

## Changes in bioavailability and tissue distribution of zinc caused by magnesium deficiency in rats

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The effect of a Mg-deficient diet (200 mg Mg/kg feed) on the bioavailability of dietary Zn and the concentration of this cation in plasma, whole blood, skeletal muscle, kidney, heart and brain of Wistar rats was studied after 7, 35, 42, 49, 56, 63 and 70 d. Mg deficiency significantly decreased Zn in whole blood on day 42 of the experiment, but there was no significant change in plasma Zn throughout the 70 d study period. The Mg-deficient diet significantly increased intestinal absorption of Zn, Zn balance, and Zn concentration in femur and kidney, but decreased Zn concentration in the heart despite the increase in dry weight of this organ. No change was found in brain Zn concentration.

**Magnesium: Zinc: Rat**

Mg is known to be essential for the metabolism of several minerals. The signs and symptoms of Mg deficiency are due, in part, to complex alterations in electrolyte balance secondary to the Mg<sup>2+</sup> deficit.

Little has been published on the relationship between Mg deficiency and Zn. The effects of Mg deficiency on Zn contents in several tissues in the gestating rat and pups have been described (Hurley & Swenerton, 1966; Hurley & Cosens, 1976); however, these reports dealt mainly with the placental transfer of Zn and the congenital malformations arising from alterations in mineral content in the fetus. Subsequent studies (Momcilovic *et al.* 1975; Forbes *et al.* 1984) examined the bioavailability of Zn in diets supplying different amounts of Ca, phytate and Mg, and suggested a possible interaction between cations during intestinal transport and in bone. More recently, Yasui *et al.* 1991 noted that a diet low in Ca and Mg caused changes in the tissue distribution of Zn; however, this study provided no information on the changes caused exclusively by Mg deficiency.

In addition to these findings, there is evidence from epidemiological studies that Mg intake in a large percentage (approximately 30) of the population in industrialized countries is below the recommended daily allowance, and that Mg deficiency, together with inadequate dietary habits, can lead to many disease states (Weston, 1987). Therefore, we designed the present study to elucidate the relationship between Mg and Zn, and to determine whether the latter indirectly contributes to the development of deficiency-related symptoms. Our approach was to examine the degree to which a Mg-deficient diet affects the bioavailability of dietary Zn and the distribution of this element in different tissues.

The present study forms part of an ongoing project aimed at characterizing the evolution of Mg deficiency in rats under the same experimental conditions: rats weighing 180 g were given, during 70 d, a diet that supplied 50% of the Mg requirements for this species (Lerma *et al.* 1993).

\* For reprints.

## MATERIALS AND METHODS

*Animals and diets*

Recently-weaned Wistar rats consumed a standard commercial diet (Panlab, Barcelona, Spain) until they reached a body weight of 180 g. Thereafter they were allowed *ad lib.* access to bidistilled water and a semi-synthetic diet deficient in Mg. The diet contained (g/kg): protein (casein) 140, DL-methionine 5, sucrose 344, maize starch 344, fibre (cellulose) 80, olive oil 40, AIN-76 mineral mix (without magnesium oxide) 35, AIN-76 vitamin mix 10, choline bitartrate 2. In all, these components provided 200 mg Mg, 53.2 mg Zn, 6200 mg Ca and 4400 mg P/kg feed, as well as 25 µg cholecalciferol/kg feed.

To study the development of Mg deficiency, ten deficient rats (five males, five females) were killed by decapitation on days 7, 35, 42, 49, 56, 63 and 70. Blood was collected and centrifuged to separate plasma. The femur, kidney, and *longissimus dorsi* muscle were also removed on days 7, 35, 42, 49, 56, 63 and 70; the heart was removed for analysis on days 7, 42 and 63. During the last 7 d of each experimental period the faeces and urine were collected for subsequent analyses, and the amount of feed ingested was recorded.

The results were compared with those for a group of control rats fed on the same diet, except that the amount of Mg was adequate to supply their nutritional requirements (465 mg/kg feed). Control animals were allowed access *ad lib.* to the diet during the first 4 weeks. Thereafter, control males were pair-fed with the deficient male having the lowest intake (11.3 g) and females with the lowest female intake (9.3 g).

All animals were kept in individual metabolism cages in a well-ventilated, temperature-controlled room ( $21 \pm 2^\circ$ ) with a light-dark period of 12 h.

We calculated biological indices apparent absorption (AA), as  $[(I-F)/I] \times 100$ , and balance, as  $I - (F + U)$ , where I is intake, F is faecal excretion, and U is urinary excretion (Food and Agriculture Organization/World Health Organization, 1966).

Changes in brain Zn content were studied in a separate experiment. Male rats were given the same Mg-deficient diet, and were killed by decapitation on days 35, 49, 63 and 70. The brains were immediately removed for analysis, and the results were compared with a group of rats fed on the control diet.

*Analytical methods*

Dry matter was determined as the material remaining after heating to  $105 \pm 2^\circ$  until weight was stable. Ash was obtained by calcination of 1 or 2 g samples at  $450^\circ$ . Zn and Mg were determined by atomic absorption spectrophotometry of a sample that had been previously mineralized by calcination at  $450^\circ$ .

The resulting residues were extracted with 6 M-HCl solution, brought up to an appropriate volume, and spectrophotometrically compared against a set of standards. Determinations in plasma were also made by atomic absorption spectrophotometry in samples that were not previously ashed.

*Statistical analysis*

The experimental data were subjected to Student's *t* test. Differences were verified at the 5% level. Differences between sexes were small and inconsistent relative to treatment effects, and the latter were analysed using pooled data from both sexes.

## RESULTS

Under our experimental conditions a faecal Zn excretion was significantly lower in rats that consumed the Mg-deficient diet than in control animals between days 28 and 70. Urinary

Table 1. *Weight changes, zinc intake, and faecal and urinary excretion of Zn of rats given a magnesium-deficient diet for 70 d†*  
(Mean values with their standard errors for ten rats)

Day of experiment	Wt changes (g/rat per d)				Zn intake (mg/rat per d)				Faecal Zn (mg/rat per d)				Urinary Zn (mg/rat per d)			
	C		D		C		D		C		D		C		D	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1-7	2.7	0.46	1.2*	0.41	0.85	0.02	0.92	0.04	0.28	0.02	0.32	0.03	0.11	0.01	0.18**	0.02
28-35	0.8	0.43	-0.4†	0.18	0.55	0.05	0.61	0.03	0.31	0.02	0.15***	0.01	0.12	0.014	0.13	0.01
35-42	2.2	0.89	-2.0***	0.43	0.55	0.05	0.60	0.03	0.33	0.03	0.13***	0.004	0.12	0.017	0.07	0.004
42-49	-1.4	0.32	-0.4**	0.23	0.55	0.05	0.57	0.03	0.36	0.04	0.18***	0.03	0.11	0.009	0.07**	0.004
49-56	1.0	0.20	0.3*	0.25	0.55	0.05	0.65	0.03	0.36	0.03	0.15***	0.005	0.10	0.01	0.09	0.01
56-63	2.3	0.37	-1.1***	0.37	0.55	0.05	0.68	0.06	0.36	0.03	0.16***	0.01	0.11	0.01	0.06***	0.01
63-70	-0.2	0.24	-1.6**	0.33	0.55	0.05	0.58	0.03	0.34	0.01	0.13***	0.007	0.10	0.008	0.12	0.03

C, control; D, deficient.

Mean values were significantly different from those for the controls: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , † $P < 0.02$ .

‡ For details of diets and procedures, see p. 316.

Table 2. Digestive and metabolic utilization of zinc for rats given magnesium-deficient diets for 70 d†  
(Mean values with their standard errors for ten rats)

Day of experiment	Zn absorbed (mg/rat per d)				Apparent absorption‡ (%)				Balance   (mg/rat per d)			
	C		D		C		D		C		D	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1-7	0.59	0.017	0.62	0.025	67.1	1.91	67.9	2.25	0.48	0.031	0.47	0.019
28-35	0.24	0.026	0.46***	0.027	43.5	3.15	72.9***	1.37	0.13	0.024	0.32***	0.034
35-42	0.23	0.029	0.47***	0.030	42.6	4.53	78.4***	1.16	0.11	0.027	0.40***	0.031
42-49	0.19	0.020	0.42***	0.023	35.0	2.91	73.4***	1.36	0.08	0.023	0.35***	0.025
49-56	0.21	0.041	0.47***	0.029	36.4	4.13	75.9***	1.29	0.10	0.017	0.41***	0.031
56-63	0.18	0.019	0.55***	0.020	33.0	4.18	77.2***	1.25	0.08	0.015	0.48***	0.016
63-70	0.20	0.014	0.46***	0.035	37.0	2.04	76.7***	2.19	0.11	0.015	0.34***	0.048

C, control; D, deficient.

Mean values were significantly different from those for controls: \*\*\* $P < 0.001$ .

† For details of diets and procedures, see p. 316.

‡  $[(I - F)/I] \times 100$ , where I is intake and F is faecal excretion.

||  $I - (F + U)$ , where I is intake, F is faecal excretion and U is urinary excretion.

Table 3. Zinc content in plasma, whole blood, longissimus dorsi muscle, femur, kidney and heart of rats given magnesium-deficient diets for 70 d†  
(Mean values with their standard errors for ten rats)

Day of experiment	Plasma (µg Zn/l)			Whole blood (µg Zn/l)			Muscle (µg Zn/g dry tissue)			Femur (µg Zn/g femur)			Kidney (µg Zn/g dry tissue)			Heart (µg Zn/g dry tissue)								
	C	D	SE	C	D	SE	C	D	SE	C	D	SE	C	D	SE	C	D	SE						
	Mean	Mean		Mean	Mean		Mean	Mean		Mean	Mean		Mean	Mean		Mean	Mean							
7	108.6	4.5	114.2	3.7	901.4	8.59	817.0	35.6	98	11	127*	3	528	29	458	29	229	10	185	19	211	18	204	1
35	107.8	2.6	105.6	5.9	870.0	17.7	850.7	28.6	99	6	149***	11	276	24	659***	29	221	15	308**	23	244	28	157***	101
42	102.4	4.1	92.3	6.6	813.9	83.3	639.9*	23.9	95	6	110	4	234	11	496***	32	242	27	483***	40	244	28	157***	101
49	100.8	4.1	93.2	7.6	894.7	15.3	614.1***	20.2	97	6	71***	3	378	23	716***	42	241	11	475***	36	244	28	157***	101
56	106.3	3.5	107.9	3.9	885.7	15.8	596.7***	32.7	94	8	96	4	397	27	623***	26	248	12	431***	6	246	23	122***	10
63	109.2	1.3	96.2	12.8	848.7	14.9	626.5***	22.4	99	9	102	4	367	17	422*	18	209	25	478***	71	246	23	122***	10
70	105.9	1.7	99.2	3.0	853.1	12.6	517.4***	39.2	85	5	93	3	306	18	422***	14	259	26	528***	37	246	23	122***	10

C, control; D, deficient.  
Mean values were significantly different from those for controls: \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.  
† For details of diets and procedures, see p. 316.

Table 4. Zinc content in brain of rats given magnesium-deficient diets for 70 d\*  
(Mean values with their standard errors for ten rats)

	Brain Zn ( $\mu\text{g/g}$ dry tissue)		
	n	Mean	SE
Control	10	580	31
Deficient: Day			
35	6	540	50
49	6	550	30
63	6	560	30
70	6	570	40

\* For details of diets and procedures, see p. 316.

Zn excretion during the first week of study was significantly higher in the experimental group; however, urinary Zn concentration tended to decrease with time, and became significantly reduced in weeks 7 and 9. After this time urinary Zn increased, reaching values slightly higher than those obtained for the controls (Table 1).

The Mg-deficient diet significantly increased Zn absorption (in mg) from day 28 to day 35. As expected, apparent absorption of Zn and Zn balance showed the same trends as net Zn absorption (Table 2).

In whole blood, Zn concentration was significantly lower in the experimental group than in controls from day 42 to the end of the experimental period, and tended to decrease slightly as Mg deficiency became prolonged (Table 3). However, plasma Zn concentrations in the two groups did not differ significantly at any time-point, although it was slightly lower in Mg-deficient animals for most of the experimental period (Table 3).

Zn content in the *longissimus dorsi* muscle was greater for the Mg-deficient group than for controls (Table 3) on days 7 and 35; after this time Zn content decreased gradually, becoming significantly lower for the experimental group on day 49. Thereafter, muscle Zn concentration in Mg-deficient rats increased, reaching values similar to those for the control group and remaining unchanged until the end of the experiment.

Zn content of the femur was significantly greater for Mg-deficient rats than for controls from day 35 until the end of the experiment (Table 3).

In the kidney (Table 3), Zn content of dry tissue was significantly higher for Mg-deficient rats from day 35 until the end of the experiment.

Heart tissues were studied on days 7, 42 and 63. Heart dry weight (mg) was increased for Mg-deficient animals on day 42 (controls 141.3 (SE 5.2) v. experimental animals 192.3 (SE 6.4);  $P < 0.001$ ) and day 63 (controls 154.8 (SE 6.8) v. Mg-deficient rats 224.1 (SE 13.2);  $P < 0.001$ ). However, Zn concentration decreased with time. The differences between Mg-deficient and control rats were significant from day 42 until the end of the experiment.

Brain Zn concentration in Mg-deficient rats did not change significantly throughout the experiment (Table 4).

#### DISCUSSION

We studied the effects of 70 d on a Mg-deficient diet on the bioavailability of Zn, and the Zn content of different organs and body compartments. Mg and Zn were analysed at weekly intervals except for weeks 2, 3 and 4, as earlier studies (Aranda *et al.* 1987, 1990)

showed that under our experimental conditions (adult Wistar rats weighing 180 g at the start of the study) there were no relevant changes during this period.

Although the mechanism of Zn absorption has not been elucidated, kinetic studies have shown the involvement of both passive and carrier-mediated processes (Hempe & Cousins, 1991), which may represent paracellular and transcellular transport pathways respectively (Hempe & Cousins, 1992). Zn absorption by the carrier-mediated process is enhanced during periods of low Zn intake. In contrast, the diffusion component of Zn absorption is unaffected by Zn deficiency, and absorption via this process is proportional to luminal Zn concentration (Cousins & Hempe, 1990). In feed-restricted control rats (Table 2), Zn absorption was reduced for most of the experiment, a finding which would not have been predicted by the previously mentioned results.

The findings in Mg-deficient animals were similar: after week 5, when Mg deficiency reduced feed intake under our experimental conditions (Lerma *et al.* 1993), Zn absorption decreased. However, because the decline in absorption in Mg-deficient rats was much less evident than in controls, Zn absorption differed significantly between the two groups from week 5 until the end of the study (Table 2).

The smaller reduction in Zn absorption in Mg-deficient animals may be related to changes in the permeability of intercellular junctions as a result of Mg depletion (Cassidy & Tidball, 1967; Lemay & Gascon-Barré, 1992). The paracellular pathway, a system of intestinal transport of great importance for many divalent cations, appears to be regulated by several nutrients such as Ca and Mg (Smith & McAllan, 1966).

Mg deficiency may also modify Zn absorption indirectly by altering the availability of other divalent cations. Gunshin *et al.* (1991) noted the existence of competition among some divalent cations for common transporters.

The reduced feed intake in control rats between days 28 and 70 did not modify urinary excretion of Zn (Table 1), a finding that supports the assumption that under normal conditions the urinary excretion of Zn does not vary appreciably as a result of dietary composition, most Zn being eliminated in gastrointestinal secretions. This makes the intestinal pathway the major route of Zn excretion in rats (Disilvestro & Cousins, 1983; Solomons, 1988).

Urinary excretion of Zn in Mg-deficient animals was similar to that recorded for controls during most weeks of the study. The fluctuations may have been related to the marked increase in mineral content in the kidney (Table 3), which in turn may have impaired renal function.

Cation retention, calculated as Zn balance (Table 2), was probably regulated mainly by Zn absorption. This was borne out by our findings which showed Zn balance to follow a pattern similar to that of Zn absorption for both experimental and control animals.

We attributed the decrease in Zn concentration of whole blood from Mg-deficient animals (Table 3) to the decrease in intra-erythrocyte Zn levels, given that plasma Zn concentrations showed no significant fluctuations during the experimental period.

Decreased erythrocyte Zn content may have many causes. Zn may be shifted to the plasma compartment in order to maintain adequate plasma concentrations (Table 3) in the face of increasing Zn deposits in the bones and kidneys of Mg-deficient animals (Table 3). Alternatively, alterations induced by Mg deficiency in erythropoiesis may have been responsible for the loss of blood cell Zn (Kenney, 1981).

Recalling that Zn is involved in the stabilization of the erythrocyte membrane (Solomons, 1988; Cousins & Hempe, 1990), the loss of erythrocyte Zn in Mg-deficient animals may contribute to the changes in fatty acid composition of the membrane (Aranda *et al.* 1989), possibly as a result of increased lipid peroxidation (Cousins & Hempe, 1990).

The Mg-deficient diet increased muscle Zn concentration during the first 5 weeks of



study, after which values declined and approached normality during week 6 (day 42), remaining unchanged thereafter until the end of the study (Table 3). These changes are suggestive of interactions between  $Zn^{+}$  and  $Mg^{2+}$  in the skeletal muscle cell, which may account for the accumulation of Zn recorded during the first weeks of the experiment. The subsequent decline may have resulted from the mobilization of Zn by muscle tissue and erythrocytes in response to the accumulation of the cation in kidney and bone, in an effort to maintain adequate plasma levels. The Mg-deficient diet significantly increased bone Zn content by day 35, despite the decline in feed intake (Table 3). However, Yasui *et al.* (1991) noted that feeding a diet with a low Ca and Mg content led to a loss of bone Zn, which was much more marked when the diet was poor in Ca and Mg and rich in Al. These findings suggest that the increase in bone deposition of Zn caused by Mg deficiency is influenced not only by interaction between the two ions, but also by other ions and/or metabolic alterations resulting from the lack of Mg.

Mg is known to increase the solubility of oxalate, given the greater solubility of magnesium oxalate, and Mg deficiency leads to the appearance of  $Ca_3(PO_4)_2$  and calcium oxalate deposits in the renal tubules (Bunce & King, 1987).

The increase in renal Zn concentration in Mg-deficient rats (Table 3) points toward the participation of other divalent cations in the appearance of renal mineral deposits. From our findings we could not determine whether increased renal Zn content was a direct consequence of the Mg deficit or of changes in tubular Ca and P re-absorption.

Hypertrophy caused by the Mg deficit (Riggs *et al.* 1992) may account for the gradual increase in heart weight with time in rats given the Mg-deficient diet. Mg deficiency also causes heart calcifications (Fischer & Giroux, 1984; Wester, 1987; Lockard & Bloom, 1991), which are apparently related to changes in coronary artery contractile response (Altura, 1979). In our Mg-deficient animals the declines in myocardial Zn concentration (Table 3) may have had the purpose of maintaining plasma Zn concentrations, or may have resulted from alterations (due to the Mg deficit) in the transport of this ion across the plasma membrane.

Despite the changes observed in various tissues from Mg-deficient rats, brain concentrations of Zn (Table 4) did not vary significantly during the 7-week experimental period, suggesting that this cation is highly stable in brain tissue (Cousins & Hempe, 1990).

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