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Magnesium deficiency improves glucose homeostasis in the rat: studies *in vivo* and in isolated islets *in vitro*

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The serum mineral levels, glucose disappearance rate (k_g), total area under the glucose (ΔG) and insulin (ΔI) curves, and static insulin secretion were compared among rats fed a Mg-deficient diet for 6 (DF-6) or 11 (DF-11) weeks, and rats fed a control diet for the same periods (CO-6 and CO-11 groups). No change in glucose homeostasis was observed among DF-6, CO-6 and CO-11 rats. DF-11 rats showed an elevated k_g and a reduced ΔG and ΔI. For evaluating the effect of supplementation, rats fed a control or Mg-deficient diet for 6 weeks were then fed a Mg-supplemented diet for 5 weeks (SCO and SDF groups respectively). The serum Mg levels in SDF rats were similar to those in CO-11 and SCO rats, but higher than in the DF-11 group. SDF rats showed similar k_g, ΔG and ΔI compared with the CO-11 and SCO groups. However, a significantly lower k_g and higher ΔG and ΔI were observed in SDF compared with DF-11 rats. Basal and 8·3 mmol glucose/l-stimulated insulin secretion by islets from DF-11 rats were higher than by islets from CO-11 rats. These results indicate that moderate Mg depletion for a long period may increase the secretion and sensitivity to insulin, while Mg supplementation in formerly Mg-deficient rats may prevent the increase in sensitivity and secretion of insulin.

Magnesium deficiency: Insulin sensitivity: Glucose homeostasis: Rats

Numerous *in vitro* studies have demonstrated a major role for Mg in insulin action and secretion (Ishizuka *et al.* 1994; Kandeel *et al.* 1996). Mg deficiency affects the activities of various enzymes which require the ion as a cofactor and use high-energy phosphate bonds in glucose metabolism (Elin, 1994). It has been shown that diabetes mellitus is the chronic disease most frequently associated with hypomagnesaemia. However, comparatively few metabolic alterations have been demonstrated unequivocally in this type of deficiency (Legrand *et al.* 1987; Suárez *et al.* 1995) and during supplementation *in vivo*, suggesting that it may be premature to assign a primary involvement to Mg in abnormal carbohydrate metabolism and insulin secretion (Garber *et al.* 1995).

Thus, as suggested by most studies presented to date, Mg may play a role in glucose homeostasis. Therefore, the present experiments were performed to study the effects of chronic Mg deficiency on the glucose tolerance and insulin secretion in rats, and to examine the influence of Mg supplementation on these responses.

Material and methods

Male Wistar rats (21-d-old, 40–60 g) bred in the animal facilities of the State University of Campinas São Paulo, Brazil, were used in all experiments. The rats were housed individually in stainless steel, wire-bottomed cages in a temperature-controlled room (22 ± 2°C) on a 12 h light–

Abbreviations: CO, control group; DF, magnesium-deficient group; k_g, glucose disappearance rate; SCO, magnesium-supplemented control group; SDF, magnesium-supplemented deficient group; ΔG, total area under glucose curve; ΔI, total area under insulin curve.

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dark cycle and were fed a commercial stock diet (Purina Paulínia, Brazil) for 7 d after arrival. Subsequently, the study was divided into two phases. In the first phase, two groups of 4-week-old rats were used: a control group (CO-6, *n* 29) consisting of rats fed a diet containing 507 mg Mg/kg for 6 weeks, and a deficient group (DF-6, *n* 40) consisting of rats fed a diet containing 70 mg Mg/kg for 6 weeks. In the second phase, some rats from each of these two groups were divided into two subgroups; those that continued with the original diet for a further 5 weeks, i.e. a total of 11 weeks (CO-11, *n* 12 and DF-11, *n* 14), and those that were repleted by changing to a Mg-supplemented diet containing 2100 mg Mg/kg (SCO, *n* 5 and SDF, *n* 10). The composition of diets was described elsewhere (Reis *et al.* 2000).

The rats had free access to deionized water and food throughout the study. Body weight of some animals was verified weekly (CO-6 *n* 12, DF-6 *n* 22, CO-11 *n* 8, DF-11 *n* 10, SCO *n* 5 and SDF *n* 10). At the end of each experimental period and after a 12 h fast, blood samples were collected from the abdominal cava vein for the measurement of serum Mg, Ca and K levels. The levels of minerals and the Mg content of the diets were determined as previously described (Reis *et al.* 2000). All experiments involving animals were approved by the State University of Campinas Ethics Committee (São Paulo, Brazil).

Intravenous glucose tolerance test

Glucose (0·75 g/kg body weight) was injected via the caudal vein 3 d before the end of each experimental period and after a 12 h fast. Plasma glucose and serum insulin concentrations were measured by the oxidase method (Trinder, 1969) and by radioimmunoassay (Scott *et al.* 1981) respectively, in samples obtained from the cut tip of the tail at 0 (basal) and at 5, 10, 15 and 30 min after injection. The glucose disappearance rate (k_g) during the intravenous glucose tolerance test was calculated using the formula $0{\cdot}693/t_{1/2}$ and the plasma glucose half-time ($t_{1/2}$) was calculated from the slope of the least square analysis of the plasma glucose concentrations during the linear phase of decline. To determine the total area under the glucose (ΔG) and insulin (ΔI) curves, the trapezoidal method was used (Matthews *et al.* 1990).

Islet isolation and insulin secretion

Islets from CO-11, DF-11 and SDF rats were isolated and the insulin secretion was measured as previously described (Latorraca *et al.* 1998). $MgCl_2$ concentrations of the incubation media were: 1·0, 0·5 or 2·0 mM for CO-11, DF-11 and SDF groups respectively.

Statistical analysis

The results are given as the means and standard deviations for the number of rats indicated. When comparing the body weight of CO-6 and DF-6 groups, Student's non-paired *t* test was used. For the remaining comparisons, Levene's test for the homogeneity of variance was initially used to check the fit of the data to assumptions for parametric ANOVA. When working with islets, data were log-transformed to correct for variance heterogeneity or non-normality. All data were then analysed by two-way ANOVA (or one-way for body weight), followed by the Tukey-Kramer test for multiples comparisons. In islets, *n* 6–11 refers to the number of experiments performed, and each experiment was performed with islets from four rats per group. Data was analysed by Statistica for Windows, release 4.3, 1993 (StatSoft, Inc. Tulsa, OK, USA).

Results

In DF-6 rats, the severity of the classic symptoms of Mg deficiency varied, with some rats developing ulcerative lesions around the head and neck and hyperaemia of the ears. In most DF-6 rats, hyperexcitability was noticed. All the skin lesions disappeared after the initial 6 weeks.

The serum Mg levels of DF-6 rats (Table 1) were lower than in DF-11, and both were lower ($P < 0{\cdot}01$) than those of CO-6 and CO-11 rats. A significant diet ($P < 0{\cdot}01$) and time ($P < 0{\cdot}01$), as well as a significant diet×time interaction ($P < 0{\cdot}01$) were found.

The mean serum Mg levels of SDF and SCO groups (0·91 (SD 0·11) mmol/l, *n* 10 and 1·00 (SD 0·09) mmol/l, *n* 5 respectively) were similar to CO-11 rats, and all had serum Mg levels significantly greater than DF-11 rats. A significant effect of the previous diet ($P < 0{\cdot}01$), supplementation ($P < 0{\cdot}01$), and a previous diet×supplementation interaction ($P < 0{\cdot}01$) were observed.

Table 1. Serum magnesium, calcium and potassium, glucose disappearance rate (k_g) and total areas under the glucose (ΔG) and insulin (ΔI) curves in rats fed control (CO-6 and CO-11) or Mg- deficient (DF-6 and DF-11) diets for 6 or 11 weeks respectively*

(Mean values and standard deviations)

	CO-6			DF-6			CO-11			DF-11			Statistical significance of effect of:		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Diet (P =)	Time (P =)	Diet×time (P =)
Mg (mmol/l)	0·93[a]	0·06	12	0·30[c]	0·04	12	0·85[a]	0·15	8	0·46[b]	0·05	10	0·000	0·009	0·000
Ca (mmol/l)	2·0[b]	0·17	12	2·4[a]	0·32	12	2·2[b]	0·10	8	2·0[b]	0·15	10	0·028	0·014	0·000
K (mmol/l)	3·02	0·32	12	3·34	0·35	12	3·47	0·21	8	3·40	035	10	NS	NS	NS
k_g (%/min	1·9[b]	0·60	6	1·9[b]	0·72	10	1·8[b]	0·35	6	3·2[a]	1·22	7	0·031	NS	0·031
ΔG (mmol/1·30 min)	445[a]	33	6	455[a]	67	10	432[a]	77	6	321[b]	50	7	0·036	0·037	0·014
ΔI (nmol/1·30 min)	6·8[ab]	0·95	6	8·6[a]	2·0	10	7·8[a]	2·5	6	4·0[b]	1·96	7	NS	0·03	0·001

[a,b] Mean values with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

* For details of composition of diets, see Reis *et al.* (2000). For details of procedures, see p. 550.

The body weight of DF-6 rats was significantly less ($P < 0{\cdot}05$) than that of CO-6 rats after 3 weeks. However, after 7 weeks of treatment there was no significant difference among rats from second phase (data not reported).

No significant effect on fasting plasma glucose and insulin concentrations were observed in the first and second phase groups (data not reported).

The diet and time effect on k_g in CO-6, CO-11, DF-6 and DF-11 groups is shown in Table 1. A significant diet effect ($P < 0{\cdot}05$), no time effect, and a diet×time interaction ($P < 0{\cdot}05$) were observed. The highest k_g was observed in DF-11 group ($P < 0{\cdot}05$).

A significant previous diet effect ($P < 0{\cdot}05$), a supplementation×previous diet interaction ($P < 0{\cdot}01$) and no supplementation effect were seen for k_g. SDF rats had a reduced mean k_g (1·9 (SD 0·42) %/min, n 9) relative to DF-11 rats ($P < 0{\cdot}05$), but it was similar to the other groups.

ΔG and ΔI in rats fed for 6 or 11 weeks with control or Mg-deficient diets are shown in Table 1. The effect of the diet ($P < 0{\cdot}05$), time ($P < 0{\cdot}01$) and the interaction between them ($P < 0{\cdot}05$) had a significant influence on ΔG. The DF-11 rats had a lower ΔG than CO-6 ($P < 0{\cdot}01$), DF-6 ($P < 0{\cdot}01$) and CO-11 rats ($P < 0{\cdot}05$). Diet had no significant effect on ΔI, whereas the time ($P < 0{\cdot}05$), and the diet×time interaction ($P < 0{\cdot}01$) affected ΔI (Table 1). DF-11 rats showed decreased ΔI relative to DF-6 ($P < 0{\cdot}01$) and CO-11 rats ($P < 0{\cdot}05$).

SDF rats had a higher mean ΔG (416 (SD 50) mmol/1·30 min, n 9) than DF-11 rats ($P < 0{\cdot}05$). A significant previous diet effect ($P < 0{\cdot}01$), supplementation ($P < 0{\cdot}05$) and a previous diet×supplementation interaction ($P < 0{\cdot}05$) were seen for ΔG.

ΔI of SDF rats (7·84 (SD 3·2) nmol/1·30 min, n 9) was higher ($P < 0{\cdot}01$) than that of DF-11 rats, but similar to other groups. A significant previous diet×supplementation interaction ($P < 0{\cdot}05$) was seen.

Determination of insulin secretion by islets from CO-11, DF-11 and SDF groups over a glucose range of 2·8 to 16·7 mmol/l revealed a significant effect of the group ($P < 0{\cdot}01$) and glucose concentration ($P < 0{\cdot}01$) as well as a group×concentration interaction ($P < 0{\cdot}05$). Mean insulin secretion in 2·8 and 8·3 mmol/l glucose by islets from DF-11 group was greater (1·83 (SD 0·87), n 8 and 3·05 (SD 1·03), n 10 ng/islet per h respectively) compared with CO-11 (0·57 (SD 0·26), n 6 and 1·00 (SD 0·58), n 9 ng/islet per h respectively; ($P < 0{\cdot}05$). At 16·7 mmol/l glucose, no difference in the insulin secretion was observed among islets of three groups. In presence of 16·7 mmol/l glucose there was an additional increment in the insulin secretion (compared with 2·8 mmol/l) in islets from CO-11 group ($P < 0{\cdot}05$), but not in those from DF-11 and SDF groups (Fig. 1).

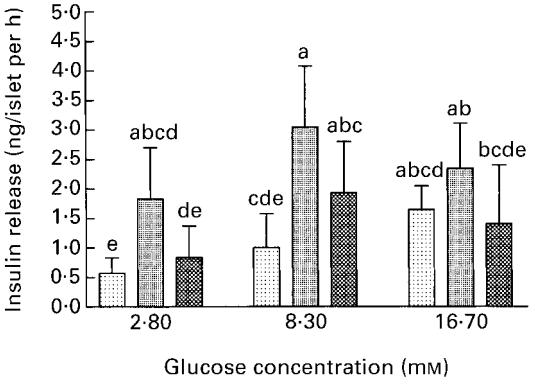

Fig. 1. Glucose-stimulated release of insulin by pancreatic islets from control (▤, CO-11), magnesium-deficient (▨, DF-11) and supplemented magnesium-deficient (▪, SDF) rats. For details of composition of diets see Reis *et al.* (2000) and for details of procedures, see p. 550. The islets were incubated with different concentrations of glucose and magnesium chloride (see p. 550). Values are means for six to eleven experiments, with standard deviations shown by vertical bars.[a,b,c,d,e] Mean values with unlike superscript letters were significantly different between groups and conditions ($P < 0{\cdot}05$).

Discussion

A time-dependent effect on glucose tolerance was observed in the present Mg-deficiency model. Mg deficiency for an initial period of 6 weeks did not affect glucose homeostasis, whereas a longer period resulted in improved glucose tolerance in DF-11 rats, as shown by the elevated k_g and low ΔG in the presence of a reduced ΔI. A previous study suggested that acute Mg depletion leads to insulin resistance, while chronic cellular Mg depletion leads to accelerated glucose uptake in muscle (Legrand *et al.* 1987). Although it may seem that the present results are not in accordance with those findings by Legrand *et al.* (1987) as 6 weeks treatment is considered a chronic depletion, the changes in glucose tolerance observed at 11 weeks suggest that in a less severe treatment regimen the alterations last longer.

Increased non-insulin-mediated glucose uptake and enhanced hepatic insulin sensitivity (Lowney *et al.* 1995) may be involved in the alterations observed in DF-11 rats. Furthermore, the reduced insulin response to glucose load (ΔI) could also play a role by upregulating insulin sensitivity (Latorraca *et al.* 1998).

The reduced k_g and consequently elevated ΔG and ΔI seen in SDF rats compared with DF-11 rats, showed that Mg supplementation prevents the increase in glucose tolerance in the former rats compared with the latter. In addition, Mg supplementation did not change glucose tolerance in SCO or SDF rats compared with CO-11 rats. Interestingly, an elevated uptake of 2-deoxyglucose (2-DG) in the diaphragm of Mg-deficient rats was promptly reversed by the addition of Mg to the incubation medium (Kahil *et al.* 1968).

The reduced ΔI in contrast to increased insulin secretion in DF-11 rats may be due to an increased hepatic insulin clearance during the intravenous glucose tolerance test and/or different Ca^{2+}:Mg^{2+} ratios during the intravenous glucose tolerance test and the insulin secretion experiments (Curry *et al.* 1977; Lowney *et al.* 1995). It has been suggested that the ratio of these ions is more important for maintaining an optimal secretory response than is the absolute concentration of either ion.

The finding that islets of DF-11 rats show an elevated insulin secretion at 2·8 and 8·3 mmol/l glucose and similar values at 16·7 mmol/l glucose in comparison with CO-11 rats could indicate a greater islet sensitivity at lower

glucose concentrations. However, according to Ishizuka *et al.* (1994) extracellular Mg may competitively inhibit Ca currents leading to decreased insulin secretion. In the absence of Mg in the extracellular space this inhibition would not occur resulting in enhanced insulin secretion. Since a lowered pancreatic insulin content was described in Mg-deficient rats (Legrand *et al.* 1987) the increased insulin release at 2·8 and 8·3 mmol/l glucose could further reduce the insulin stores and consequently decrease the insulin response to higher glucose concentration as observed in the ΔI of DF-11 rats.

Mg supplementation for SDF rats apparently prevented some of the Mg deficiency effects on β-cells, probably due to the direct inhibitory effect of Mg on these cells (Ishizuka *et al.* 1994). A normal islet glucose sensitivity, hepatic insulin clearance and insulin content are suggested, since similar results between CO-11 and SDF rats were recorded.

In conclusion, our results show that Mg is involved in glucose homeostasis in rats, so that Mg depletion leads to increased insulin secretion, which is prevented by Mg supplementation. In diabetes mellitus hypomagnaesemia may act by improving β-cell glucose sensitivity and thus participating in the exacerbated pancreatic response to hyperglycaemia.

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References

Curry DL, Joy RM, Holley DC & Bennett LL (1977) Magnesium modulation of glucose-induced insulin secretion by perfused rats pancreas. *Endocrinology* **101**, 203–208.

Elin RJ (1994) Magnesium. The fifth but forgotten electrolyte. *American Journal of Clinical Pathology* **102**, 616–622.

Garber A, Blackard WG, Feinglos M, Haffner S, Lasagna L, Maryniuk M, Rivlin R & Sussman KE (1995) Consensus statement. Magnesium supplementation in the treatment of diabetes. *Diabetes Care* **18**, Suppl. 1, 83–85.

Ishizuka J, Bold RJ, Townsend CM Jr & Thompson JC (1994) In vitro relationship between magnesium and insulin secretion. *Magnesium Research* **7**, 17–22.

Kahil ME, Simons EL & Brown H (1968) Magnesium deficiency and sugar transport in muscle. *Diabetes* **17**, 673–678.

Kandeel FR, Balon E, Scott S & Nadler JL (1996) Magnesium deficiency and glucose metabolism in rat adipocytes. *Metabolism* **45**, 838–843.

Latorraca MQ, Carneiro EM, Boschero AC & Mello MAR (1998) Protein deficiency during pregnancy and lactation impairs glucose-induced insulin secretion but increases the sensitivity to insulin in weaned rats. *British Journal of Nutrition* **80**, 291–297.

Legrand C, Okitolonda W, Pottier AM, Lederer J & Henquin JC (1987) Glucose homeostasis in magnesium-deficient rats. *Metabolism* **36**, 160–164.

Lowney P, Hannon TS & Baron AD (1995) Magnesium deficiency enhances basal glucose disposal in the rat. *American Journal of Physiology* **268**, E925–E931.

Matthews JNS, Altman DG, Campbell MJ & Royston P (1990) Analysis of serial measurements in medical research. *British Medical Journal* **27**, 230–235.

Reis MAB, Reyes FGR, Saad MJA & Velloso LA (2000) Magnesium deficiency modulates the insulin signaling pathway in liver but not muscle of rats. *Journal of Nutrition* **130**, 133–138.

Scott AM, Atwater I & Rojas E (1981) A method for the simultaneous measurement of insulin released and β-cell membrane potential in single mouse islets of Langerhans. *Diabetologia* **21**, 407–475.

Suaréz A, Pulido N, Casla A, Casanova B, Arrieta FJ & Rovira A (1995) Impaired tyrosine-kinase activity of muscle insulin receptors from hypomagnesaemic rats. *Diabetologia* **35**, 1262–1270.

Trinder P (1969) Determinations of blood glucose using on oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology* **22**, 158–161.