

Changes in Bioavailability and Tissue Distribution of Copper Caused by Magnesium Deficiency in Rats

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The effect of an Mg-deficient diet (200 mg of Mg/kg of feed) on the bioavailability of dietary Cu and the concentration of the cation in plasma, whole blood, skeletal muscle, kidney, heart, and liver of Wistar rats after 7, 35, 49, and 70 days was studied. The Mg-deficient diet significantly increased the amount of Cu absorbed during the 10th (final) week of the experiment. A direct linear correlation was found between Cu absorbed and Cu retention (balance) (pair-fed control $r = 0.98$, $p < 0.001$; Mg-deficient rats $r = 0.64$, $p < 0.001$). Mg deficiency significantly reduced Cu content in whole blood on all sampling days and increased liver Cu from day 35 until the end of the experiment. Plasma, muscle, and renal Cu levels were significantly increased only at the end of the study (day 70). However, there was a significant decrease on day 70 in Cu content in heart tissue. These findings show that chronic Mg deficiency increases Cu absorption and the concentrations of this mineral in plasma, muscle, kidney, and liver. Under the experimental conditions used in this study, absorption appears to play a significant role in the regulation of the tissue distribution of Cu.

Keywords: Magnesium deficiency; copper; rat

INTRODUCTION

Magnesium deficiency is known to be linked with cardiovascular alterations and renal, gastrointestinal, neurological, and muscular disorders (Shils, 1996). The symptoms and signs of Mg deficiency have been traced, in large part, to complex electrolytic interactions secondary to the mineral deficit.

Few studies have related Mg deficiency with Cu metabolism. In rats fed an Mg-deficient diet for 2 weeks, Kimura and Itokawa (1989) found a decrease in liver Cu content but no significant changes in the Cu content of brain, spinal cord, heart, spleen, kidney, testicle, muscle, bone, blood, or feces. This study also found no significant changes in the Zn/Cu ratio. Uehara et al. (1992) found that feeding a diet containing a large amount of P and a low level of Mg for 21 days increased urinary Cu excretion; however, this study provided no information on the changes caused exclusively by Mg deficiency. Another recent study in persons with acute myocardial infarction reported reduced plasma concentrations of Mg and increased concentrations of Cu during the first week postinfarction (Zeana et al., 1993).

In addition to these findings there is evidence from epidemiological studies that Mg intake in a large proportion of the population in industrialized countries is below the recommended daily allowances and that Mg deficiency, together with inadequate dietary habits, can lead to many disease states (Wester, 1987). In their study of 4859 German children, Schimatschek and Classen (1993) found hypomagnesemia in 12% of the children younger than 1 year and an incidence of 30.5%

in adolescents aged 16–18 years. According to Wong et al. (1983), approximately 10% of patients admitted to large city hospitals are hypomagnesaemic.

In previous studies based on the same experimental procedures as reported here, we fed rats for 70 days with a diet that supplied 50% of their Mg requirements and found that Mg deficiency altered the bioavailability and tissue distribution of Ca, P, and Zn ions (Planells et al., 1993, 1994, 1995). The aim of the present study was to elucidate the relationship between Mg and Cu and to determine whether the latter indirectly contributes to the development of Mg deficiency-related symptoms. We examined the degree to which a Mg-deficient diet affected the bioavailability of dietary Cu and the distribution of this element in different tissues.

MATERIALS AND METHODS

Animals and Diets. Recently weaned Wistar rats (male, female) consumed a standard commercial diet (Panlab, Barcelona, Spain) until they reached a body weight of 180 g. They were then moved to individual metabolic cages (day 0).

(1) *Mg-Deficient Group.* Forty rats (20 males, 20 females) were allowed access ad libitum to double-distilled water and a semisynthetic diet deficient in Mg. The diet contained (per kilogram of diet) 140 g of protein (casein) (Musal & Chemical, Granada, Spain), 5 g of DL-methionine (Roche SA, Madrid, Spain), 344 g of sucrose (Musal & Chemical), 344 g of wheat starch (Musal & Chemical), 80 g of fiber (cellulose), 40 g of olive oil, 35 g of AIN-76 mineral mix (without magnesium oxide) (National Research Council, 1979), 10 g of AIN-76 vitamin mix (National Research Council, 1979), and g of 2 choline bitartrate (Merck). In all, these components provided 200 mg of Mg and 7.4 mg of Cu/kg of food.

To study the evolution of Mg deficiency, one group of 10 deficient rats (5 males, 5 females) was killed by decapitation on days 7, 35, 49, and 70. Blood was collected (heparin as an anticoagulant) and centrifuged at 3000g for 15 min to separate plasma; aliquots of whole blood were taken for digestion before centrifuging. The liver, kidney, heart, and longissimus dorsi muscle were also removed on each day. During the last 7 days of each experimental group, the feces and urine were collected every 24 h and stored for subsequent analyses. The amount of food ingested was recorded.

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Table 1. Mg and Cu Content in Plasma and Whole Blood of Rats Given a Mg-Deficient Diet for 70 Days^a

day of expt	plasma Mg, mg/dL		whole blood Mg, mg/dL		plasma Cu, μ g/dL		whole blood Cu, μ g/dL	
	control	deficient	control	deficient	control	deficient	control	deficient
7	2.05 \pm 0.03	1.93 \pm 0.07	9.20 \pm 0.26	8.20 \pm 0.35*	133 \pm 6.3	119 \pm 3.4	288 \pm 14.0	175 \pm 16.6***
35	2.25 \pm 0.09	1.91 \pm 0.06*	9.00 \pm 0.24	4.90 \pm 0.28***	138 \pm 9.2	126 \pm 5.0	258 \pm 20.3	143 \pm 16.8***
49	2.30 \pm 0.09	1.77 \pm 0.03***	8.90 \pm 0.42	4.50 \pm 0.15***				
70	2.20 \pm 0.08	1.62 \pm 0.03***	8.70 \pm 0.34	3.60 \pm 0.12***	131 \pm 6.7	155 \pm 7.1*	248 \pm 12.8	125 \pm 10.0***

^a Mean values for 10 rats \pm SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Significant difference between control and deficient for each day of the experiment.

Table 2. Food and Cu Intake and Fecal and Urinary Excretion of Cu in Rats Given a Mg-Deficient Diet for 70 Days^a

days of expt	daily food intake, g/rat		daily Cu intake, μ g/rat		daily fecal Cu, μ g/rat		daily urinary Cu, μ g/rat	
	control	deficient	control	deficient	control	deficient	control	deficient
1–7	16.2 \pm 0.27	18.4 \pm 0.65**	113.4 \pm 1.9	136.2 \pm 4.8***	77.6 \pm 2.1	101.5 \pm 1.7***	9.0 \pm 0.7	27.9 \pm 0.6***
28–35	10.3 \pm 0.33	11.2 \pm 0.50	72.0 \pm 2.7	82.3 \pm 3.7*	48.4 \pm 3.6	53.6 \pm 3.3	5.5 \pm 0.2	14.8 \pm 2.5**
42–49	10.3 \pm 0.33	10.3 \pm 0.53	72.0 \pm 2.7	76.6 \pm 3.9	51.9 \pm 3.0	52.7 \pm 3.5	5.8 \pm 0.6	5.1 \pm 0.3
63–70	10.3 \pm 0.33	10.6 \pm 0.33	72.0 \pm 2.7	78.5 \pm 2.4	56.4 \pm 2.5	56.6 \pm 2.8	5.1 \pm 0.4	5.8 \pm 0.6

^a Mean values for 10 rats \pm SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Significant difference between control and deficient for each range of days of the experiment.

(2) *Control Group.* Forty rats (20 males, 20 females) were allowed access ad libitum to the diet during the first 4 weeks. From the 5th week [when food intake in Mg-deficient rats decreased (Lerma et al., 1993)] until the end of the experiment (10th week), control rats were pair-fed with the deficient group having the lowest intake (7th week). The control group was fed the same diet, except that the amount of Mg was adequate to cover nutritional requirements (465 mg of Mg and 7.00 mg of Cu/kg of food). As in the Mg-deficient group, 10 control rats (5 males, 5 females) were killed by decapitation on days 7, 35, 49, and 70 and the same tissues were removed for analysis. Food intake also was recorded, and feces and urine were collected during the last 7 days of each experimental period.

Throughout the 70 days of the experimental period the control rats and the Mg-deficient rats were housed in individual metabolic cages in a well-ventilated, temperature-controlled room (21 ± 2 °C) with a light/dark period of 12 h.

We calculated the biological indices net absorption as $I - F$ and balance as $I - (F + U)$, where I = intake, F = fecal excretion, and U = urinary excretion (FAO/WHO, 1966).

Analytical Techniques. Magnesium content in diets and whole blood was determined by flame ion atomic absorption spectroscopy (AAS) (Perkin-Elmer 1100B spectrometer) of samples previously ashed at 450 °C, until the weight was stable, in a Nabertherm furnace (Germany) with increments of 0.3 °C/min from room temperature to 450 °C. Ashed samples were then extracted with a 6 N solution of HCl (Merck) and 0.1–1.0% lanthanum chloride (Merck), brought up to an appropriate volume, and spectrophotometrically compared against a set of standards.

Copper in wet diets, excretes, and tissue was determined by AAS of a sample that had been previously wet-ashed with nitric oxide and perchloric acid (both Merck) according to the following technique: The sample was placed in a glass vessel, and 10 mL of concentrated nitric acid was added. The vessel was placed in a sand bath, and the volume of nitric acid was kept constant until decoloration was complete and the emission of nitrous vapors had stopped. Two milliliters of concentrated perchloric acid was then added, and the mixture was concentrated to a volume of about 3 mL (until white vapors appeared). The solution was then cooled, 5 mL of concentrated HCl was added, and demineralized water was added to a total volume of 25 mL (Palacios et al., 1985).

Mg and Cu in plasma were determined by AAS in samples that had been previously diluted but not ashed.

Bovine muscle (certified reference material CRM 184, Community Bureau of References, Brussels, Belgium) yielded a Cu value of 2.29 ± 0.05 μ g/g (mean \pm SEM of five determinations) (certified value 2.36 ± 0.06 μ g/g). This material was used for Cu quality control assays. Copper in CRM 184 was determined by AAS of wet-ashed samples.

Table 3. Digestive and Metabolic Utilization of Cu for Rats Given a Mg-Deficient Diet for 70 Days^a

days of expt	daily Cu absorption, μ g/rat		daily balance, μ g/rat	
	control	deficient	control	deficient
1–7	35.6 \pm 2.2	34.5 \pm 4.3	26.0 \pm 2.0	6.5 \pm 4.1***
28–35	23.6 \pm 3.7	28.8 \pm 3.6	18.1 \pm 3.9	14.0 \pm 2.9
42–49	20.1 \pm 4.4	23.4 \pm 3.5	14.3 \pm 4.6	17.0 \pm 3.9
63–70	15.3 \pm 1.3	22.2 \pm 3.0*	10.2 \pm 1.7	16.0 \pm 3.7

^a Mean values for 10 rats \pm SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Significant difference between control and deficient for each range of days of the experiment. ^b $I - (F + U)$, where I is intake, F is fecal excretion, and U is urinary excretion.

Statistical Analysis. Control vs Mg-deficient data were compared for each time period with Student's t test. In both the control and Mg-deficient groups, linear regression analyses were done.

Differences between sexes were also analyzed with Student's t test. Because these differences were small and inconsistent relative to the treatment effects, analyses were done using pooled data from both sexes. Interactive effects between diet and sex and between time and sex were evaluated with two-way analysis of variance and were also found to be small and inconsistent.

All analyses were done with the SPSS/PC software package. Differences were verified at the 5% probability level.

RESULTS

During the 70 days of the experimental period we recorded the changes in the bioavailability and tissue distribution of Cu caused by dietary Mg deficiency. Table 1 summarizes the changes in Mg concentrations in plasma and whole blood during the experimental period. The concentration of Cu in plasma and whole blood was measured on days 7, 35, and 70. The only difference in plasma was the higher Cu concentration in Mg-deficient rats on day 70. However, in whole blood, Mg deficiency decreased Cu concentration on all three days (Table 1).

Copper intake was significantly higher in Mg-deficient animals than in controls in weeks 1 (days 1–7) and 5 (days 28–35), but no significant differences were found thereafter (Table 2). In feces and urine, the differences in Cu concentrations between the two groups were similar to those found for Cu intake, although the increase in fecal Cu in week 5 did not reach significance (Table 2). In both control and Mg-deficient rats, Cu absorption tended to decrease with time (Table 3). The Mg-deficient diet increased Cu absorption, and the

Table 4. Cu Content in Longissimus Dorsi Muscle, Heart, Kidney, and Liver of Rats Given a Mg-Deficient Diet for 70 Days^a

day of expt	muscle, $\mu\text{g/g}$ of wet tissue		heart, $\mu\text{g/g}$ of wet tissue		kidney, $\mu\text{g/g}$ of wet tissue		liver, $\mu\text{g/g}$ of wet tissue	
	control	deficient	control	deficient	control	deficient	control	deficient
7	1.10 \pm 0.03	1.20 \pm 0.08	2.90 \pm 0.12	2.45 \pm 0.19	6.28 \pm 0.54	6.62 \pm 0.70		
35	0.93 \pm 0.01	1.10 \pm 0.11	2.41 \pm 0.16	2.95 \pm 0.38	5.91 \pm 0.94	5.69 \pm 0.65	6.28 \pm 0.23	7.64 \pm 0.45**
49	0.95 \pm 0.10	1.02 \pm 0.13	1.75 \pm 0.16	2.47 \pm 0.54	5.96 \pm 0.70	5.22 \pm 0.69	5.96 \pm 0.16	9.08 \pm 0.26***
70	0.79 \pm 0.05	1.20 \pm 0.15*	1.61 \pm 0.09	1.22 \pm 0.15*	5.51 \pm 0.36	7.64 \pm 0.91*	6.38 \pm 0.19	9.83 \pm 0.60***

^a Mean values for 10 rats \pm SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Significant difference between control and deficient for each day of experiment.

Table 5. Correlation Coefficients between Cu Intake, Cu Absorption and Balance, and Cu Absorption and Cu Content in Muscle and Heart in Control and Mg-Deficient Rats

	Cu intake ^a	Cu absorbed ^a	Cu balance ^a
control			
Cu absorbed	$r = 0.71$ ***		$r = 0.98$ ***
Cu muscle	$r = 0.44$ **	$r = 0.50$ **	$r = 0.45$ **
Cu heart	$r = 0.62$ **	$r = 0.54$ **	$r = 0.49$ **
Mg-deficient			
Cu absorbed	$r = 0.50$ ***		$r = 0.64$ ***
Cu muscle	ns	ns	ns
Cu heart	ns	ns	ns

^a ns, no significant correlation. **, $p < 0.01$; ***, $p < 0.001$.

difference with respect to control values was significant in week 10 (days 63–70).

Copper balance (retention) was significantly lower in Mg-deficient rats during the first week. However, retention subsequently increased to above control values in weeks 5, 7, and 10, although none of the differences was significant (Table 3).

Muscle concentrations of Cu were significantly higher in the Mg-deficient group on day 70; before this time, no significant differences between the two groups were evident (Table 4).

In both groups the concentration of Cu in the myocardium tended to decrease with time. The concentration was significantly reduced in Mg-deficient animals on day 70 (Table 4) in comparison with the control group.

Copper concentrations in the kidney of rats fed the Mg-deficient diet fluctuated throughout the experimental period. On day 70, renal Cu levels were significantly higher in this group than in control animals (Table 4).

Copper concentration in the liver was measured on days 35, 49, and 70. In rats fed the Mg-deficient diet, Cu concentrations were significantly higher than in control animals, and these concentrations tended to increase with time (Table 4).

Table 5 summarizes the most relevant correlation coefficients and significance levels.

DISCUSSION

The increase in Cu intake during the first week in animals fed the deficient diet (Table 2) reflects the increase in food intake during this period. The difference between the two groups in the fifth week, in contrast, reflects the slightly higher Cu content in the deficient diet than in the control feed (see Animals and Diets), and the slight (although nonsignificant) increase in food intake (Table 2).

In general, the changes in Cu absorbed, expressed as micrograms (Table 3), paralleled food intake in control rats, with a clear decrease in week 5 (days 28–35), when pair-feeding was started. These results are consistent with the fact that the absorption of Cu depends on the chemical form (Linder, 1996) and the dietary Cu content

(Turnlund, 1994). Turnlund et al. (1989) reported that fecal Cu excretion varied in a manner that paralleled Cu intake. However, during the pair-feeding period (weeks 5–10) Cu absorbed in control animals did not stabilize but showed a slight tendency to decrease (Table 3) as a result of the slight increase in fecal excretion with time (Table 2). This finding may be related with decreased protein intake caused by restricted food consumption. Greger and Snedeker (1980) found that fecal loss of Cu increased as protein intake decreased.

In our experiment the changes in Cu balance (Table 3) in control animals paralleled those in Cu absorbed, as expected. There was a significant linear correlation between Cu absorption and balance ($r = 0.98$) (Table 5). However, the correlation between Cu absorbed and urinary Cu excretion failed to reach significance. These results indicate that Cu cation retention is dependent mainly on absorption (Turnlund et al., 1989; Turnlund, 1994).

The effect of decreased protein intake on Cu absorption was also evident in rats fed the Mg-deficient diet (Table 3). However, Mg deficiency increased Cu absorption during the final week of the study. This finding appears to reflect the slightly greater intake in this group, as fecal losses (Table 1) were practically identical in weeks 5–10. The lower correlation between Cu intake and absorption in Mg-deficient animals than in controls (Table 5) suggests that the lower Mg intake interfered with the behavior of Cu in the gastrointestinal tract, causing a slight increase in absorption.

The increase in Cu absorption in Mg-deficient rats may also be related to changes in the permeability of intercellular junctions as a result of Mg depletion (Cassidy and Timball, 1967; Lemay and Gascon-Barre, 1992). Mg deficiency may also modify Cu absorption indirectly by altering the availability of other divalent cations (Gunshin et al., 1991; Planells et al., 1993, 1994).

The increased urinary Cu excretion in weeks 1–5 is a result of Mg depletion (Uehara et al., 1992). Later, during weeks 6–10, urinary losses decreased to control levels, apparently as a consequence of the decrease in food intake and the increase in Cu retention in muscle, kidney, and liver tissues (Table 4).

Under our experimental conditions, Cu retention (measured as Cu balance) in Mg-deficient animals (Table 3) also appeared to be regulated mainly by absorption. However, the linear correlation between Cu absorption and retention (Table 5) was not as close as in control animals. The difference may be related to changes in urinary Cu excretion in the latter group (Table 2).

We assume that the abrupt decrease in Cu concentrations in whole blood in rats fed the Mg-deficient diet (Table 1) reflects intraerythrocyte losses, as the slight changes in plasma Cu levels were insufficient to account for the entire decrease. The decrease in intraerythro-

cyte Cu may be related to alterations caused by Mg deficiency in cytoplasmic membrane transport systems.

According to the model proposed by Menkes and Wilson (Vulpe and Packman, 1995), the Cu transporter is probably a P-ATPase. Copper transport by this molecule requires phosphorylation and subsequent dephosphorylation by a phosphatase. Because Mg deficiency decreases alkaline phosphatase activity (Risco and Traba, 1994), it may also have inactivated the phosphatase required for Cu transport.

In previous studies we showed that Mg deficiency also alters the fatty acid composition of the erythrocyte membrane (Lerma et al., 1995) and decreases intraerythrocyte levels of other ions such as Zn (Planells et al., 1994), Se (Jimenez et al., 1997), and P, while increasing Ca cation levels (Planells et al., 1995). The membrane modifications, together with the intraerythrocyte ion imbalance caused by Mg deficiency, may also have influenced the behavior of Cu in these cells. Plasma concentrations of Cu showed a slight tendency to increase with time (Table 1), possibly because of the considerable losses from erythrocytes.

Copper concentrations in muscle tissue from control rats decreased with time (Table 4), most likely as a result of pair-feeding. As noted above, the restriction in food intake reduced net absorption and retention of the ion. This hypothesis is supported by the significant linear correlations between Cu concentration in muscle and Cu intake, absorption, and retention (Table 5). Muscle Cu may have been mobilized in control animals to maintain homeostasis. In this group there was a clear decrease in Cu balance (Table 2), although we found no changes in plasma concentrations of the ion. These findings suggest that muscle tissue may act as a reservoir of mobilizable Cu, as it does for Mg (Lerma et al., 1993).

Feeding the Mg-deficient diet significantly increased muscle and kidney Cu concentration on day 70. This effect may be related with the loss of intraerythrocyte Cu (Table 3) and with the slight increase in absorption (Table 2) in this group. In the kidney this finding may also have been influenced by the high rate of Ca and P deposition in the renal tubules caused by Mg deficiency (Planells et al., 1995).

In control animals, changes in Cu concentrations in the myocardium (Table 3) in time paralleled those in skeletal muscle. Significant linear correlations were found between heart Cu and Cu ingested, absorbed, and retained (Table 5). This is probably traceable to the same mechanisms as noted above for skeletal muscle.

Mg deficiency can lead to cardiac hypertrophy (Planells et al., 1994). If hypertrophy is not accompanied by an increase in Cu deposits, it may account for the decrease in Cu seen at the end of the experiment (Table 3). Reduced dietary intake of Mg also causes calcification and loss of phosphates and Zn in cardiac muscle (Planells et al., 1994, 1995), an effect suggestive of an alteration in the Cu ion transport system under our experimental conditions.

The increase in liver concentrations of Cu in Mg-deficient animals (Table 4) reflects a mechanism aimed at avoiding an increase in plasma Cu, which result from reduction in erythrocyte levels of Cu. The increase at the end of the experiment (day 70) may also have been influenced by the increase in Cu absorption (Table 3). After being absorbed by the intestine, Cu is rapidly deposited, mainly in the liver and kidney (Linder, 1996).

In conclusion, chronic Mg deficiency increased Cu absorption and concentrations of this mineral in plasma, muscle, kidney, and liver. Under our experimental conditions absorption appears to play a significant role in the regulation of the tissue distribution of Cu.

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LITERATURE CITED

- Cassidy, M. M.; Tidball, C. S. Cellular mechanism of intestinal permeability: alterations produced by chelation depletion. *J. Cell Biol.* **1967**, *32*, 685–698.
- FAO/WHO (Food and Agriculture Organization/World Health Organization). *Protein Needs*; Nutrition Meeting 37; FAO/WHO: Rome, 1966.
- Greger, J. L.; Snedeker, S. M. Effect of dietary protein and phosphorus level on the utilization of zinc, copper and manganese. *J. Nutr.* **1980**, *110*, 2243–2253.
- Gunshin, H.; Noguchi, T.; Naito, H. Effect of calcium on the zinc uptake by brush border membrane vesicles isolated from the rat small intestine. *Agric. Biol. Chem.* **1991**, *55*, 2813–2816.
- Jimenez, A.; Planells, E.; Aranda, P.; Sánchez-Viñas, M.; Llopis, J. Changes in bioavailability and tissue distribution of selenium caused by magnesium deficiency in rats. *J. Am. Coll. Nutr.* **1997**, *16*, 175–180.
- Kimura, M.; Itokawa, Y. Inefficient utilization of iron and minerals in magnesium deficient rats. In *Magnesium in Health and Disease*; Itokawa, Y., Durlach, J., Eds.; John Libbey: London, 1989; pp 95–102.
- Lemay, J.; Gascon-Barré, M. Responsiveness of the intestinal 1,25-dihydroxyvitamin-D3 receptor to Mg-depletion in the rat. *Endocrinology* **1992**, *5*, 2767–2777.
- Lerma, A.; Planells, E.; Aranda, P.; Llopis, J. Evolution of magnesium deficiency in rats. *Ann. Nutr. Metab.* **1993**, *37*, 210–217.
- Lerma, A.; Planells, E.; Aranda, P.; Llopis, J. Effect of magnesium deficiency on fatty acid composition of the erythrocyte membrane and plasma lipid concentration in rats. *J. Nutr. Biochem.* **1995**, *6*, 577–581.
- Linder, M. C. Copper. In *Present Knowledge in Nutrition*; Ziegler, E. E., Filer, L. J., Eds.; ILSI Press: Washington, DC, 1996; pp 307–319.
- National Research Council. Committee on Laboratory Animal Diets. Control of diets in laboratory animal experimentation. *Nutr. Abstr. Rev.* **1979**, *11*, 413–419.
- Palacios, M. A.; Arévalo, I.; Cámara, C. Determinación de Se en muestras biológicas por absorción atómica mediante generación de hidruros. *Quim. Anal.* **1985**, *4*, 320–325.
- Planells, E.; Aranda, P.; Peran, F.; Llopis, J. Changes in calcium and phosphorus absorption and retention during long-term magnesium deficiency in rats. *Nutr. Res.* **1993**, *13*, 691–699.
- Planells, E.; Aranda, P.; Lerma, A.; Llopis, J. Changes in bioavailability and tissue distribution of zinc caused by magnesium deficiency in rats. *Br. J. Nutr.* **1994**, *72*, 315–323.
- Planells, E.; Llopis, J.; Perán, F.; Aranda, P. Changes in tissue calcium and phosphorus content and plasma concentration of parathyroid hormone and calcitonin after long-term magnesium deficiency in rats. *J. Am. Coll. Nutr.* **1995**, *14*, 292–298.
- Risco, F.; Traba, M. L. Possible involvement of a magnesium dependent mitochondrial alkaline phosphatase in the regulation of the 25-hydroxyvitamin D3-1 α and 25-hydroxyvitamin D3-24R-hydroxylases in LLC-PK1 cells. *Magnesium Res.* **1994**, *7*, 169–178.
- Schimatschek, H. F.; Classen, H. G. Age, sex and seasonal effects on plasma and calcium levels of 4859 children. *Magnesium Res.* **1993**, *6*, 65–66.

- Shils, M. E. Magnesium. In *Present Knowledge in Nutrition*; Ziegler, E. E., Filer, L. J., Eds.; ILSI Press: Washington, DC, 1996; pp 307–319.
- Turnlund, J. R. Copper. In *Modern Nutrition in Health and Disease*; Shils, M. E., Olson, J. A., Shike, M., Eds.; Lea & Febiger: Philadelphia, 1994; pp 231–241.
- Turnlund, J. R.; Keyes, W. R.; Anderson, H. L.; Acord, L. L. Copper absorption and retention in young men at three levels of dietary copper by use of the stable isotope ^{65}Cu . *Am. J. Clin. Nutr.* **1989**, *49*, 870–878.
- Uehara, M.; Suzuki, K.; Goto, S. The effects of dietary phosphorus and magnesium levels on iron, copper and zinc utilization in rats. *Magnesium Res.* **1992**, *6*, 193 (Abstr.).
- Vulpe, C. D.; Packman, S. Cellular copper transport. *Annu. Rev. Nutr.* **1995**, *15*, 293–322.
- Wester, P. O. Magnesium. *Am. J. Clin. Nutr.* **1987**, *45*, 1305–1312.
- Wong, E. T.; Rude, R. K.; Singer, F. R. A high prevalence of hypomagnesemia and hypermagnesemia in hospitalized patients. *Am. J. Clin. Pathol.* **1983**, *79*, 348–352.
- Zeana, C.; Chirulescu, Z.; Suci, A.; Pirvulescu, R.; Carseli, C.; Zeana, C. C. Plasma Mg, Zn and Cu in acute myocardial infarction (AMI). *Magnesium Res.* **1993**, *6*, 85–86.

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