohydrates [49]. It has also been shown that chronic sucrose feeding alters the activity of specific enzymes regulating hepatic carbohydrate metabolism, and both a decrease in the activity of glucokinase [49, 50] and an increase in glucose-6-phosphatase [51] have been described. Furthermore, fructose feeding has been shown to lead to a decrease in the ability of insulin to suppress activation of glucose-6-phosphatase and fructose-1,6-bisphosphatase activity [51, 52]. These findings, along with several other observations, seem to indicate that fructose feeding would lead to a decrease in both hepatic glucose and glycogen synthesis, stimulation of glycogenolysis and gluconeogenesis, as well as interference with the effect of insulin on hepatic glucose metabolism.

Similarly, reduced dietary magnesium intake and subsequently depressed serum magnesium have been associated with insulin resistance and reduced glucose uptake by target tissues. Low serum magnesium levels have been implicated in the induction of insulin resistance. It has been reported that hypomagnesemia induced in rats by feeding a low magnesium diet produced a decrease in the submaximal insulin-stimulated glucose uptake by the perfused hindquarters, which could be prevented by supplementation with magnesium [53]. Many other in vitro studies have shown a reduction in insulin-mediated glucose uptake during magnesium deficiency [54-56]. Rosolova et al. [57] have reported that relatively low magnesium concentrations in non-diabetic subjects are associated with a decrease in insulin-mediated glucose disposal. Earlier studies suggest several possible mechanisms whereby low serum magnesium levels may lead to the development of

**Figure 3.** Time course changes in magnesium concentrations in red blood cells of rats fed different experimental diets. Mean values with their standard deviations, n = 6. HS, high sucrose; LM, low magnesium; HSLM, high sucrose low magnesium * significantly different from C group, p < 0.05; # significantly different from HS group, p < 0.05.
type 2 diabetes. First of all, it is an essential cofactor in reactions involving phosphorylation, thus magnesium deficiency could impair the insulin signal transduction pathway [58, 59]. Secondly, low serum or erythrocyte magnesium levels may affect the interaction between insulin and insulin receptors by decreasing hormone receptor affinity or by increasing membrane micro viscosity [60]. Finally, magnesium can also be a limiting factor in carbohydrate metabolism, since many of the enzymes in this process require magnesium as a cofactor during reactions that utilize the phosphorus bond [58, 59, 61, 62].

Conclusion
Magnesium deficiency is fairly common and is a potential risk factor for the development of insulin resistance. Sucrose feeding has also been independently implicated in the induction of diet-induced insulin resistance. Combined together, a diet rich in sucrose and deficient in magnesium could possibly be used as a tool to study and characterize the development of insulin resistance. A high sucrose low magnesium diet has been reported to induce fasting hyperglycemia, hyperinsulinemia and hypertriacylglycerolemia in rats. The present study clearly demonstrates that a high sucrose low magnesium diet reduces in vitro glucose uptake in target tissues of rats, thereby inducing insulin resistance in rats. Therefore, it may be concluded that a high sucrose low magnesium diet produces insulin resistance by decreasing the entry of glucose in the target tissues of rats.

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Figure 4. Effect of low magnesium diet on glucose uptake in target tissues of sucrose fed rats. Mean values with their standard deviations, n = 6. HS, high sucrose; LM, low magnesium; HSLM, high sucrose low magnesium *Mean values were significantly different from C group, p < 0.05; # significantly different from HS group, p < 0.05; significant different from LM group, p < 0.05.
References


