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Influence of magnesium deficiency on the bioavailability and tissue distribution of iron in the rat

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We investigated the effect of dietary magnesium (Mg) deficiency on the nutritive utilization and tissue distribution of iron (Fe). Wistar rats were fed an Mg-deficient diet (56 mg/kg) for 70 days. Absorbed Fe, Fe balance, number of the erythrocytes [red blood cells (RBC)] and leukocytes white blood cells (WBC)], hemoglobin (Hb), and Fe content were determined in samples of plasma, whole blood, skeletal muscle, heart, kidney, liver, spleen, femoral bone, and sternum obtained on experimental days 21, 35, and 70. The Mg-deficient diet significantly increased Fe absorption and Fe balance from week 5 until the end of the experimental period. This effect was accompanied by a significant decrease in the concentration of RBC and Hb from day 35, which caused the decrease in whole blood Fe seen on day 70. However, WBC were significantly increased from day 21 until the end of the experimental period. Mg deficiency significantly increased plasma and liver Fe at all three time points investigated. Spleen, heart, and kidney Fe were significantly increased only at the end of the study. However, on day 70, Fe concentration in the sternum had decreased significantly. No changes were found in skeletal muscle or femur Fe content. Mg deficiency led to increased intestinal absorption of Fe and decreased RBC counts, possibly as a result of increased fragility of the erythrocytes. Intestinal interactions between Fe and Mg, together with activation of erythropoiesis as a result of hemolysis, favored intestinal absorption of Fe. This situation gave rise to an increase in plasma Fe levels, which in turn favored Fe uptake and storage by different organs, especially the liver and spleen. However, despite the increased Fe content seen in the tissues of rats fed the Mg-deficient diet, these animals were unable to compensate for the hemolysis caused by this nutritional deficiency. (J. Nutr. Biochem. 11:103–108, 2000) © *Elsevier Science Inc. 2000. All rights reserved.*

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Introduction

Magnesium (Mg) deficiency is known to be associated with many alterations in neurologic, muscular, cardiovascular, gastrointestinal, renal, and other systems.[1] The symptoms and signs of Mg deficiency have been traced, in large part, to complex electrolytic interactions secondary to Mg deficit.

There is evidence from epidemiologic studies that Mg intake in a large proportion of the population in industrialized countries is below the recommended daily allowance and that Mg deficiency can lead to many disease states.[2] An incidence of hypomagnesemia of 30.5% has been found in German adolescents aged 16 to 18 years.[3] Low intakes of Mg for long periods may be responsible for the appearance of symptoms that have thus far remained unexplained. Examples include the relationship between low erythrocyte concentrations of Mg and sleep alterations,[4] chronic fatigue syndrome,[5] dementia of the Alzheimer type,[6] depression,[7] and increased levels of free radicals.[8]

Mg deficiency is known to increase iron (Fe) content in different tissues.[9] It has been suggested that Mg deficiency leads to increased tissue levels of Fe, possibly through mechanisms such as increased fragility and destruction of erythrocytes.[10] Hemolytic anemias are observed in some but not all chronic experimental Mg deficiencies.[11]

Increased Fe absorption may also contribute to Fe overload. Increased Mg absorption has been found in Fe-deficient rats.[12] Therefore, the opposite relationship

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between these two elements can also be hypothesized; that is, a deficient dietary intake of Mg might increase the intestinal absorption of Fe. However, Schümann et al.[13] found no change in Fe absorption in rats fed an Mg-deficient diet for 30 days.

Recent debate has focused on the chronic toxicity of Fe, particularly with regard to its relationship to cancer, atherosclerosis, and neurodegenerative disorders. This hypothetical relationship is based on the ability of Fe to generate reactive oxygen species (via Fenton and Huber-Weiss reactions) and/or to stimulate lipid peroxidation, modify proteins, or cause damage to nucleic acids. These reactions have been categorized as "oxidative stress."[14]

To shed light on the mechanisms by which Mg deficiency leads to increased tissue deposits of Fe, we designed the present in vivo experiment to follow the changes in Fe absorption and content in different tissues in rats fed an Mg-deficient diet for 10 weeks.

Methods and materials

Animals and diets

Recently weaned male Wistar rats ($n = 60$) consumed a standard commercial diet (Panlab, Barcelona, Spain) until they reached a body weight of 100 g. Thereafter they were allowed access ad libitum to double-distilled water and a semisynthetic diet deficient in Mg. The diet contained (in g/kg): casein (Musal & Chemical, Granada, Spain) 200; DL-methionine (Roche SA, Madrid, Spain) 3; sucrose (Musal & Chemical) 315; wheat starch (Musal & Chemical) 315; fiber (cellulose; Musal & Chemical) 80; olive oil 40; AIN-76 [15] mineral mix (without magnesium oxide) 35; AIN-76[15] vitamin mix 10; and choline bitartrate (Merck). In all, these components provide 56 mg Mg and 35 mg Fe per kilogram of feed.

Eight rats from the Mg-deficient and control groups were euthanized by decapitation on experimental days 21, 35, and 70. At each sampling time blood was collected (with heparin as an anticoagulant) and centrifuged at 3,000 × g for 15 minutes to separate plasma; aliquots of uncentrifuged whole blood were saved for digestion prior atomic absorption spectrophotometry (AAS). At each time point the longissimus dorsi muscle, heart, kidney, liver, spleen, femur, and sternum also were removed, weighed, and stored at −20°C for analysis. The technique of Weiser[16] was used to isolate the whole enterocyte cell populations from the duodenum and proximal-most segment of the jejunum. During the last 5 days of each experimental period the entire feces and urine output were collected every 24 hours and stored at −20°C for subsequent analysis. The amount of food ingested was recorded throughout the experimental period.

The results in Mg-deficient rats were compared with those for a group of control animals fed the same diet, except that the amount of Mg was adequate to cover their nutritional requirements (450 mg/kg food). Control animals were pair-fed with the deficient rats.

Throughout the 70-day experimental period, the control and 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 hours.

We calculated the biological indices absorbed Fe as $I - F$ and Fe balance as $I - (F + U)$, where I is intake, F is fecal excretion, and U is urinary excretion.

Table 1 Magnesium (Mg) content in plasma and whole blood of rats fed an Mg-deficient diet

Day	Mg plasma (mg/dL)		Mg whole blood (mg/dL)	
	Control	Deficient	Control	Deficient
21	2.48 ± 0.07	1.74 ± 0.06[a]	8.88 ± 0.62	5.41 ± 0.33[a]
35	2.46 ± 0.04	1.74 ± 0.04[a]	8.62 ± 0.06	4.76 ± 0.18[a]
70	2.77 ± 0.06	1.65 ± 0.05[a]	9.52 ± 0.68	3.81 ± 0.22[a]

Values are mean ± SEM, $N = 8$ rats at each time point.
[a]Significantly different (Student's t-test) from the control value $*P < 0.001$).

Analytical techniques

Erythrocyte [red blood cells (RBC)] and leukocyte [white blood cells (WBC)] counts and hemoglobin level were determined using a Symex CC-130 automatic cell counter (Japan). Mean cell hemoglobin (MCH) was calculated from the hemoglobin and RBC values.

Dry matter was determined as the material remaining after heating to 105 ± 2°C until weight was stable.

Magnesium content in diets and whole blood was determined by flame ionization AAS (Perkin Elmer 1100B spectrometer, Norwalk, CT USA) of samples previously ashed at 450°C (Nabertherm Furnace, Bremen, Germany) until the weight was stable. The resulting residues were extracted with 5 N HCl (Merck) and 0.1% lanthanum chloride (Merck), brought up to an appropriate volume and spectrophotometrically compared against a set of standards (Perkin-Elmer).

The content of Fe in diets, whole blood, and tissues was determined by AAS of samples previously ashed at 450°C and extracted with a 5 N solution of HCl (Merck). Plasma Mg and Fe were also determined by AAS in samples that were not previously ashed.

Precinorm U (Ref 180 509; Boehringer Mannheim, Barcelona, Spain) was used for quality control of Mg as the reference standard for Mg content. Bovine muscle (Certified Reference Material CRM 184, Community Bureau of Reference, Brussels, Belgium) yielded an Fe value 78.5 ± 3.2 μg/g (mean ± SEM of five determinations; certified value 79 ± 2 μg/g). This material was used for Fe quality control assays as the reference standard for Fe content.

Control versus 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. All analyses were done with the SPSS software package (Chicago, IL USA). Differences were considered significant at the 5% probability level.

Results

Table 1 summarizes the changes in Mg concentration in plasma and whole blood during the experimental period. Body weight in rats fed the Mg-deficient diets was significantly lower than that of controls at the three time points (day 21, 182 ± 2 g control versus 160 ± 8 g deficient, $P < 0.05$; day 35, 253 ± 9 g control versus 216 ± 5 g deficient, $P < 0.01$; day 70, 362 ± 5 g control versus 273 ± 7 g deficient, $P < 0.001$). In both deficient and control animals Fe intake declined during the end of the study (days 66–70; *Table 2*) in comparison with earlier periods (days 17–21 and 31–35). The lower Fe intake in Mg-deficient animals reflected the lower food intake as a result of anorexia caused

Table 2 Iron (Fe) intake and fecal and urinary excretion of Fe in rats fed a magnesium-deficient diet

Days of experiment	Fe intake (μg/rat/day)		Fecal Fe (μg/rat/day)		Urinary Fe (μg/rat/day)	
	Control	Deficient	Control	Deficient	Control	Deficient
17–21	542 ± 0.0	524 ± 30.1	230 ± 8.7	186.3 ± 12.8[a]	10.9 ± 1.46	7.6 ± 0.50[a]
31–35	525 ± 0.0	532 ± 19.7	259 ± 4.0	223.8 ± 3.7[c]	10.5 ± 0.31	5.2 ± 0.22[c]
66–70	369 ± 0.0	372 ± 22.3	203 ± 12.8	146.6 ± 4.8[b]	7.8 ± 0.25	3.1 ± 0.27[c]

Values are mean ± SEM, $N = 8$ rats at each time point.
Significant difference (Student's *t*-test) from the control value; [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

by the Mg-deficient diet.[17] In control animals the lower food intake was the result of pair-feeding. In both groups the reduced food intake from day 66 to day 70 led to decreases in Fe absorbed and Fe balance in comparison with earlier periods (*Table 3*). The relationship between food intake and Fe status is supported by the high linear correlation coefficients in both groups for Fe intake and absorbed Fe in both groups ($r = 0.87$, $P < 0.001$ in controls; $r = 0.88$, $P < 0.001$ in Mg-deficient rats) and for Fe intake and Fe balance ($r = 0.88$, $P < 0.001$ in controls; $r = 0.92$, $P < 0.001$ in Mg-deficient rats).

In Mg-deficient rats, fecal and urinary Fe excretion were significantly lower than in control animals throughout the study (*Table 2*). The lower fecal Fe excretion in Mg-deficient animals reflected the increased intestinal absorption of this element. The increased enterocyte content of Fe on day 70 (*Table 4*) supports these results. Under our experimental conditions the mean values of absorbed Fe in Mg-deficient rats were higher than in controls at all time points, although the difference reached significance only on days 31 through 35 and 66 through 70 (*Table 3*). The increased Fe absorption together with the lower urinary Fe excretion in Mg-deficient rats (*Table 2*) accounted for the increased Fe retention (balance) seen during the last two periods of the study (*Table 3*).

Under our experimental conditions the Mg-deficient diet led to a significant reduction in RBC counts from day 35 until the end of the study (*Table 5*). However, Mg deficiency clearly increased the WBC counts at all time points (*Table 5*). The decrease in RBC numbers in deficient rats resulted in significantly lower Hb concentrations in these animals from day 35 until the end of the study. We found no significant differences in MCH between control and Mg-deficient rats (*Table 5*).

In Mg-deficient rats, plasma Fe levels (*Table 5*) were significantly higher than in controls and showed a clear tendency to increase with time. However, the figures for whole blood Fe showed the opposite tendency: At the end of the study (day 70) Fe levels were significantly lower than in control rats (*Table 5*). The Mg-deficient diet had no significant effect on tissue concentrations of Fe in muscle or femur (*Table 4*). In the liver, Fe accumulation was initially seen on day 21 and tended to increase with time. Levels of Fe had also showed significant increases in the enterocytes, heart, kidney, and spleen by day 70. The differences seen in heart tissue apparently reflect the decrease in Fe content in control animals during the last period of the experiment.

In control animals we found no significant correlation between Fe concentration in liver and any other digestive or metabolic parameter, whereas in rats given the Mg-deficient diet we observed an inverse correlation between Fe content in the liver and urinary Fe excretion ($r = -0.61$, $P < 0.02$). Magnesium deficiency significantly reduced the Fe concentration found in the sternum on day 70 (*Table 4*).

Discussion

Under our experimental conditions the greater intestinal absorption of Fe seen from days 31 through 35 until the end of the study in animals fed with the Mg-deficient diet (*Table 3*) may have been caused by different mechanisms. Many studies have reported interactions between Mg and Fe in the intestine.[12,18,19] However, the mechanism of interaction has not been established; current theories have suggested a common transport system. Stonell et al.[20] have shown that Fe can be taken up by RBC via the Na^{+}/Mg^{2+} antiport system.

In addition to the role of intraluminal and mucosal factors, Fe absorption is also influenced by somatic factors such as erythropoiesis.[14,21,22] Increased plasma iron turnover is also seen in disorders such as hemolytic and sideroblastic anemias.[22] Under our experimental conditions we observed a significant increase in Fe absorption and a

Tabe 3 Digestive and metabolic utilization of iron (Fe) in rats given a magnesium-deficient diet

Days of experiment	Fe absorption (μg/rat/day)		Balance (μg/rat/day)	
	Control	Deficient	Control	Deficient
17–21	312.5 ± 8.2	338.8 ± 28.4	300.3 ± 7.9	330.0 ± 31.5
31–35	266.0 ± 3.4	311.3 ± 20.0[a]	255.0 ± 3.3	303.8 ± 21.0[a]
66–70	166.0 ± 12.8	225.4 ± 21.3[a]	156.3 ± 13.4	223.2 ± 21.5[a]

Values are mean ± SEM, $N = 8$ rats at each time point.
[a]Significantly different (Student's *t*-test) from the control value ($P < 0.05$).

Table 4 Iron content in enterocytes (mg/g protein), l*ongissimus dorsi* muscle, heart, kidney, liver, spleen, femur, and sternum (μg/g dry tissue) in rats fed a magnesium-deficient diet

	Day of experiment					
	21		35		70	
Tissue	Control	Deficient	Control	Deficient	Control	Deficient
Enterocytes	—	—	—	—	2.4 ± 0.1	5.0 ± 0.8^{b}
Muscle	74.0 ± 6.3	83.0 ± 13.8	90 ± 18	90 ± 16	88.3 ± 5.2	120 ± 15
Heart	326 ± 36	301 ± 16	302 ± 32	369 ± 32	226 ± 23	355 ± 30^{b}
Kidney	112 ± 10	120 ± 9	110 ± 7	134 ± 12	112 ± 11	143 ± 7^{a}
Liver	201 ± 11	402 ± 59^{c}	205 ± 8	447 ± 6^{c}	203 ± 7	554 ± 16^{c}
Spleen	—	—	946 ± 124	825 ± 87	1193 ± 71	2979 ± 378^{c}
Femur	78.5 ± 7.6	80.8 ± 15.6	75.7 ± 7.0	83.8 ± 4.8	75.0 ± 3.1	83.6 ± 2.7
Sternum	—	—	78.8 ± 1.2	79.6 ± 6.2	78.0 ± 4.9	36.5 ± 1.5^{c}

Values are mean ± SEM, $N = 8$ rats at each time point.
Significant difference (Student's *t*-test) from the control value: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

significant decrease in RBC counts from week 5 until the end of the study.

The structural and functional changes in the erythrocyte caused by Mg deficiency probably account for the decrease in RBC and in Hb concentrations. The fact that no significant differences were found in MCH supports the notion that the decrease in Hb concentration reflected mainly a decrease in the numbers of RBC (*Table 5*).

Among the effects of Mg deficiency are modifications in the fluidity[23] and composition of fatty acids in the erythrocyte membrane,[24] and notable changes in ion concentrations in the erythrocyte, which might affect membrane stability. Magnesium deficiency leads to calcification of the erythrocyte,[25] and activation of the calcium-binding protein calmodulin might lead to disruption of the cytoskeletal structure. Upon binding, calcium alters the structure of the protein molecule, allowing it to activate a number of structural and enzymatic proteins including phosphodiesterases, adenylate cyclase, Ca^{2+}, Mg^{2+}-ATPase, and phospholipase A2 among others.[26]

Mg deficiency is known to decrease intraerythrocyte levels of zine (Zn)[27] and copper (Cu).[28] Zn deficiency made erythrocytes more fragile[29] and affected the organization of microtubules and microfilaments that make up the cytoskeleton.[26] Although Zn deficiency did not alter erythrocyte superoxide dismutase activities, Cu deficiency decreased this activity.[29] Mg deficiency also led to a decrease in tissue vitamin E levels.[30] The effects of Mg deficiency on Cu and vitamin E levels may favor hemolysis by increasing oxidative damage.

Increased hemolysis in Mg-deficient animals may have been responsible for the steady decrease in whole-blood Fe levels (*Table 5*). The increase in plasma Fe levels in Mg-deficient rats appeared to be a consequence of the increased release of Fe to the plasma as a result of destruction of RBCs and increased Fe absorption and balance (*Table 3*).

Mg deficiency results in a generalized "pro-inflammatory state." The finding of large increases in the macrophage-derived cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor-α[31] in the plasma of Mg-deficient rats suggests that hypomagnesemia activates macrophages. All three of these cytokines are involved in a wide variety of biological activities, including cell growth and differentiation. We believe that the effects of these cytokines may play an important role in the increase in WBC counts (*Table 5*), and in fact, other researchers have reported increases in WBC counts in relation with Mg deficiency.[11]

The Mg-deficient diet led to increases in Fe concentration in most of the tissues we analyzed. Like Günther et al.,[32] we found that the greatest changes occurred in the liver and spleen (*Table 4*).

Although we noted a tendency for Fe to increase in skeletal muscle tissue, the difference between control and experimental rats was not significant at the end of the study (day 70). This finding contrasts with the results of Günther et al.,[32] who found significant increases in skeletal muscle Fe levels in rats that had consumed an Mg-deficient diet for 21 days. The discrepancy between these findings may have been due to differences between the breeds of rat (Wistar

Table 5 Erythrocytes (RBC), leukocytes (WBC), hemoglobin (Hb), plasma, and whole blood iron (Fe) in rats fed a magnesium-deficient diet

Day of experiment	RBC × 10^6/mm^3		WBC × 10^3/mm^3		Hb (g/dL)		MCH (pg)		Plasma Fe (μg/dL)		Whole blood (mg/dL)	
	Control	Deficient	Control	Deficient	Control	Deficient	Control	Deficient	Control	Deficient	Control	Deficient
21	6.4 ± 0.2	6.4 ± 0.1	8.5 ± 0.8	12.8 ± 1.0^{b}	14.0 ± 0.4	14.5 ± 0.2	21.9 ± 0.4	22.8 ± 0.3	208 ± 15	293 ± 30^{a}	45.0 ± 4.2	48.5 ± 3.4
35	6.9 ± 0.2	6.1 ± 0.2^{a}	6.1 ± 0.4	10.6 ± 1.0^{b}	15.9 ± 0.3	14.1 ± 0.5^{b}	23.3 ± 0.4	23.3 ± 0.3	210 ± 17	283 ± 22^{a}	47.3 ± 4.5	43.6 ± 4.3
70	7.1 ± 0.3	6.0 ± 0.2^{b}	8.6 ± 0.5	12.3 ± 1.3^{b}	16.8 ± 0.4	14.1 ± 0.5^{b}	23.8 ± 1.4	23.8 ± 0.8	228 ± 15	515 ± 21^{c}	48.1 ± 1.5	42.7 ± 1.9^{a}

Values are mean ± SEM, $N = 8$ rats at each time point.
Significant difference (Student's *t*-test) from the control value: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

versus Sprague-Dawley), because the amount of Mg supplied by the deficient diet was similar in both studies (56 ppm versus 80 ppm).

Erythrocytes recognized as senescent by changes in membrane structure are catabolized by Kupffer cells and spleen macrophages, and this probably accounts for the increases in the final period of the experiment in tissue uptake and storage of Fe, especially in the liver and spleen. However, we noted a significant increase in liver Fe concentration on day 21, before changes in RBC count and Hb concentration appeared. Schümann et al.[13] found no decrease in RBC numbers, Hb levels, or the percentage of reticulocytes in animals that had consumed an Mg-deficient diet for 30 days. The slight increase in Fe absorption from days 17 to 21 (*Table 3*), although not statistically significant, may mean that there were small daily increments in absorption, which might have had the cumulative effect of increasing plasma Fe levels. The increase in liver stores of Fe seen on day 21 might reflect a compensatory mechanism that removed excess Fe from the circulation. The liver is the main storage site for Fe and contains approximately 60% of the entire pool of body ferritin.[14] This may account for the early appearance of Fe deposition in this organ. Increases in tissue Fe content are a factor that helps regulate Fe homeostasis by removing excess Fe from the plasma. Because the other tissues we studied contain less ferritin,[33] any increase that may have occurred in Fe levels in these tissues would have gone undetected during the early part of the study (days 21 and 35).

The high plasma levels of Fe caused by the increase in Fe absorption and hemolysis gave rise to the increased liver Fe concentrations and an Fe overload in the heart, kidney, and spleen observed on the final day of the experiment (day 70; *Table 4*). As previously noted, the ferritin content of the kidney and heart is lower than that of the liver.[33] The large increase in spleen Fe levels resulted from the fact that erythrocytes are degraded mainly in this organ.

However, Mg deficiency did not have the same effect in the sternum and femur as in other tissues (*Table 4*). Because the sternum marrow plays an important role in hematopoiesis, we believe the decrease in Fe concentration on day 70 was the result of greater metabolic activity in the bone marrow, serving as a mechanism to increase erythropoiesis in an attempt to compensate for the hemolysis induced by Mg deficiency. Because the femur is less important for hematopoiesis (which is limited to epiphyseal marrow), this compensatory phenomenon, if it occurred, would not be detected in this type of bone under our experimental conditions.

In conclusion, Mg deficiency under our experimental conditions led to increased intestinal absorption of Fe and decreased RBC counts, possibly as a result of increased fragility of the erythrocytes. Intestinal interactions between Fe and Mg, together with the activation of erythropoiesis as a result of hemolysis, favored the intestinal absorption of Fe. This situation gave rise to an increase in plasma Fe levels, which in turn favored Fe uptake and storage by different organs, especially the liver and spleen. However, despite the increased Fe content in some tissues of rats fed the Mg-deficient diet, these animals were unable to compensate for the hemolysis caused by this nutritional deficiency.

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