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Effect of magnesium deficiency on fatty acid composition of the erythrocyte membrane and plasma lipid concentration in rats

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We studied the effects of a magnesium (Mg)-deficient diet fed to rats (200 mg/kg of feed) on plasma lipid concentrations on days 7, 35, 42, 56, 63, and 70 and analyzed the changes in fatty acid composition of the erythrocyte membrane on day 70. Dietary Mg deficiency significantly increased free cholesterol, triglyceride, and phospholipid concentrations from day 7 of treatment and led to significant increases in total cholesterol from day 35 of treatment until the end of the experimental period. On day 70, Mg deficiency had reduced oleic acid (OA) (18:1n9) and arachidonic acid (AA) (20:4n6) in the erythrocyte membrane, and had increased eicosatrienoic (20:3n6), docosapentaenoic (22:5n6), and eicosapentaenoic acid (20:5n3). Our findings suggest that the decrease in AA after long-term (70 days) dietary Mg deficiency was not due to decreased Δ-6-desaturase activity, in contrast with earlier studies. (J. Nutr. Biochem. 6:577–581, 1995.)

Keywords: erythrocyte; membrane fatty acid; Mg deficiency; plasma; phospholipids; rat; total cholesterol; free cholesterol; triglycerides

Introduction

Substantial epidemiologic and animal experimental evidence indicates that magnesium (Mg) deficiency is an important factor in the origin of cardiovascular diseases.[1–6] Studies with short-term experimental Mg deficiency in rodents have found hyperlipidemia, mainly due to hypertriglyceridemia, together with increased concentrations of free cholesterol.[7,8] These findings were not accompanied by significant changes in total cholesterol.[9] Other short-term studies have reported marked increases in plasma phospholipid concentrations.[10]

Mg deficiency is thought to produce these results by reducing lecithin-cholesterol acyltransferase (LCAT)[7] and lipoproteinlipase (LPL) activity,[11] decreasing LPL concentrations in adipose tissue,[12] modifying lipoprotein content and composition,[10] or decreasing total uptake, receptor binding, and internalization of low density lipoprotein.[13] All these factors may well limit lipoprotein metabolism decreasing HDL, with a resultant increase of the LDL/HDL ratio; according to Rayssiguier et al.,[14] the resultant increase in lipoprotein concentrations gives rise to hyperlipidemia and increasing membrane fluidity.[15–17] However, a study with long-term Mg deficiency found lesser degrees than expected of hypertriglyceridemia and hyperphosphatemia, together with increased levels of total cholesterol.[18]

The changes in plasma lipid concentrations in Mg-deficient animals were accompanied by variations in plasma fatty acid concentrations. One short-term study reported increased levels of oleic (18:1n9) and linolenic acid (18:2n6), with decreases in stearic (18:0) and arachidonic acid (20:4n6).[19] In this connection, a long-term study found that plasma fatty acid concentrations, like those of other plasma lipids, changed in different directions and to different degrees in rabbits subjected to Mg deficiency. Moreover, the changes in plasma fatty acid composition caused by Mg deficiency can differ markedly from the changes observed

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in tissues.[18] However, no information is available on changes in fatty acid composition of the membrane, which may be related to functional alterations noted in erythrocytes in Mg deficiency.[9]

To clarify these contradictory findings, we designed the present experimental study in rats to investigate the changes in plasma lipid concentrations during chronic Mg deficiency and to record the changes in fatty acid composition of the erythrocyte membrane. Magnesium deficiency was caused by feeding a diet that covered 50%[20] of this species' requirements, for a period of 10 weeks. This long time frame was used so that our experimental model would more nearly approximate the situation described in Western populations.[8,21]

Methods and materials

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 access ad libitum to bidistilled water and a semisynthetic diet deficient in Mg. The diet contained (g/kg) protein (casein) (Musal & Chemical, Granada, Spain) 140; DL-methionine (Roche SA, Madrid, Spain) 5; sucrose (Musal & Chemical) 344; wheat starch (Musal & Chemical) 344; fiber (cellulose) (Musal & Chemical) 80; olive oil 40; AIN-76 mineral mix (without magnesium oxide) 35; AIN-76 vitamin mix 10; and choline bitartrate (Merck) 2. In all, these components provided 200 mg of Mg, 6,200 mg of Ca, 4,400 mg of P, and 25 μg of cholecalciferol/kg of feed.

To study the effect of Mg deficiency on plasma lipids, 10 deficient rats (5 males, 5 females) were killed by decapitation on experimental days 7, 35, 42, 49, 56, 63, and 70. Blood was collected and centrifuged to separate plasma and erythrocyte. Any plasma sample that showed signs of hemolysis was discarded and replaced with a sample from a different animal that had been subjected to the same experimental procedure.

Changes in the fatty acid composition in erythrocyte membrane total lipids were studied only on experimental day 70.

The results were compared with those for a group of control rats fed the same diet, except that the amount of Mg was adequate to cover their nutritional requirements (465 mg/kg of food). Control animals were allowed access ad libitum 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).[22]

Analytical techniques

Blood samples were centrifuged at 3,000*g* for 10 min to separate plasma from cells. Plasma magnesium was determined by atomic absorption spectrophotometry with a Perkin Elmer 1100B AAS (Perkin Elmer Co., Norwalk, CT).

Plasma concentrations and total and free cholesterol, triglycerides, and phospholipids were determined with commercial enzyme colorimetric kits from Boehringer Mannheim GmbH (Germany).

Erythrocyte ghosts were obtained according to the method described by Hanahan and Ekholm.[23] Red blood cell membrane lipids were extracted with chloroform/methanol (2:1) containing the antioxidant 2,6-di-tert-butyl-p-cresol (BHT) (50 mg/L).[24] Fatty acids were methylated according to the method of Morrison and Smith,[25] and were determined by gas chromatography with a Perkin Elmer 8310 Chromatograph equipped with a flame ionization detector. The sample was placed on a 4 m-long metallic column measuring ⅛ inch in internal diameter. The stationary phase was 10% Sp-2330 on 100/120 mesh Chromosorb HAW (Technocroma, Barcelona, Spain). Fatty acids were identified by comparing their stopping times with those of a standard solution containing an 0.2% mixture of individual fatty acid methyl esters (Sigma, St. Louis, MO USA; Supelco, Bellefonte, PA USA).

The data were analyzed with one-way analysis of variance (ANOVA). Differences between sexes, also evaluated by one-way ANOVA, were small and inconsistent relative to treatment effects. Interactive effects between diet and sex, and between time and sex, evaluated with two-way ANOVA, were also small and inconsistent. Differences between the means were considered significant at $P < 0.05$.

Results

Table 1 summarizes the changes in plasma Mg during the experimental period. Under our experimental conditions, feeding an Mg-deficient diet (200 mg/kg) for 70 days significantly increased plasma concentrations of free cholesterol (*Figure 1*) after day 7, and total cholesterol after day 35 (*Figure 2*). These values remained elevated until the end of the experimental period. Total cholesterol in Mg-deficient rats showed only small fluctuations throughout the experiment and tended to stabilize at significantly higher values than in the control group. However, free cholesterol increased sharply between days 49 and 56, then remained elevated until day 70 (*Figure 1*).

As shown in *Figures 3* and *4*, plasma triglyceride and phospholipid concentrations were significantly higher in Mg-deficient rats than in controls throughout the duration of the experiment. Triglyceride (*Figure 3*) levels were lowest on day 35; phospholipid (*Figure 4*) values were lowest on day 42; after this time, the concentrations of both lipids tended to increase, although phospholipids showed a tendency to stabilize from day 49 onward.

Table 2 gives the changes in fatty acid composition of erythrocyte membrane lipids during the 70-day period of dietary Mg deficiency. Under our experimental conditions, oleic (18:1n9) and arachidonic acid (20:4n6) decreased significantly, while eicosatrienoic (20:3n6), docosapentaenoic (22:5n6), and eicosapentaenoic acid (20:5n3) increased.

Discussion

Under our experimental conditions, the slight decrease on day 35 in free cholesterol, triglycerides, and phospholipids

Table 1 Mg content in plasma (22)

Day	Plasma Mg, mg/dl	
	Control	Mg-Deficient
7	2.05 ± 0.03	1.93 ± 0.07
35	2.25 ± 0.09	1.91 ± 0.06*
42	2.10 ± 0.11	1.81 ± 0.05*
49	2.30 ± 0.09	1.77 ± 0.03*
56	2.20 ± 0.13	1.59 ± 0.06*
63	2.10 ± 0.09	1.61 ± 0.05*
70	2.20 ± 0.08	1.62 ± 0.03*

Values shown are means for 10 rats ± SEM. *$p < 0.05$ vs control.

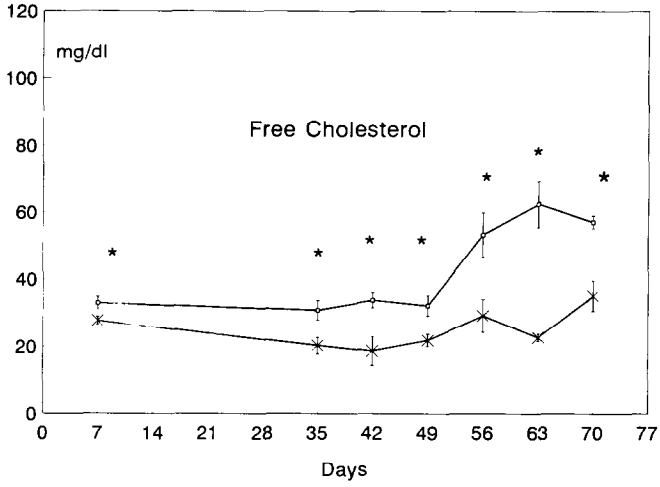

Figure 1 Plasma concentrations of free cholesterol during 70 days of Mg deficiency in rats. (■) Mg-deficient, (×) Controls. *p < 0.05.

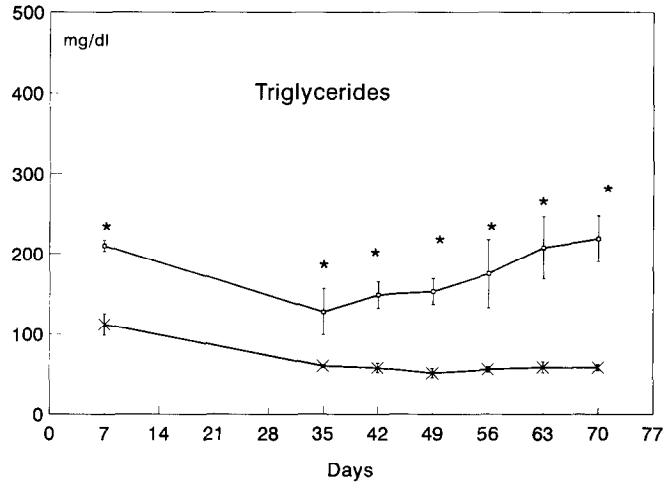

Figure 3 Plasma concentrations of triglycerides during 70 days of Mg deficiency in rats. (■) Mg-deficient, (×) Controls. *p < 0.05.

in both control animals and Mg-deficient rats, and the decline in total cholesterol in controls (*Figures 1, 3,* and *4*), may reflect anorexia caused by Mg deficiency itself. In an earlier study in our laboratory under similar conditions, decreased food intake was seen after 28 days in Mg-deficient rats.[22] In control animals, the decrease is traceable to pair-feeding.

The increased concentration of free cholesterol in Mg-deficient animals (*Figure 1*) is thought to be traceable to impaired LCAT activity. Magnesium deficiency impairs LCAT activity; because the role of this enzyme is to prevent unesterified cholesterol from accumulating in plasma,[7] decreased activity could lead to increased circulating concentrations of free cholesterol[7,11] together with a decrease in HDL concentration.[14]

The increase in free circulating cholesterol found on day 7 (*Figure 1*) did not significantly affect total cholesterol concentration, a finding also reported in a short-term study.[9] The increases in free cholesterol account for only 30.5% of the increase in total cholesterol seen on day 35, 45.4% on day 42, and 15.6% on day 49. During this part of the study, the increments in free cholesterol may have been accompanied by increases in esterified cholesterol. However, after day 49, the increases in free cholesterol accounted for 70% of the increase in total cholesterol on day 56 and for the entire increase recorded on days 63 and 70 (*Figures 1* and *2*).

During the first 7 weeks of Mg deficiency, LCAT may have been only partially inhibited, and part of the cholesterol may have been esterified. This, together with the lipolytic effect of Mg deficiency[11] and impaired lipoprotein metabolism,[9,10,13,14] may have led to an increase in both free and total fractions of cholesterol. Zhou et al.[17] also found that free and esterified cholesterol were increased in rabbits fed an Mg-deficient diet for 50 days.

After day 49, tissue Mg depletion[22] may have also contributed to the increased inactivation of LCAT. Moreover, the reduction in adipose tissue as a result of the lipolytic

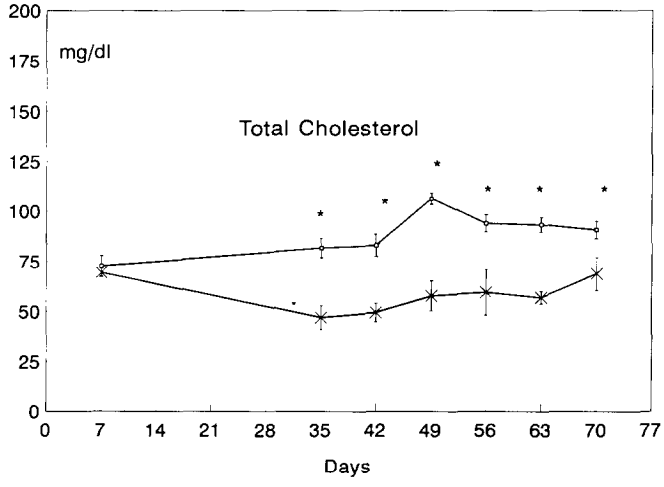

Figure 2 Plasma concentrations of total cholesterol during 70 days of Mg deficiency in rats. (■) Mg-deficient, (×) Controls. *p < 0.05.

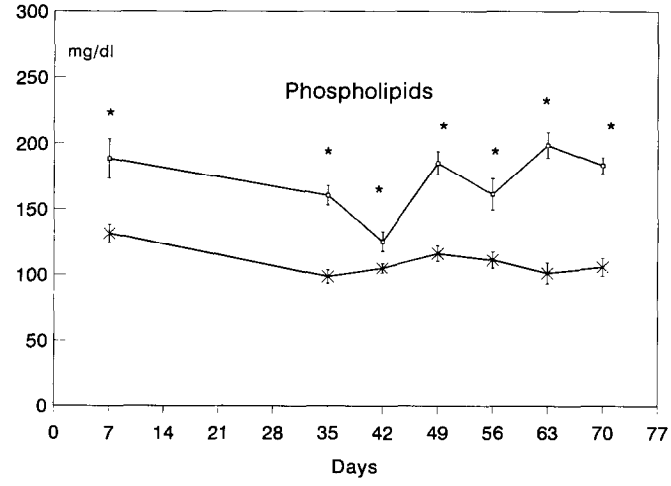

Figure 4 Plasma concentrations of phospholipids during 70 days of Mg deficiency in rats. (■) Mg-deficient, (×) Controls. *p < 0.05.

Table 2 Changes in fatty acid composition of total lipids in the erythrocyte membrane in Mg-deficient rats

Fatty acids (% total fatty acids)	Control	Mg-Deficient (70 days)
C12	0.08 ± 0.01	0.22 ± 0.12
C14	0.97 ± 0.22	0.50 ± 0.13
C16	40.53 ± 1.89	41.71 ± 1.21
C16:1n7	1.29 ± 0.19	0.77 ± 0.24
C18	14.44 ± 1.44	16.65 ± 1.38
C18:1n9	17.60 ± 1.88	9.65 ± 0.87*
C18:2n6	3.93 ± 0.33	5.08 ± 0.96
C18:3n6	0.65 ± 0.02	1.91 ± 0.62
C18:3n3	0.73 ± 0.24	1.33 ± 0.25
C20:3n6	0.55 ± 0.11	4.80 ± 0.31*
C20:4n6	15.32 ± 1.67	8.30 ± 0.70*
C20:5n3	0.30 ± 0.08	1.24 ± 0.13*
C22:4n6	1.67 ± 0.22	2.55 ± 0.42
C22:5n6	0.69 ± 0.10	2.75 ± 0.42*
C22:5n3	0.63 ± 0.15	0.90 ± 0.11
C22:6n3	1.38 ± 0.29	1.47 ± 0.09

Values shown are means for 10 rats ± SEM. *$p < 0.05$ vs control.

effect of Mg depletion[11] may have reduced the availability of fatty acids for the formation of esters. In fact, during the final weeks of the experimental period used here, Mg-deficient rats almost completely lacked adipose tissue. These findings may explain why, during the last weeks of Mg deficiency, the increase in free cholesterol accounted for the entire increase in total cholesterol concentration, as noted previously. However, other enzyme systems may also be involved: Golf et al.[26] suggested that Mg deficiency may keep hydroxy-methyl-glutaryl coenzyme A reductase (HMG CoA) in an activated state and thus favor cholesterol biosynthesis.

The increase in circulating triglyceride levels (*Figure 3*) may have been a consequence of altered LPL behavior.[11] Alternatively, this change may have reflected a decrease in LPL concentration in adipose tissue,[12] together with alterations in lipoprotein metabolism[9,10,13,14] and changes in the amount of adipose tissue.

Little is known about the effects of prolonged consumption of a Mg-deficient diet on phospholipid metabolism. In rats, Mg deficiency increases the plasma concentration of phospholipids (*Figure 4*).[10,18] In rabbits, however, there is no change in total plasma phospholipids, although some individual phospholipids such as phosphatidylcholine, phosphatidic acid, phosphatidylinositol, and sphingomyelin are increased, a finding Zhou et al.[17] attributed to increased synthesis of hepatic phospholipids. Species differences may be responsible for the discrepancy between these findings.

The changes in the fatty acid composition of total lipids in the erythrocyte membrane (*Table 2*) of Mg-deficient rats differed from the modifications reported in plasma[18,19] and from the alterations found in the liver, kidney, heart, and aorta.[18] However, like Rayssiguier et al.,[19] we found a marked decrease in AA (20:4n6) (*Table 2*).

The causes for the decrease in OA (18:1n9) in the erythrocyte membrane are unknown. Weis[27] noted that the incorporation of OA into tissue lipids was independent of tissue Mg content. A plausible explanation for this finding is that after 70 days of Mg deficiency, our rats showed evident signs of protein-energy malnutrition, and because this fatty acid is more quickly oxidized than others,[28] it may have been used for energetic metabolism.

Studies of hepatic microsomes have suggested that Mg deficiency decreases Δ-6-desaturase activity.[29] However, we found no significant changes in linolenic acid (18:2n6) in the erythrocyte membrane; this result, together with the significant increase in eicosatrienoic acid (20:3n6) (*Table 2*), speaks against this hypothesis, since 20:3n6 and eicosapentaenoic acid (20:5n3) could not have increased if Δ-6-desaturase activity had been diminished. The findings of Cunnane et al.[18] in serum, liver, kidney, heart, and aorta likewise fail to support increased Δ-6-desaturase activity in these tissues. The effects of Mg deficiency may thus be dependent on the duration of the deficit and on the type of tissue analyzed.[18]

The decrease in 20:4n6 content in Mg-deficient rats appeared to be due to causes other than the decline in Δ-6-desaturase activity. The greater 22:5n6 content in comparison with control animals (*Table 2*) suggests that the metabolism of 20:4n6 to long-chain n6 derivatives was enhanced, although the increases in these compounds accounted for only a small part of the decrease in 20:4n6. Weis[27] found that Mg depletion indirectly reduced the activities of coenzyme A synthetase and acyl transferase by modifying protein kinase activity[30] and thus reduced the incorporation of arachidonic acid into tissue phospholipids[27] making more arachidonic acid available to cyclo-oxygenase or lipoxygenase.[30] In this connection, Soma et al.[31] observed that isolate perfused mesenteric vascular beds of Mg-deficient rats produced more 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin E_2, and thromboxane B_2 (a metabolite of thromboxane A_2) than those of control animals. Moreover, Mg depletion increases the susceptibility of platelets to thrombin-induced aggregation.[19]

The 20:4n6 and 20:5n3 fatty acids are known to compete for cyclo-oxygenase and lipoxygenase in the formation of different eicosanoids. If more 20:4n6 is available, more prostaglandin E_2 and thromboxane A_2 (stimulant of platelet aggregation) are produced. The increased availability of 20:4n6 thus curtails the metabolism of 20:5n3 (which gives rise to eicosanoids with strong antiplatelet aggregation properties).

Under our experimental conditions, AA (20:4n6) in the membrane of erythrocytes from Mg-deficient rats is decreased, partly because of the diminished incorporation of phospholipids in tissues.[27,30] As a result, more AA is available for metabolism by cyclo-oxygenase and lipoxygenase. This facilitates the formation of eicosanoid derivatives of AA, a result that partly accounts for the increased platelet aggregation in Mg-deficient rats and their increased susceptibility to vascular lesions.[19]

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