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Effect of increased magnesium intake on plasma cholesterol, triglyceride and oxidative stress in alloxan-diabetic rats

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Summary

Cardiovascular disorders are the primary causes of morbidity and mortality in patients with diabetes mellitus (DM). Agents that improve lipid profile and reduce oxidative stress have been shown to reduce the ensuing risk factors. In the present study, we investigated whether increased magnesium intake could improve hyperglycaemia, dyslipidaemia, and reduce oxidative stress in alloxan-induced diabetic rats. Male Wistar rats were divided into non-diabetic (ND), diabetic (DM) and diabetic fed on a high magnesium diet (DM-Mg) groups. Plasma concentrations of thiobarbituric acid reactive substances (TBARS) were used as markers of oxidative stress. Plasma levels of ascorbic acid, magnesium and calcium were also determined. Diabetes was induced by injecting alloxan (100mg/kg B.W). The fasting blood glucose levels were significantly lower in the DM-Mg rats than in the DM rats. Plasma total cholesterol, triglyceride, TBARS levels were significantly higher while plasma HDL-cholesterol, HDL-cholesterol/total cholesterol ratio, ascorbic acid levels were significantly lowered in DM rats compared with the ND rats. Increased intake of magnesium significantly abrogated these alterations. There were no significant differences in the plasma levels of magnesium and calcium between the DM and ND groups. However, plasma levels of magnesium but not calcium were significantly elevated in DM-Mg rats when compared with other groups. In conclusion, these results suggest that diet rich in magnesium could exert cardioprotective effect through reduced plasma total cholesterol, triglyceride, oxidative stress and ameliorated HDL-cholesterol/total cholesterol ratio as well as increased plasma ascorbic acid and magnesium in diabetic rats.

Keywords: *Diabetes mellitus, hypercholesterolemia, magnesium, oxidative stress*

Résumé

Les désordres cardiovasculaires sont les causes primaires de la morbidité et la mortalité chez les patients ayant le diabète mellite. Les agents pouvant améliorer le profil des lipides et de réduire les oxydations sont connus réduisant les facteurs à risque. Cette étude évalue si l'augmentation du magnésium pourrait améliorer l'hyperglycémie, la dyslipidemie et la réduction des oxydations chez les rats diabétiques induites par l'alloxane. Les rats males étaient divisés en groupe : non diabétiques, diabétiques et diabétiques nourris avec un régiment concentré en magnésium. Les concentrations plasmiques des substances des réactions d'acide thiobarbituriques étaient utilisées comme marqueurs des réactions d'oxydation. Les taux plasmiques d'acide ascorbique, de magnésium et de calcium étaient déterminés. Le diabète était induit en injectant l'alloxane (100mg/kg). Les taux de glucose à jeune étaient significativement plus faibles au groupe diabétique à régiment élevé de magnésium qu'aux groupes de rats diabétiques. Les taux totaux du cholestérol, des triglycérides et des marqueurs étaient significativement plus élevés alors que les taux d'acide ascorbique et de la lipoproteines-cholestérole étaient significativement bas aux rats diabétiques comparés aux non diabétiques. L'augmentation de la consommation du magnésium influençait ces changements ; il n'y avait pas de différence significative entre les taux de magnésium et de calcium plasmiques entre les groupes diabétiques et non diabétiques. En conclusion, ces résultats suggèrent que les régiments riches en magnésium pourraient exercer un effet cardioprotective par la réduction du total cholestérol plasmique, des triglycérides, des réactions d'oxydations et améliorer les proportions des lipoprotéines cholesterolique bien que l'augmentation de l'acide ascorbique et du magnésium plasmique chez les rats diabétiques.

Introduction

Diabetes mellitus (DM) has been identified as a primary risk factor for cardiovascular complications and altered lipid metabolism [1]. Studies have demonstrated an excessive production of free radicals and a reduction in enzymatic and non-enzymatic antioxidants in diabetes and cardiovascular disorders [2,3]. Lipid peroxidation is a key event in the initiation, progression and rupture of atheromatous plaque. Lipid peroxidation results from exaggerated

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oxidative stress, and there is accumulating evidence that this also accounts for hypercholesterolemia-induced vasculopathies [2]. A decreased plasma level of water-soluble antioxidant vitamin, ascorbic acid has been reported in patients [4,5] and experimental animal models of DM [6,7]. Increased urinary excretion of ascorbic acid [7] and impaired enzymatic regeneration of ascorbic acid [6] has been implicated. Extensive efforts are under way to determine agents that may have the potential to improve or prevent cardiovascular complications of DM. Interventions that improve lipid profile, especially those capable of lowering cholesterol, as well as preventing lipid peroxidation reduce cardiovascular disease morbidity and mortality in patients with DM [2,3].

Magnesium is the fourth most abundant cation in the organism and it is an important cofactor in all kinases, and other ATP-related enzymes and channels regulating insulin action [8]. Epidemiological studies have demonstrated that magnesium intake has a role in reducing the incidence of cardiovascular complications [9]. It has been reported that low dietary magnesium is associated with adverse alterations in the plasma lipid profile [10-12]. Increased magnesium intake, on the other hand, has been shown to prevent the development of atherosclerosis in cholesterol-fed animals by inhibiting cholesterol accumulation in the aortic wall [13].

Several potential beneficial actions of magnesium supplementation have been reported in human and experimental type 2 DM, including increased insulin sensitivity [14,15], improved glucose disposal [14] and improvement of metabolic control [8,15,16]. However, studies on the effect of increased magnesium intake on plasma lipid profile, oxidative stress, in type 1 DM have not been conducted. Therefore, the present study investigated the effect of increased dietary magnesium intake on fasting blood glucose, lipids, oxidative stress, ascorbic acid, magnesium and calcium in the alloxan-induced diabetic rats.

Materials and methods

Animals and treatments

Male albino Wistar rats weighing 180-200g were obtained from the Animal House, College of Medicine, University of Ilorin (Ilorin, Nigeria). Animals were housed in wire-bottomed stainless steel cages in a well ventilated room on 12-h light/ 12-hr dark cycle at 22±2°C and supplied with standard pellet diet (Bendel Feeds and Flourmills Ltd; Benin city, Nigeria) *ad libitum*. Procedures involving animals and their care were conducted in conformity with the institutional guideline of the College of Medicine, University of Ilorin (Ilorin, Nigeria) and in accordance with the American Physiological Society's "Guiding principles for Research Involving Animals" The animals were age- and weight-matched and divided into three experimental groups i.e. non-diabetic rats (ND; n=8), diabetic rats (DM; n=8) and diabetic rats whose diet was enriched with magnesium (DM-Mg; n=8). The ND and DM rats continued on chow containing 0.1% (w/w) magnesium, whereas DM-mg rats were on 1% (w/w) magnesium. Extra magnesium was supplied in chloride salt otherwise the chows were identical. Diabetes was induced by a single intravenous injection of alloxan (ALX; 100mg/kg B.W, Sigma, St Louis, MO, USA) dissolved in cold 0.9% saline immediately before use. The ND rats were injected with 0.9% saline (as vehicle). Diabetes was verified 72h post-injection based on a blood glucose level higher than 300mg/dl (16.7mmol/l). Blood glucose levels were determined with a blood glucometer (Diascam-S; Home Diagnostics Inc. Eaton town NJ). Rats were started on a high magnesium diet 48h after injection of ALX. The blood glucose concentration was monitored in venous blood from the tail vein, every week after 12h fast. After 4 weeks of diabetes induction, the rats were anaesthetize with ether following a 12h overnight fast and blood was collected from the abdominal aorta in heparinized tubes and centrifuged at 3000rpm for 10min. The plasma was frozen for determination of other biochemical parameters.

Plasma lipid, lipid peroxidation and ascorbic acid levels

Plasma levels of total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride were determined by the methods of Allain *et al* [17], Hino *et al* [18] and Buccolo and David [19] respectively, using assay kits obtained from Randox Laboratory Ltd (Co. Antrim, U.K). Plasma low-density lipoprotein (LDL) cholesterol level was calculated with the use of Friedewald's formula [20]. Plasma levels of thiobarbituric acid reactive substances (TBARS), products of malonyldialdehyde were assayed by measuring the pink-coloured chromophore formed by the reaction of thiobarbituric acid with malonyldialdehyde [21]. Plasma ascorbic acid concentration was determined by a colorimetric procedure that involves generation of a complex of

ferrous iron with á á¹ dipyridyl after reduction of ferric to ferrous iron by ascorbic acid [22].

Plasma magnesium and calcium

Plasma levels of magnesium [23] and calcium [24] were determined by colorimetric method, using assay kits supplied by PPC Pharm-tec GmbH (Essen, Germany).

Statistical analysis

All values were expressed as means±SEM of measurements. A one-way analysis of variance (ANOVA) was used, followed by Duncan's multiple range tests for pair-wise comparison. All statistical comparisons and tests were performed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL., USA) for Windows. $P<0.05$ was accepted as significant.

Results

Survival and blood glucose

After 4 weeks of treatment, the survival rate was 100, 60 and 90% in ND, DM and DM-Mg groups, respectively. It appears that diet rich in magnesium reduced the mortality rate in diabetic rats. Injection of ALX led to a significant increase in blood glucose levels throughout the duration of the experimental period. However, the blood glucose levels were significantly lower in DM-Mg group compared with the DM group in the 3rd and 4th weeks of treatment (Fig. 1).

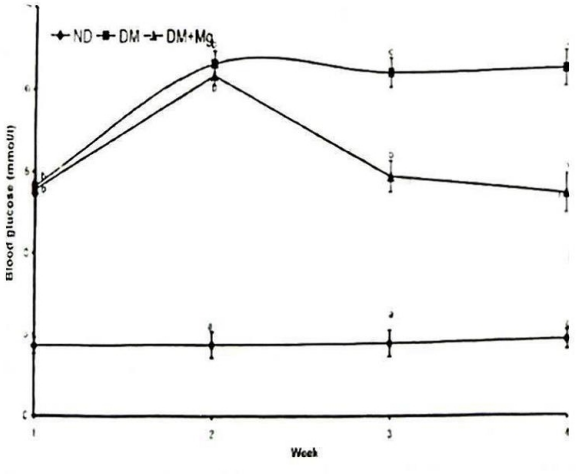

Fig. 1: Blood glucose levels in the non-diabetic (ND), alloxan-diabetic (DM) and alloxan-diabetic+Mg (DM-Mg) rats (mean+/-SEM); Groups not sharing common letter are significantly different ($P<0.05$).

Plasma lipids, lipid peroxidation and ascorbic acid

As shown in tables 1 and 2, the levels of plasma total cholesterol, triglyceride, and TBARS were significantly increased while the plasma levels of HDL-cholesterol, HDL-cholesterol/total cholesterol ratio and ascorbic acid were significantly decreased in DM rats when compared with the ND rats. A high magnesium diet abrogated all these alterations. Compared with ND rats, DM-Mg rats had significantly lower levels of total cholesterol, triglyceride, LDL-cholesterol and TBARS and significantly higher HDL-cholesterol/LDL-cholesterol and ascorbic acid (Tables 1 and 2).

Plasma magnesium and calcium

Table 3 summarizes the levels of plasma magnesium and calcium in all 3 experimental groups. Plasma levels of magnesium in DM rats were similar to those of the ND rats. However, DM-Mg group had significantly higher values ($p<0.05$) of plasma magnesium than those observed in the ND and DM groups. Plasma calcium levels were comparable in all the three groups.

Discussion

There has been an increased usage of dietary supplements for glycaemic control in DM due to the side effects associated with the existing conventional hypoglycaemic drugs [25]. In the present study, Type 1 DM was induced in rats by intravenous injection of alloxan. The subsequent increases in fasting blood glucose, plasma cholesterol, triglyceride, lipid peroxidation and decreases in LDL-cholesterol and ascorbic acid, were comparable to those observed previously in experimental animals [6,7,26] and patients [1,4] with Type 1 DM. The increases in total cholesterol and triglyceride and reductions in HDL-cholesterol, HDL-cholesterol/total cholesterol ratio in the diabetic group were abrogated by a high magnesium diet. These results are consistent with previous studies regarding magnesium supplementation and lipid profile [13,27]. The lipid-lowering effect of magnesium supplementation may be due to the ability of magnesium to reduce hepatic lipogenic enzymatic activity, increase lipoprotein lipase activity or enhance insulin action [28,29]. It has been known that Type 1 DM is due to insulin deficiency. Insulin deficiency leads to alterations in the metabolism of carbohydrate, lipids and central feature of the syndrome is chronic hyperglycaemia.

Table 1: Plasma lipid concentrations in the non-diabetic (ND), alloxan-diabetic (DM) and alloxan-diabetic+Mg (DM-Mg) rats

	ND	DM	DM-Mg
Total cholesterol (mmol/l)	2.39 ± 0.07^a	2.88 ± 0.07^b	1.77 ± 0.06^c
Triglyceride (mmol/l)	0.75 ± 0.02^a	0.99 ± 0.04^b	0.69 ± 0.04^c
LDL-cholesterol (mmol/l)	0.91 ± 0.06^a	0.79 ± 0.08^a	0.40 ± 0.03^b
HDL-cholesterol (mmol/l)	0.69 ± 0.03^a	0.47 ± 0.04^b	0.67 ± 0.02^a
HDLcholesterol/ LDL-cholesterol ratio	0.75 ± 0.06^a	$0.59 + 0.08^a$	1.68 ± 0.06^b
HDL-cholesterol/ total cholesterol ratio	0.29 ± 0.04^a	$0.15 + 0.04^b$	0.37 ± 0.06^a

Values are mean ± SEM, n=8 per group. Means in rows sharing different superscript letters^{a,b,c} are significantly different at p<0.05.

Table 2: Plasma levels of lipid peroxidation index, thiobarbituric-reactive substances (TBARS) and ascorbic acid in non-diabetic (ND), alloxan-diabetic (DM) and alloxan-diabetic+Mg (DM-Mg) rats

	ND	DM	DM-Mg
TBARS (ìmol/l)	2.21 ± 0.04^a	2.79 ± 0.05^b	1.78 ± 0.08^c
Vitamin C (ìmol/l)	$86.6 \pm 2.2.1^a$	73.5 ± 2.6^b	139.8 ± 6.1^c

Values are presented as means ± SEM, n=8 per group. Means in rows sharing different superscript letters^{a,b,c} are significantly different at p<0.05.

Table 3: Plasma concentrations of magnesium and calcium in non-diabetic (ND), alloxan-diabetic (DM) and alloxan diabetic+Mg (DM+Mg) rats

	ND	DM	DM-Mg
Magnesium (mmol/l)	0.82 ± 0.04^a	0.88 ± 0.05^a	1.09 ± 0.07^b
Calcium (mmol/l)	2.32 ± 0.06^a	2.35 ± 0.07^a	2.37 ± 0.05^a

Values are presented as means ± SEM, n=8 per group. Means in rows sharing different superscript letters^{a,b} are significantly different at p<0.05.

Insulin also increases the transcription of lipoprotein lipase (LPL) gene [30]. Thus, in Type 1 DM, where insulin is deficient, the reduction in LPL activity predisposes to hyperlipidaemia because of reduced catabolism of circulating lipoproteins [30]. It is important to note that the occurrence of vascular disease accounts for the majority of the clinical complications in DM [1]. Studies have shown an exaggerated degree of oxidative stress in DM, this condition leads to inactivation of nitric oxide, thus resulting in vascular disease [2,3]. Plasma levels of TBARS have been proposed to be reliable markers of lipid peroxidation [5,21] and in the present study; plasma level of TBARS was increased in untreated diabetic rats compared with the non-diabetic rats. Increased lipid peroxidation is thought to contribute to cardiovascular complications associated with DM [5,30]. Indeed, reduction in plasma lipid peroxidation with increased antioxidant vitamins, such as vitamin C [31] and vitamin E [30] decrease cardiovascular complications in patients with DM. One possible explanation for the pathophysiological role of lipid peroxidation may be that this free radical can inactivate nitric oxide and decrease prostacyclin formation, hence impair the endothelium dependent vasodilation [30].

A study from this environment by Anetor *et al* [32] showed that serum level of magnesium was significantly lower in patients with type 2 DM than in non-diabetics. The importance of magnesium on

glucose metabolism has been described in patients with type 2 DM [8,15,16,]. However, the benefits of magnesium supplementation, especially on risk factors for cardiovascular disease associated with DM remains to be fully evaluated [16]. The results from this study showed that diabetic rats treated with magnesium-enriched diet demonstrated remarkable increase in plasma magnesium levels that was accompanied by improvement in hyperglycaemia, hypercholesterolaemia, hypertriglycaemia and reduced lipid peroxidation. These results provide evidence for the potential utility of oral magnesium supplementation to bring about an increase in plasma magnesium levels and to prevent hyperglycaemia-related hyperlipidaemia in Type 1 DM, even when plasma magnesium is within a physiological range.

Previous studies have shown that diabetic rats exhibit similar abnormalities in vitamin C metabolism that are seen in patient with DM. Such abnormalities include reduced plasma ascorbic acid; decrease ascorbate/dehydroascorbate ratio and increased urinary excretion of ascorbate [6,7]. In the present study, diabetic animals had reduced plasma ascorbic acid concentrations in comparison with the non-diabetic animals. The possible explanation for this observation may be due to the increased utilization, increased urinary excretion, decreased regeneration of ascorbic acid or may relate to free radicals. Ascorbic acid is a water-soluble antioxidant, said to be more potent than other plasma components, such as vitamin E and plays a critical role at suppressing formation of advanced glycoscylating end-products (glycation) in plasma and tissues, inhibiting glyco-oxidation, and preventing lipid peroxidation by trapping the peroxyl radicals in the aqueous phase before it can interact with lipids in plasma and cell membrane [33]. Decreased plasma ascorbic acid and increased lipid peroxidation observed in untreated DM rats in this study suggests that lipids, glucose and proteins in the plasma and/or tissues are not protected against oxidation. This finding is in agreement with reports of other studies [2-7].

In the present study, diabetic animals fed on magnesium-enriched diet had significantly increased plasma ascorbic acid and this effect is accompanied by a decrease in plasma lipid peroxides. These results imply that a high magnesium diet could improve anti-oxidant capacity and reduce susceptibility of lipids in plasma or in tissue to oxidation. The improvements in ascorbic acid levels could be attributed to a sparing effect of magnesium on ascorbic acid by attenuating the oxidation of ascorbic acid [34]. This finding is in consonance with the study in non-diabetic subjects with chronic fatigue, where supplementation with magnesium led to significant increases in plasma vitamin E concentration and reduction in susceptibility of lipid to peroxidation [35]. Furthermore, HDL is an important antioxidant, which may improve the availability of nitric oxide by decreasing the formation of peroxynitrite [36]. The results from the present study show that increased intake of magnesium significantly increased plasma levels of HDL-cholesterol, HDL-cholesterol/total cholesterol ratio and HDL-cholesterol/LDL-cholesterol ratio. Hence improved lipid profile. The increased HDL-cholesterol levels especially may also play significant role in the cardioprotective effect of magnesium supplementation in patients with DM. Therefore, the ability of magnesium supplementation to increase plasma level of ascorbic acid, improve lipid profile and reduce lipid peroxidation may at least, partially, justify the potential utility of oral supplementation of magnesium or food-enriched with magnesium (such as fruits and vegetables) as adjunct agent in the management of DM [32].

Interestingly, in the ALX-diabetic rats not supplemented with magnesium, the plasma magnesium and calcium levels seem to be unchanged when compared with the non-diabetic rats. This finding is consistent with other studies in experimental animals [14] and humans [16] with Type 2 DM. In contrast, we found elevation in the plasma levels of magnesium but not in the plasma calcium in ALX-diabetic rats fed on a magnesium-enriched diet. This finding implies that either alloxan-induced DM or increased magnesium intake in alloxan-induced DM rats would not alter plasma calcium homeostasis. Thus, altered plasma calcium concentrations may not be linked to alterations in glucose and lipid metabolism, and oxidative stress associated with DM. This study also suggests that Type 1 DM may not be associated with depressed plasma levels of magnesium (Table 3). The elevated levels of plasma magnesium levels in diabetic rats fed on a high magnesium diet is in consonance with the reports of studies in patients with Type 2 DM [15] that used MgCl as employed in this study. Nevertheless, the study of Lima *et al* [16] that used MgO supplementation did not reveal increase in plasma magnesium levels in patients with Type 2 DM when compared with the non-diabetic control

subjects. The discrepancies in these studies might be due to differences in the magnesium salts or doses used. However, this study provides further evidence that a high-magnesium diet or oral supplementation with magnesium may be a necessity to achieve an elevated plasma magnesium concentration required to prevent the development of cardiovascular complications in patients with DM, even without depressed plasma magnesium levels.

In conclusion, the results of the present study suggest that Type 1 DM in animal models is associated with detrimental plasma lipid profile, increased lipid peroxidation and depressed plasma ascorbic acid levels. The beneficial effect of increased intake of magnesium could thus be due to reduced total cholesterol, triglyceride, lipid peroxidation, and enhanced HDL-cholesterol/total cholesterol ratio, HDL-cholesterol/LDL-cholesterol ratio and increased plasma ascorbic acid levels.

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