Magnesium supplementation in streptozotocin-induced diabetes: Comparison of metabolic control and tissue magnesium status in male and female Wistar rats

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Zusammenfassung

Es wurden die Wirkung der Magnesiumzufuhr (10 mM Mg in der Trinkflüssigkeit) auf den Mg-Gehalt der Knochen und des Gewebes sowie Anzeichen für einen Status Glycaemicus bei STZ-diabetischen männlichen und weiblichen Wistar-Ratten untersucht. Die Mg-Plasmaspiegel waren bei den diabetischen Tieren weder erniedrigt noch korrelierten sie mit den Blutglukose- oder Fruktosaminkonzentrationen oder HBA1c-Spiegeln. Bei den männlichen, nicht aber bei den weiblichen Tieren verringerte die Mg-Zufuhr den mit STZ-Diabetes verbundenen Verlust an Körpermasse. Der femorale Mg-Gehalt war bei männlichen und weiblichen zusatzernährten Kontrolltieren erniedrigt. Bei weiblichen diabetischen Ratten zeigte sich ein erhöhter vertebraler Mg-Gehalt, wobei allerdings keine Veränderungen im Mg-Gehalt der Rippen beobachtet wurden. Bei den diabetischen Ratten war, mit Ausnahme der Skelettmuskeln, das Verhältnis von Gewebemasse zur Körpermasse diabetischer Tiere erhöht. Erhöhungen des Mg-Gehaltes wurden in den Geweben in Bezug auf das Nassgewicht nicht aber bezogen auf den DNS-Gehalt beobachtet.

Summary

The effects of magnesium (Mg) supplementation (10 mM in drinking fluid) on bone and tissue Mg content and indices of glycaemic status were investigated in STZ-diabetic male and female Wistar rats. Plasma Mg levels were not reduced in diabetic animals nor did they correlate with blood glucose or fructosamine concentration or HbA1c levels. In male, but not female, animals Mg supplementation reduced the weight loss associated with STZ-diabetes. Femoral Mg content was reduced in male and female supplemented controls, vertebral Mg content was increased in female diabetic rats but no changes in rib Mg content were observed. In the diabetic rats tissue weight to body weight ratios were increased in all tissues except skeletal muscle. Increases in Mg content were observed in diabetic tissues when expressed to tissue wet weight but not when expressed to tissue DNA content.

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Introduction

Diabetes mellitus has been associated with electrolyte abnormalities in both humans and animal models of diabetes [1]. Magnesium homeostasis is affected, with hypomagnesaemia being consistently reported in type I and type II diabetic patients and in experimentally-induced diabetes in animals [2–5]. Hypomagnesaemia is commonly attributed to renal tubular magnesium wasting associated with glucose diuresis and inhibition of tubular magnesium reabsorption [5].

Some authors have related hypomagnesaemia to the aetiology of diabetic complications and various diabetically-related pathologies [6–7]. The degree of hypomagnesaemia has been associated with glycaemic status. Poor glycaemic control can be related to a reduction in serum magnesium concentration [8]. Indicators of short-term and long-term glycaemic status, such as blood fructosamine concentrations and percentage glycated haemoglobin (HbA1c) together with blood glucose concentration are better indicators of chronic glycaemic status than estimation of blood glucose concentration alone. Inverse correlations between magnesium concentration in serum and both fructosamine concentration or percentage HbA1c have been reported [9].

However, serum or plasma magnesium concentration is not a reliable index of whole body magnesium status and the effect of factors that regulate mineral metabolism, for instance parathyroid hormone, may mask intracellular or tissue magnesium depletion. Analysis of red blood cell, mononuclear blood cell or tissue magnesium concentrations are better predictors of magnesium sufficiency or depletion [10–11].

Magnesium in bone accounts for over 50 % of body magnesium stores [12] and acts as a reservoir which can be mobilised to the extracellular fluid in times of magnesium deficiency [13]. Chronic magnesium depletion or increased renal excretion could be balanced in extracellular fluid by replenishment of magnesium from bone stores with a consequent reduction in bone magnesium content [14–15].

Dietary magnesium supplementation to counteract renal magnesium wasting has been advocated in diabetic patients and studied in some animal models of induced diabetes [16–17].

This study was undertaken to test in a streptozotocin-induced diabetic model in rats: firstly, the effects of dietary magnesium supplementation by measurement of soft tissue and bone magnesium status; secondly, to correlate magnesium status with indicators of glycaemic control; and thirdly, to assess differences in response to diabetes and magnesium supplementation in male and female rats.

Materials and methods

Animals

Age and weight matched Wistar rats were randomly assigned to one of four groups (n = 12, 6 male + 6 female): control (C); control plus magnesium supplementation (CM); STZ-diabetic (D); or STZ-diabetic plus magnesium supplementation (DM). Animals were housed individually in plastic bottomed cages at constant temperature (19–21°C) and humidity (55 ± 5 %) with a 14/10 light/dark cycle. Food and fluids were provided *ad libitum.*

Baseline body weight and food and fluid intake were monitored for two weeks prior to the start of the experiment. Thereafter, they were measured daily for the first week and then twice weekly to the end of the experiment. All animals were checked daily for their general well-being.

Our previous studies with this model have shown that STZ-induction of diabetes is associated with polyphagia and polydipsia [18]. This greatly increased food intake could compensate for the effect of diabetes on magnesium status, therefore, magnesium reduced food was used in the STZ-diabetic groups to stabilise magnesium intake in food to that of control animals.

Induction of Diabetes

Diabetes was induced in groups D and DM, after the baseline period, by injecting the appropriate dose of STZ (65 mg kg^{-1} body weight) in citrate buffer (pH 4.5). No more than 0.2 ml was injected intraperitoneally and dosage volumes were calculated accordingly. The control animals (C and CM) were sham injected with buffer only. Glucose solution (2.5 % w/v) replaced water as drinking fluid for the first 48h in order to counteract the hyperinsulinaemia caused by the destruction of the pancreatic β cells. After this time, 10 mM magnesium chloride solution was given as drinking fluid in groups CM and DM.

On day 5, blood was collected from anaesthetised (Xylonor spray) tail tips prior to the nocturnal feed. Blood glucose concentration was measured using glucose indicator strips. Rats with blood glucose concentrations below 16 mmol l^{-1} were excluded from the study.

Diabetic status was confirmed at the end of the experiment by immunohistological examination of paraffin wax sections of pancreas. The absence of insulin-positive β cells was taken as confirmation of diabetic status.

Experimental methods

On completion of the experiment, blood was collected by cardiac puncture under barbiturate anaesthesia. Blood glucose concentration was analysed by the Trinder method [19]. Glycated haemoglobin was determined using a kit method and calibrated against commercially available standards (Sigma). Fructosamine concentration in whole blood was determined spectrophotometrically using a kit method (Sigma, cat.no. 465A). Magnesium concentrations in plasma or tissue digests were measured by Atomic Absorption Spectroscopy.

Body organs and tissues were dissected out, weighed and stored at –20 °C until analysis. Tissue protein and DNA concentrations were determined on tissue homogenates by the methods of *Lowry* [20] and *Sterzel* [21] respectively.

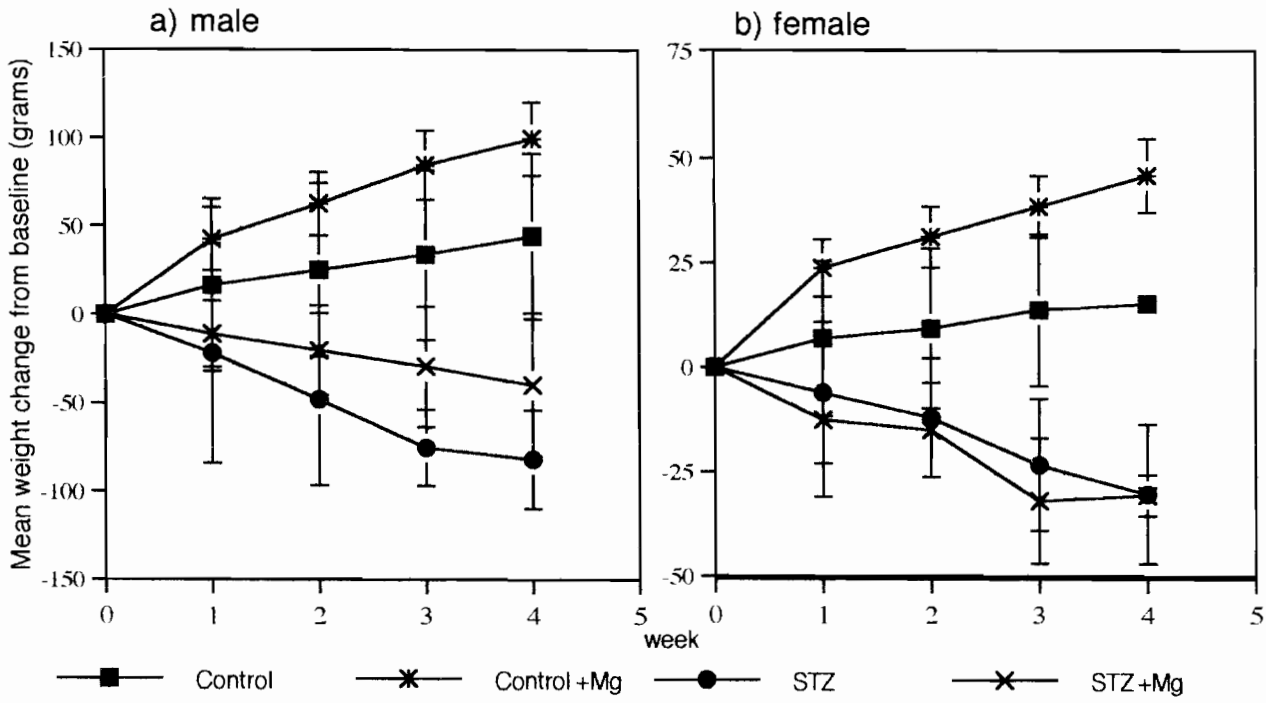

Fig. 1: Mean (± Standard deviation) weight changes from baseline in male and female experimental groups.

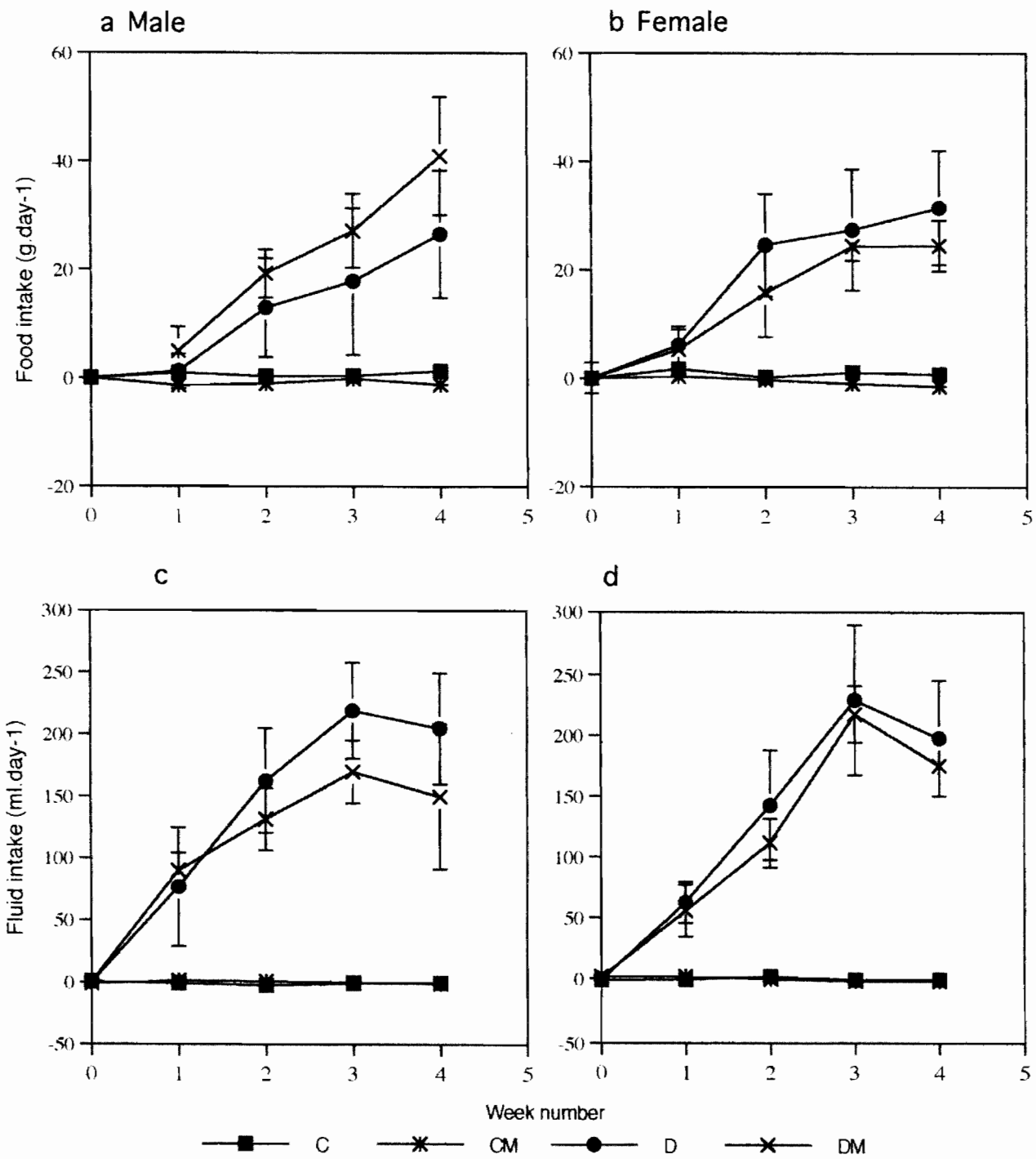

Fig. 2: Mean (± Standard deviation) daily food and fluid intake per week.

Statistical analysis included factorial ANOVA for independent data and a repeated measures ANOVA for changes over time. A Kruskall Wallis test was used on non-parametric data. The level of significance was taken as 5 %.

Results

Body weight

Body weight changes over the course of the study have been reported previously [18]. In brief, control animals gained weight whereas STZ-diabetic animals lost varying degrees of body weight compared to baseline. Repeated measures ANOVA in male and female animals revealed significant changes in body weight between groups and over time ($P < 0.0001$). STZ-diabetic male rats lost proportio-

nately more weight than the corresponding female animals, however, this difference was not significant.
In the male STZ-induced diabetic groups magnesium supplementation diminished the weight loss seen in the non-supplemented diabetic groups ($P < 0.05$). There was no apparent protective effect of magnesium supplementation in female SZT-diabetic animals. See Fig. 1.

Food and fluid intake

These results have been reported previously [18]. In summary, STZ-diabetic rats became polyphagic irrespective of magnesium supplementation. STZ-diabetes increased daily food intake ($P < 0.01$) compared to baseline data. Magnesium supplementation had no effect on polyphagia associated with the induction of diabetes in male or female animals. Male and female STZ-diabetic animals became polydipsic and the addition of magnesium to the drinking fluid did not affect fluid consumption in either male or female controls. Compared to baseline values, non-supplemented STZ-diabetic animals increased their fluid intake more than the corresponding supplemented group. However, this difference was not significant in either male or female rats. See Fig. 2.

Total magnesium intake

Total magnesium intake was calculated as follows:
Weight food consumed x magnesium concentration in food = daily intake from food
Volume of fluid imbibed x magnesium concentration of fluid = daily intake from fluid
Then, total magnesium intake per day = daily intake from food + daily intake from fluid
Absolute magnesium intake was standardised to body weight of the animal and expressed as: g day^{-1} kg^{-1} body weight.
STZ-diabetes significantly increased ($P < 0.001$) magnesium intake in both male and female animals. Although measures were taken to stabilise magnesium intake at baseline levels in diabetic animals to counteract the effects of polyphagia, magnesium intake still increased with time following induction of diabetes. The food intake increased so much, that in some STZ-diabetic animals magnesium intake from the reduced magnesium food alone was significantly higher than that before the induction of diabetes ($P < 0.002$ in males; $P < 0.001$ in females). Nonetheless, these values were lower than projected intake levels if reduced magnesium food had not been substituted for normal diet. See Tab. 1.

Indices of glycaemic control

Blood glucose concentrations were significantly increased (> 30 mmol l^{-1}) in male ($P < 0.006$) and female ($P < 0.0046$) STZ-diabetic groups compared to control (< 8 mmol l^{-1}). Magnesium supplementation did not significantly reduce blood glucose concentrations in either control or STZ-diabetic animals. The percentage glycated haemoglobin levels were elevated in STZ-diabetic groups (> 3.8 %) compared to those in controls (< 2.8 %). The trends were similar in male and female animals with increases in all STZ-diabetic groups. The fructosamine concentration across groups was significantly increased in male (> 1.8 mmol l^{-1}; $P < 0.0049$) and female (> 2.2 mmol l^{-1}; $P < 0.0008$) animals respectively compared to controls (< 1.4 mmol l^{-1}).
These indices together reflect chronic perturbations in glycaemic control in STZ-diabetic animals which was not corrected by magnesium supplementation.

Tab. 1: Mean (± SD) daily magnesium intake

Week of Study		Mean (± SD) Daily Magnesium Intake (mg / day)			
		C	CM	D	DM
Female	Week 1	33.6 (2.4)	51.8 (4.3)	37.0 (6.3)	36.0 (2.6)
	Week 2	34.2 (3.8)	52.5 (3.8)	48.0 (11.0)	35.0 (9.7)
	Week 3	31.9 (1.1)	49.7 (4.1)	43.0 (15.0)	33.0 (1.2)
	Week 4	32.9 (3.4)	49.6 (2.9)	42.0 (8.0)	31.0 (3.8)
Mean daily intake		33.0 (0.9)	51.0 (1.4)	42.0 (11.0)	34.0 (5.2)
Projected daily intake[a]		NA	NA	50.8	45.5
Male	Week 1	38.2 (1.7)	52.5 (2.1)	57.0 (9.1)	53.0 (8.1)
	Week 2	36.9 (1.2)	53.1 (2.8)	66.0 (2.1)	79.0 (8.2)
	Week 3	35.6 (1.6)	54.6 (2.2)	51.0 (13.0)	57 (7.2)
	Week 4	34.8 (1.4)	52.7 (3.0)	58.0 (14.0)	59 (9.1)
Mean daily intake		36.0 (1.9)	53.0 (2.5)	58.0 (15.0)	62.0 (13.00)
Projected daily intake		NA	NA	64.0	78.8

[a] Projected magnesium intake if standard food eaten instead of reduced magnesium food
NA = Not applicable SD = Standard Deviation C = Control D = Diabetic
CM = Control + magnesium supplementation DM = Diabetic + magnesium supplementation

Plasma magnesium concentration
Plasma magnesium concentrations did not differ between groups apart from means in magnesium supplemented and non-supplemented control males where magnesium concentration was significantly reduced in the supplemented group ($P = / < 0.05$). There were no statistically significant correlations between plasma magnesium concentration and any of the indices of glycometabolic control.

Magnesium concentration in bone
The magnesium content of acid digests of whole femur, 4th rib and 4/5th lumbar vertebra was determined. Magnesium concentration was expressed as wet weight of bone tissue.
STZ-diabetes did not alter magnesium concentrations in femur. The femur magnesium concentration in both male and female rats was significantly reduced in the magnesium supplemented controls compared to non supplemented controls ($P < 0.0001$) and to magnesium supplemented STZ-induced diabetic groups ($P < 0.0001$ in males; $P < 0.05$ in females).
There were no significant changes in mean vertebral magnesium concentration between treatment groups except for mean magnesium concentration in the female STZ-diabetic group which was significantly greater than control ($P \geq 0.05$). No significant changes in rib magnesium concentration were observed. These results are summarised in Fig. 3.

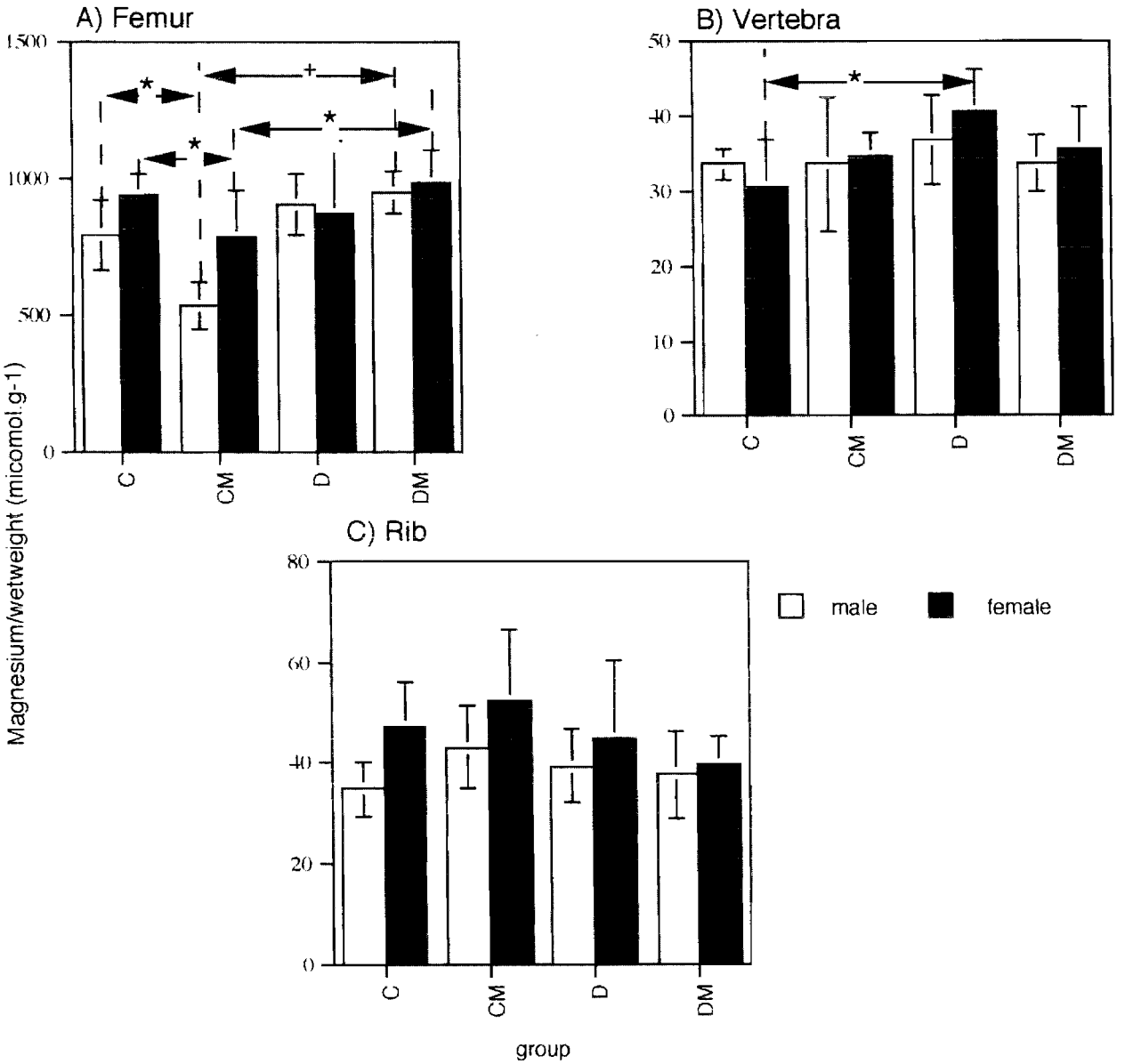

Fig. 3: Mean (± Standard deviation) bone magnesium concentration. * $P < 0.05$; ' $P < 0.0001$.

Soft tissue magnesium concentration

The tissues studied were lung, heart, kidney, liver and skeletal muscle. Tissue magnesium content was expressed in relation to wet weight of tissue; protein concentration; or DNA content. In uncontrolled diabetes, tissue weight is an unstable index as weight will be changing according to the intracellular hydration status and cell volume. With hyperglycaemia, tissue dehydration occurs as water moves out of the cell down the osmotic gradient established by the high glucose concentrations and water wasting in the kidney. Uncontrolled STZ-diabetes is characterised by catabolism of proteins therefore, cellular protein concentration may be reduced. The magnesium concentration expressed to protein content data must be interpreted with caution because of this potential protein wastage. DNA was chosen as a stable reference index. When results were expressed to wet weight of tissue there were no changes in mean magnesium content following induction of diabetes (C vs D) in either male or female animal tissues except for increases in male STZ-diabetic animals in the liver and muscle ($P < 0.05$). Magnesium supplementation (C vs CM) did not affect magnesium concentration in male or female control group tissues. Magnesium supplementation (CM vs DM) increased magnesium concentrations in all tissues in the female and all tissue except heart in the male magnesium supplemented STZ-diabetics compared to the supplemented controls ($P < 0.05$). There were no changes in tissue magnesium concentration in the diabetic groups (D vs DM) with magnesium supplementation. See Fig. 4.

When results were expressed to protein concentration there were no significant changes following induction of diabetes (C vs D) except an increase in heart of female animals ($P < 0.0001$). Supplementation of control groups (C vs CM) reduced magnesium concentration in male heart, female liver and female muscle ($P < 0.05$) but increased magnesium concentration in female kidney ($P < 0.05$). Comparison of magnesium supplemented control and STZ-diabetic groups (CM vs DM) revealed increased magnesium concentrations in the male and female diabetic heart and male diabetic kidney ($P < 0.05$) but reduced magnesium concentration in female diabetic kidney ($P < 0.0001$), liver and lung ($P < 0.05$). Dietary magnesium supplementation in the STZ-diabetic animals (D vs DM) caused no changes in tissue magnesium except for reduced concentration in female kidney ($P < 0.05$). See Fig. 5.

No changes in magnesium concentration for any tissue with induction of diabetes or with magnesium supplementation in either male or female animals were revealed when expressed

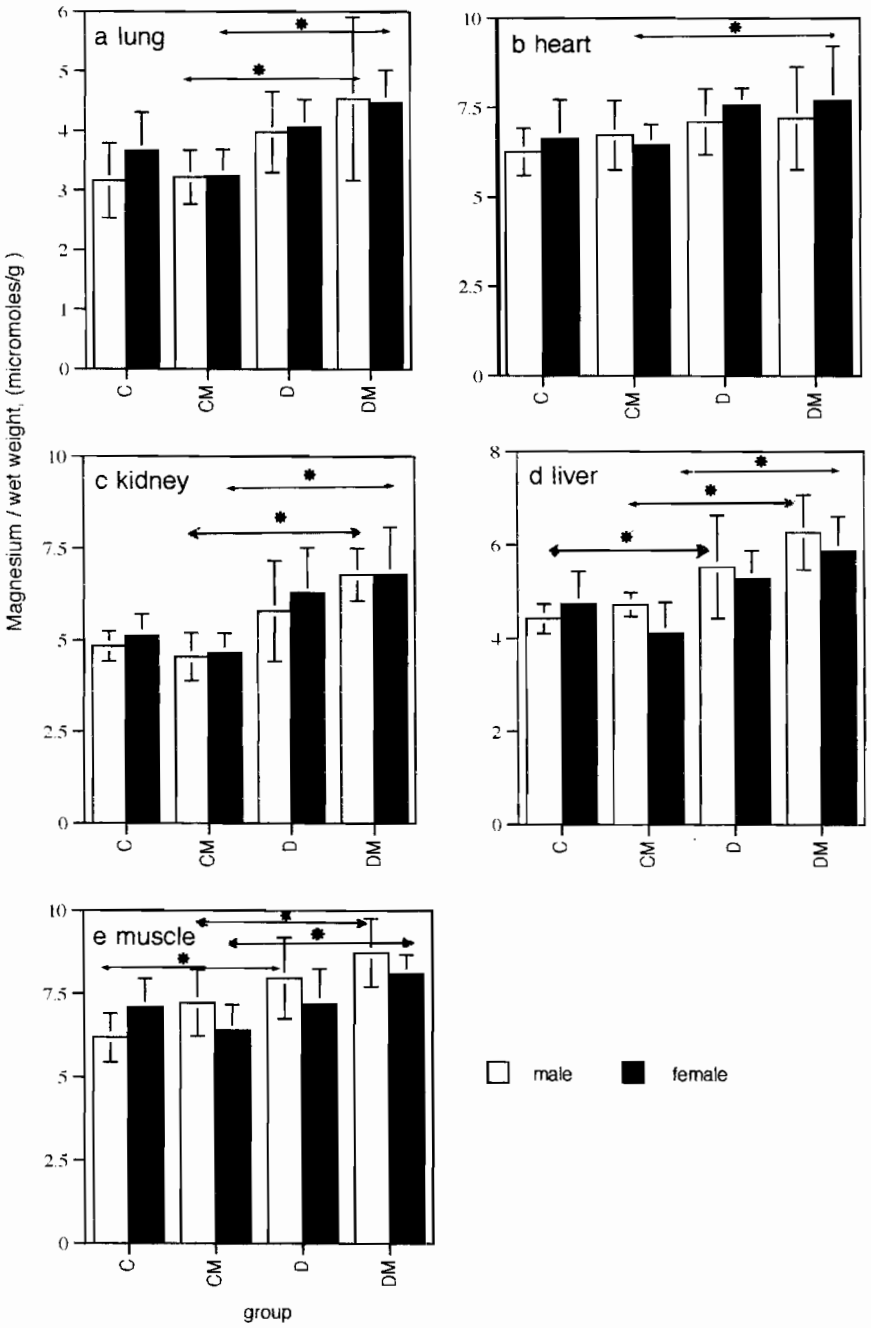

Fig. 4: Mean (± Standard deviation) tissue magnesium concentration expressed to wet weight of tissue. * $P < 0.05$.

to DNA content except for an increase in female DM lung compared to that in magnesium supplemented controls (1.4 mmol g^{-1} *vs* 0.8 mmol g^{-1}; $P < 0.05$).

Organ weights

Tab. 2 presents organ weight data as a percentage of body weight. Examination of the data reveals that in both male and female animals all organ weights, expressed as a percentage of body weight, were increased in diabetic animals compared with their respective controls with the exception of muscle where the organ weight to body weight ratio was significantly reduced in male and female animals compared to controls. There were no significant changes in the lung weight to body weight ratio in male animals but a significant increase was seen in female diabetic groups compared to control.

Discussion

The induction of STZ-diabetes in male and female rats caused significant increases in serum glucose concentrations, polyphagia, polydipsia and polyurea. From the blood glucose concentrations it was apparent that all the rats injected with STZ became diabetic and destruction of pancreatic β-cells was confirmed immunohistologically. Total magnesium intake was increased in STZ-diabetic animals, irrespective of magnesium supplementation, because of the great increase in food intake. An attempt to peg magnesium intake to control levels, by feeding reduced magnesium food, was only partially successful in that, whilst magnesium intake was lower than projected from the weight of normal laboratory diet consumed, it was still higher than in controls.

STZ-diabetes was associated with marked morbidity, especially in male animals. *Kromann* and co-workers (1982) found that in a low dose STZ model (40 mg kg^{-1}) of type I diabetes, the male mice developed hyperglycaemia but the females remained euglycaemic. This effect in the females was counteracted by testosterone injection [22].

Dietary magnesium supplementation increased the growth rate of male and female control rats. In STZ-diabetic rats a protective effect against the associated weight loss was not exhibited in the females, where the DM group lost weight more quickly. However, a partial protective effect was suggested in male animals. This effect could have been due to sex difference and or the fact that their actual intake of dietary magnesium was less than that for the non-supplemented STZ-diabetic group.

Contrary to other published reports [2–5], in this study the induction of STZ-diabetes was not associated with hypomagnesaemia in either male or female animals. It is possible that diabetes would need to be maintained for longer time periods than here.

All indices of glycometabolic control were increased in STZ-diabetic animals. Dietary magnesium supplementation was not associated with reductions in blood glucose, fructosamine or glycated haemoglobin concentrations. Plasma magnesium concentration was not related to either blood glucose or fructosamine concentration or to the percentage glycated haemoglobin level.

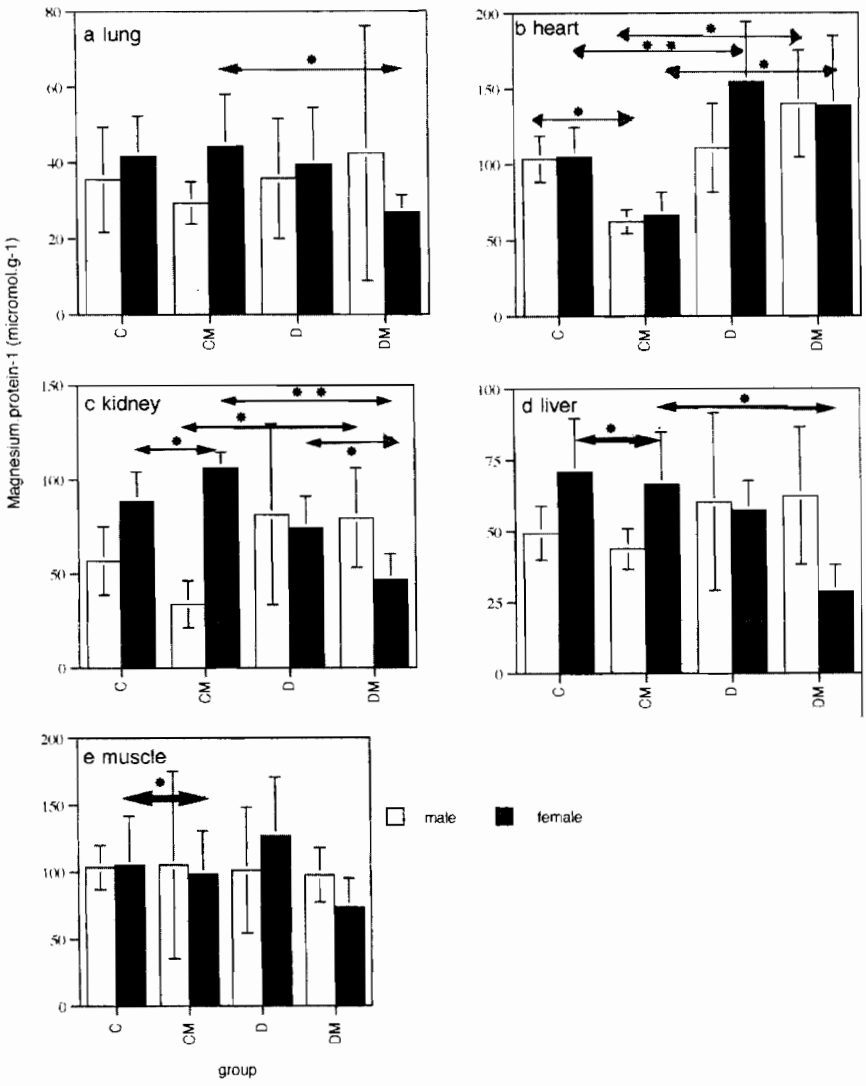

Fig. 5: Mean (± Standard deviation) tissue magnesium concentration expressed to protein concentration. * $P < 0.05$; ** $P < 0.0001$.

Tab. 2: Mean (± Standard Deviation) organ weights expressed as a percentage of body weight

Tissue	Experimental Group			
	C	CM	D	DM
Female				
BW Change	↑	↑↑	↓↓	↓↓
Lung**	0.453 (0.058)	0.592 (0.058)	0.543 (0.039)	0.525 (0.044)
Heart**	0.342 (0.021)	0.375 (0.034)	0.427 (0.028)	0.410 (0.022)
Liver***	3.78 (0.496)	4.328 (0.265)	6.48 (0.379)	5.78 (0.204)
Kidney**	0.413 (0.098)	0.370 (0.022)	0.685 (0.059)	0.613 (0.053)
Skeletal Muscle**	0.798 (0.077)	0.818 (0.105)	0.615 (0.079)	0.585 (0.132)
Male				
BW Change	↑	↑↑	↓↓	↓
Lung	0.430 (0.046)	0.490 (0.075)	0.385 (0.031)	0.422 (0.044)
Heart***	0.278 (0.008)	0.303 (0.023)	0.338 (0.017)	0.370 (0.013)
Liver***	3.815 (0.364)	4.013 (0.454)	5.318 (0.313)	4.958 (0.418)
Kidney**	0.335 (0.016)	0.328 (0.022)	0.665 (0.017)	0.585 (0.045)
Skeletal Muscle*	0.726 (0.097)	0.698 (0.088)	0.660 (0.085)	0.563 (0.057)

↑ Body weight (BW) increased over time, ↑↑ Greater body weight increase over time compared with control, ↓ Body weight decrease over time, ↓↓ Body weight decreased over time compared to DM group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, C = Control, CM = Control + magnesium supplementation, D = Diabetic, DM = Diabetic + magnesium supplementation.

McNair and colleagues found that serum magnesium concentration was inversely correlated to fasting blood glucose concentration together with urinary excretion rates of magnesium and glucose [5]. *Bertollini* proposed that metabolic control was factor related to serum magnesium concentration in human diabetes and that there was an inverse relationship between serum levels of magnesium and glycated haemoglobin [8].

Examination of tissue magnesium levels revealed a paradox: if magnesium is lost in the urine at a higher rate than normal in diabetes then it would be reasonable to suppose that extracellular fluid magnesium must be replenished from soft tissue and bone stores resulting in a reduction in intracellular magnesium content.

Examination of the data presented here, revealed that tissue magnesium concentrations were mainly increased in STZ-diabetic tissues compared with those in controls when content was expressed to wet weight of tissue or to protein concentration. Whole body physiology is changed in uncontrolled diabetes as the body attempts to find alternative energy sources to glucose. One of these energy sources is provided by the catabolism of protein and tissues high in protein, for instance muscle, will inevitably lose weight. It is possible that protein catabolism may occur differentially in vital organs such as the heart and lungs to that in skeletal muscles. In addition, cell volume will be altered by loss of cell water caused by the increased osmolality of extracellular fluid.

When organ weights were compared to body weight, differential changes were observed dependent upon the organ involved and the experimental group. Both male and female organ weight to body weight ratios were increased in the STZ-diabetic groups compared to their respective controls, with the exception of skeletal muscle where the ratio was significantly reduced, compared to controls in both male and female animals.

If the body weight is decreased by the end of the experiment but organ weight is static or increased then this will be reflected by an increase in the organ weight to body weight ratio. This could be a possible explanation for the changes seen here for heart, liver and kidney. On the other hand if changes in organ weight parallel changes in body weight then no change in ratios would be observed. However, if proportionally more weight is lost from a particular organ compared to overall body weight changes then the ratio will be reduced as can be observed here for skeletal muscle in both male and female STZ-diabetic animals, irrespective of magnesium supplementation. This suggests that much of the body weight loss in STZ-diabetic animals is from loss of skeletal muscle mass whilst other organs are relatively spared in terms of organ weight loss. This observation lends weight to the interpretation of changes in index parameters in vital organs but skeletal muscles results must be interpreted with caution.

We have investigated the use of tissue DNA as a stable reference parameter and when expressed to this, magnesium concentrations did not differ significantly between groups, except in female STZ-diabetic supplemented lung compared with supplemented control tissues.

Over 50 % of body magnesium is in bone tissues [12]. Bone magnesium

content may be a better indicator of magnesium depletion and reductions in bone magnesium content have been reported in diabetic patients [14] and rats receiving a low magnesium diet [15]. Conversely, magnesium excess has been shown to have an adverse effect on bone and in mice this magnesium excess stimulated bone resorption independently of parathyroid hormone [12].

In this study, dietary magnesium supplementation resulted in significant decreases in femoral magnesium in both male and female control animals which may be due to increased resorption and hence loss of bone magnesium. However, there were no differences in STZ-diabetic tissues compared to control. In vertebra, supplementation in female STZ-diabetic animals caused an increase in vertebral magnesium content compared to controls. No other effects were seen and no changes in rib bone magnesium status were noted in male or female control or STZ-diabetic animals, irrespective of magnesium supplementation.

It is possible that STZ-diabetes would need to be maintained for longer than the time used here before effects on tissue or bone magnesium status become apparent. In addition, in this model of untreated STZ-induced diabetes, the tissue chosen for the assessment of magnesium status is important. Skeletal muscle analysis may reflect more the changes in muscle protein and cell water than changes in magnesium content. The use of DNA as a reference parameter may overcome the difficulties in finding a stable reference parameter.

Acknowledgements

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