Magnesium Supplementation of STZ-Diabetic Rats. Lack of Effect on Diabetic Cardiomyopathy

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Zusammenfassung

Die Studie befaßt sich mit der Wirkung von Magnesiumgaben bei Wistar-Ratten, die mittels Streptozotozin-Injektionen zu Diabetikern gemacht wurden, auf ihre Glukose-Toleranz hin und das Eintreten von Kardiomyopathie. MgO_4 wurde im Trinkwasser in Konzentrationen von 0.3 g/l für diabetische Ratten und 0.9 g/l für nicht-diabetische Ratten verabreicht. Diese Einstellung resultierte in gleichen eingenommenen Magnesiummengen für beide Teile. Die Plasma-Mg-Werte wurden bei den nicht behandelten diabetischen Ratten signifikant reduziert, während sie andererseits bei den behandelten Ratten wieder in normale Höhe gestellt wurden. Die während der Behandlung gemessenen Glykämien sowie die Flächen unter den Glykämiekurven der Glukosetoleranztests waren nicht signifikant verschieden zwischen den behandelten und nicht behandelten Diabetikergruppen. Die myokardische Funktion, welche bei der nicht behandelten diabetischen Gruppe im Vergleich zur nicht diabetischen (behandelt und nicht behandelt) gesenkt war, wurde durch die Magnesiumsupplementierung nicht verbessert.

Summary

The effects of Mg supplementation on glucose tolerance and diabetes-induced cardiomyopathy were investigated in STZ-diabetic, male Wistar rats. $MgSO_4$ was administered in the drinking water at a concentration of 0.3 g/l for diabetic, and 0.9 g/l for nondiabetic, supplemented rats. This adjustment resulted in similar supplemental Mg intakes for both groups. Plasma Mg levels were significantly depleted in untreated diabetic rats, but were restored to normal levels in the Mg-treated, diabetic animals. Blood glucose levels at termination and the areas under the curve of glucose tolerance test responses were not significantly different between the treated and untreated diabetic groups. Myocardial function, depressed in the untreated diabetic group compared to both treated and untreated controls, was also not improved by Mg supplementation.

Résumé

Les effets d'un traitement par magnésium sur la tolérance au glucose et la cardiomyopathie induite par le diabète ont été étudiés chez le Rat Wistar rendu diabétique par la streptozotocine. La supplémentation en magnésium pendant 10 semaines a permis une restauration des taux plasmatiques en magnésium des rats diabétiques. Elle n'a cependant induit aucune amélioration de la réponse intégrée au test de tolérance au glucose déterminée par les surfaces sous les courbes de glycémie, ou de la fonction cardiaque déterminée au niveau ventriculaire gauche sur modèle de coeur isolé "travaillant" par la mesure de la pression développée, de la force de contraction et de la vitesse de relaxation.

Introduction

Hypomagnesemia and hypermagnesuria are consistent findings in both human and experimental animal studies of diabetes-induced changes in metal ion metabolism [1]. It has been suggested that magnesium deficiency may be implicated in the pathogenesis and prognosis of ischemic heart disease [2]. In humans, atherosclerosis, a common secondary complication of diabetes, is associated with hypomagnesemia; while in rats diabetes is accompanied by a characteristic cardiomyopathy within 6 to 8 weeks of induction of diabetes, as well as hypomagnesemia and hypermagnesuria [3–5].

Despite the fact that serum magnesium is generally considered a poor indicator of magnesium status, the frequency with which diabetic patients develop low serum magnesium suggests the possibility that it may be related to the deficiency in heart function which also accompanies diabetes [6]. Hypomagnesemia has also been shown to be a risk factor for development of diabetic retinopathy [7].

Results of a number of studies, as analyzed by metaanalysis [8], have shown that magnesium replacement reduces mortality from postmyocardial infarction. Although the studies included in the metaanalysis were not focussed on diabetic subjects, there is no reason to believe that these effects would be substantially different in diabetic patients compared to nondiabetic patients. In fact, recent studies of magnesium supplementation in noninsulin dependent diabetic subjects have shown improved insulin response and glucose handling [9]. These findings prompted us to investigate the possible prevention of diabetic cardiomyopathy by oral supplementation with magnesium in the drinking water.

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Material and Methods

Animals

Thirty-two male, Wistar rats (Charles River, Quebec, Canada), weighing 190–200 g were randomly allocated to 1 of 4 groups: control (C), magnesium-supplemented (CT), diabetic (D), and magnesium-supplemented diabetic (DT). Animals were housed in polyethylene cages (2 rats/cage), at constant temperature (21 ± 1 °C) on a 12 hour light/dark cycle. Food (Purina rat chow) and drinking water were available ad libitum. Purina rat chow contains 2.1 gm Magnesium per kg.

Diabetic induction

Diabetes was induced by a single tail vein injection of streptozotocin (STZ, Sigma, St. Louis, MO), 55 mg/kg body weight, freshly prepared in saline solution and administered under light halothane anesthesia (Fluothane, Ayerst, New York, NY). Control animals received a comparable volume of saline via the same route. Four days after STZ administration, blood was obtained by tail snipping for determination of blood glucose concentration using a Glucometer II (Ames, Miles Elkhart, IN). Rats with a blood glucose level below 11 mM (four rats) were excluded from the study.

Magnesium supplementation

Magnesium supplementation consisted of providing magnesium sulfate, 0.3 mg/ml (2.5 mM) in the drinking water for CT and DT groups. Treatment was started one week after administration of STZ. Magnesium sulfate concentration for CT was increased to 0.9 mg/ml (7.5 mM) after 3 weeks of treatment in order to compensate for the higher fluid intake in DT.

Blood glucose and glucose tolerance testing

Blood glucose levels (non-fasting) were determined weekly in tail-vein blood. Glucose tolerance testing, in which a 1 g/kg body weight oral glucose load was administered by gavage at time 0 to 12-hour fasted rats, and blood samples collected at 0, 10, 20, 30 and 60 minutes, was performed on all diabetic rats. The results were plotted and the areas under the curve of plasma glucose levels were determined.

Plasma magnesium assay

Plasma magnesium concentrations were determined prior to supplementation, and at week 3, 6 and 10 of the experiment, by atomic absorption spectrophotometry following wet ashing with concentrated nitric acid (BDH, ultrapure, London, Ontario, Canada).

Collection of tissues

At the end of week 10, rats were anesthetized by the intraperitoneal injection of pentobarbital and killed by cardiac excision. Kidneys and liver were blotted dry, flash frozen in liquid nitrogen and stored at −70 °C for magnesium assay (< 2 weeks post-autopsy). Blood was collected and centrifuged at 3.000 rpm for 15 minutes at 4 °C in a tabletop clinical centrifuge. Plasma was removed and stored at −70 °C until analyzed.

Heart function tests

Myocardial performance of the rats was examined using the isolated working heart apparatus as described by *Neely* et al. [10] with minor modifications [11]. Cardiac function data, including left ventricular developed pressure (LVDP), rate of force development (+dP/dT), rate of ventricular relaxation (−dP/dT), and heart rate were collected using a Grass 79D polygraph (Grass Instrument Co., Quincy, Mass.).

Statistical analysis

Data are expressed as means ± SEM. Between group differences were analyzed for statistical significance by ANOVA, followed by Newman-Keuls test for within group variation. A probability of < 0.05 was accepted as statistically significant.

Results

The general characteristics of the rats are outlined in tab. 1. Diabetic rats were lighter than non-diabetic, as expected from previous studies. Magnesium treatment had no effect on body weight. Heart weights, and heart to body weight ratios, were also significantly affected by STZ-diabetes, but not by magnesium supplementation (tab. 1).

Plasma glucose levels of the diabetic rats, both D and DT, were significantly higher than those of the control groups, C and CT (tab. 1). Plasma triglyceride and cholesterol levels were higher, and plasma insulin levels significantly lower, in diabetic compared to non-diabetic rats; however, magnesium supplementation had no significant effect (data not shown).

Magnesium intake was significantly higher in DT than in CT, even with the adjustment to a higher concentration of Mg in the CT group.

The area under the curve of glucose levels, with baseline subtracted, in response to oral glucose tolerance testing was not different between C and CT. Both diabetic and DT curves were higher than controls, reflecting the diabetic state, but were not significantly

Tab. 1: Effects of STZ-diabetes and magnesium supplementation on body weights, blood glucose levels, heart weights, and heart/body weight ratios.

	C	CT	D	DT
(n)	8	8	6	6
Body weight (g)	591 ± 8.8	577 ± 14.1	443 ± 26.8*	425 ± 11.4*
Plasma glucose (mM)	5.5 ± 0.2	5.2 ± 0.2	18.3 ± 1.0*	17.4 ± 1.2*
Mg supplement (mg/day)	0	71.7 ± 1.0	0	88.8 ± 3.2*
Heart weight (g)	2.33 ± 0.04	2.28 ± 0.02	1.93 ± 0.06*	1.91 ± 0.07*
Heart/Body weight ratio (g x 10^3)	3.9 ± 0.1	4.0 ± 0.1	4.4 ± 0.2*	4.5 ± 0.1*

Values are means ± SEM. Magnesium (Mg) supplement is the calculated additional intake from magnesium sulfate in the drinking water at concentrations of 0.3 g/l for DT and 0.9 g/l for CT groups. STZ, streptozotocin; D, diabetic rats; C, control; CT, nondiabetic rats supplemented with magnesium sulfate; DT, diabetic rats supplemented with magnesium sulfate; n, number of animals. *$P < 0.05$ diabetic vs. control.

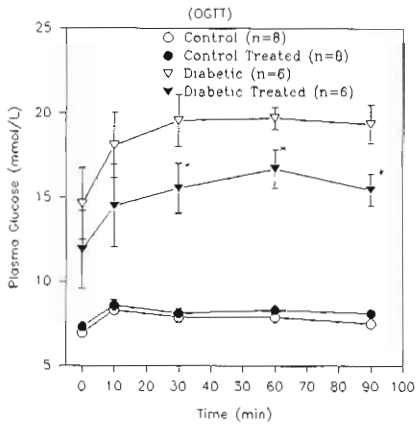

Fig. 1: Plasma glucose concentrations before and after oral administration of glucose (1 g/kg) in the four groups of experimental animals following ten weeks of treatment. Values are means ± SEM. *p < 0.05 vs. diabetic untreated.

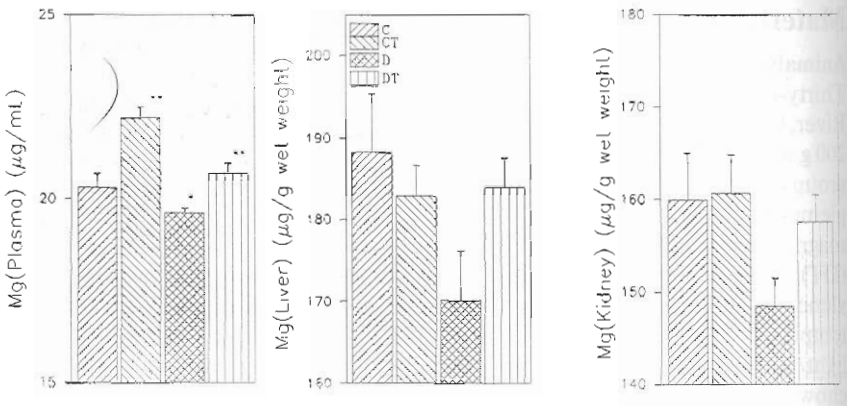

Fig. 2: Plasma, liver and kidney Mg concentrations in treated and untreated, diabetic and nondiabetic rats following treatment for 10 weeks. Treatment consisted of $MgSO_4$ in the drinking water, 0.3 g/l for diabetic, and 0.9 g/l for nondiabetic rats. C, control (n = 8), CT, Mg-treated, non-diabetic (n = 8); D, diabetic (n = 6), DT, Mg-treated, diabetic (n = 6). Mg was measured in wet ashed samples by AAS. Values are means ± SEM. *p < 0.05 vs. control; **p < 0.01 vs. control and diabetic untreated.

different from each other (fig. 1). However, plasma glucose levels at 30, 60, and 90 minutes, when evaluated individually, were significantly higher in D compared with DT rats (fig. 1).
Magnesium levels in plasma of D were significantly lower than in C, and were corrected by magnesium supplementation ($P < 0.05$), although the effect appeared to be slight (fig. 2).
There were not significant differences between magnesium levels in the four groups in liver or kidney magnesium content (fig. 3a and b).
LVDP and +dP/dT were significantly depressed in both diabetic groups (D and DT) at higher filling pressures (fig. 4). Magnesium supplementation had no ameliorative effect. Rates of relaxation (−dP/dT) were significantly greater in CT compared to D at higher filling pressures, but were otherwise unchanged between groups.

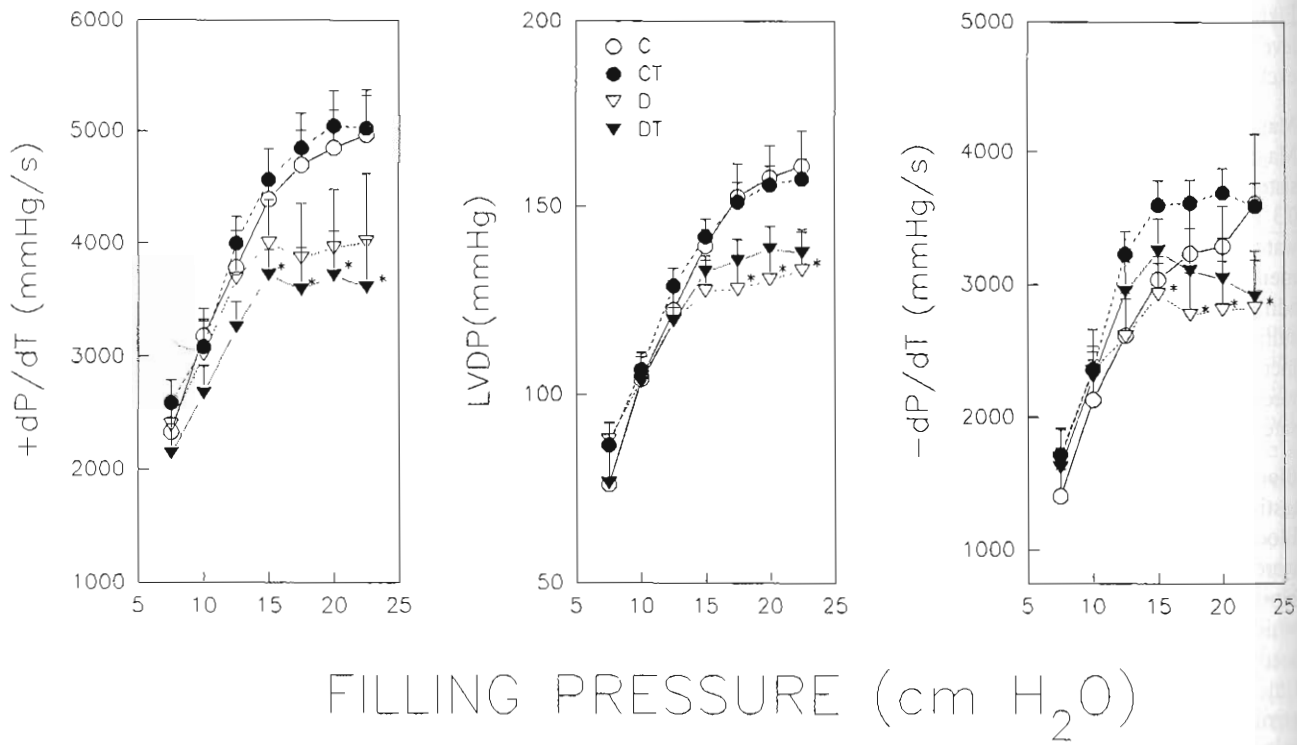

Fig. 3: Mechanical function of isolated perfused working heart. The working heart preparation was used to assess myocardial function in the four groups of rats, as described for fig. 2. Three indices of isolated heart function: a) left ventricular developed pressure (LVDP), b) rate of contraction (+dP/dT), and c) rate of relaxation (−dP/dT) were recorded at atrial filling pressures between 7.5 and 22.5 cm H_2O. Values are means ± SEM. *p < 0.05 vs. nondiabetic.

Discussion

That STZ-diabetes in the rat is accompanied by a form of cardiomyopathy characterized by diminished rate of contraction and lower left ventricular developed pressure had been shown in a number of investigations [3, 4, 12]. In this study, magnesium supplementation did not appear to prevent the characteristic diabetes-induced cardiomyopathy. While it is possible that the time-frame was too short to demonstrate this effect (a deficiency of magnesium was not shown in either liver or kidney in the diabetic untreated group); it is nonetheless clear that magnesium, at a concentration in the drinking water that was high enough to reverse the hypomagnesemia, is not as effective as either carnitine [13] or vanadyl sulfate [14] in preventing cardiac dysfunction in diabetic rats.

Indications of a modest improvement in glucose tolerance, as shown by significant reductions in plasma glucose at individual time-points following an oral glucose challenge, suggest that magnesium supplementation may be a useful adjunct in diabetic treatment [9]. Since a majority of studies demonstrating a positive effect of magnesium supplementation in diabetes have dealt with type II diabetics, a more appropriate model to examine these effects might be the Zucker rat, or the ob/ob mouse, both of which more closely approximate the human noninsulin dependent diabetic state [15].

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References

[1] *Grafton, G.; Baxter, M.A.:* The role of magnesium in diabetes mellitus. A possible mechanism for the development of diabetic complications. J. Diab. Comp. 6 (1992) 143-149.

[2] *Levin, G.E.; Mather, H.M.; Pilkington, T.R.E.:* Tissue magnesium status in diabetes mellitus. Diabetologia 21 (1981) 131-134.

[3] *Vadlamudi, R.V.S.V.; Rodgers, R.L.; McNeill, J.H.:* The effect of chronic alloxan- and streptozotocin-induced diabetes on isolated rat heart performance. Can. J. Physiol. 60 (1982) 902-911.

[4] *Lopaschuk, G.D.; Tahiliani, A.G.; Vadlamudi, R.V.S.V.; Katz, S.; McNeill, J.H.:* Cardiac sarcoplasmic reticulum function in insulin- or carnitine-treated diabetic rats. Am J. Physiol. 245 (1983) H969-H976.

[5] *Bhimji, S.; Godin, D.V.; McNeill, J.H.:* The status of magnesium in rabbits with experimentally-induced diabetes mellitus. Horm. Metab. Res. 18 (1986) 734-738.

[6] *Elamin, A.; Tuvemo, T.:* Magnesium and insulin-dependent diabetes mellitus. Diab. Res. and Clin. Practice 10 (1990) 203-209.

[7] *McNair, P.; Christiansen, C.; Madsbad, S.; Lauritzen, E.; Faber, O.; Binder, C.; Transbol, I.:* Hypomagnesemia, a risk factor in diabetic retinopathy. Diabetes 27 (1978) 1075-1077.

[8] *Teo, K.K.; Yusuf, S.; Collins, R.; Held, P.H.; Peto, R.:* Effects of intravenous magnesium on suspected acute myocardial infarction: overview of randomized trials. Br. Med. J. 303 (1991) 1499-1503.

[9] *Paolisso, G.; Sgambato, S.; Pizza, G.; Passariello, N.; Varricchio, M.; D'Onofrio, F.:* Improved insulin response and action by chronic magnesium administration in aged NIDDM subjects. Diabetes Care 12 (1989) 265-269.

[10] *Neely, J.R.; Leibermeister, H.; Battersby, E.J.; Morgan, H.E.:* Effect of pressure development on oxygen consumption by isolated rat heart. Am. J. Physiol. 212 (1967) 804-814.

[11] *Dai, S.; McNeill, J.H.:* Myocardial performance of STZ-diabetic DOCA-hypertensive rats. Am. J. Physiol. 263 (Heart Circ. Physiol. 32) (1992) H1798-H1809.

[12] *Fein, F.S.; Kornstein, L.B.; Strobeck, J.E.; Capasso, J.M.; Sonnenblick, E.H.:* Altered myocardial mechanics in diabetic rats. Circ. Res. 47 (1980) 922-933.

[13] *Rodrigues, B.; Xiang, H.; McNeill, J.H.:* Effect of L-carnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. Diabetes 37 (1988) 1358-1364.

[14] *Ramanadham, S.; Cros, G.H.; Mongold, J.J.; Serrano, J.J.; McNeill, J.H.:* Enhanced in vivo sensitivity of vanadyl-treated diabetic rats to insulin. Can. J. Physiol. Pharmacol. 68 (1990) 486-491.

[15] *Bell, Jr., R.H.; Hye, R.J.:* Animal models of diabetes mellitus: physiology and pathology. J. Surg. Res. 35 (1983) 433-460.

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