Effect of Magnesium Deficiency on Post-Heparin Lipase Activity and Tissue Lipoprotein Lipase in the Rat

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Previous studies have provided evidence that Mg deficiency affects lipid metabolism. The present experiments were designed to assess whether the hypertriglyceridemia associated with Mg deficiency was related to alterations in post-heparin lipase activity (PHLA). Mg-deficient and control diets were pair-fed to weanling Wistar rats for eight days and plasma lipoproteins were separated into various density classes by sequential preparative ultracentrifugation. Triglycerides were significantly increased in chylomicrons and in the very low density lipoprotein, low density lipoprotein and high density lipoprotein (HDL) fractions. Cholesterol and phospholipid levels were significantly lower in the HDL fraction. PHLA in deficient rat was substantially lower than in control rats. The inverse correlation between plasma triglyceride concentration and PHLA strongly suggests that hypertriglyceridemia is the result of defective lipolysis of plasma triglycerides in Mg-deficient rats. Further examination of the PHLA was carried out by salt-mediated inhibition of lipoprotein lipase (LPL) and by heparin sepharose affinity chromatography and purified rat LPL antiserum. The results indicate that hepatic lipase is significantly decreased in Mg-deficient rats but the low PHLA is due mainly to a decline in LPL. However, total LPL activity, that is, both the intracellular and the extracellular pools of LPL in adipose tissue, heart and diaphragm, were unaffected by Mg deficiency. The results suggest that the decrease of LPL activity in the plasma of Mg-deficient rats may be due to a selective decrease in the heparin-releasable pool of enzyme.
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Several studies have provided evidence that magnesium deficiency affects lipid metabolism (1–5). Magnesium deficiency can have pronounced effects on the physical state of membrane bilayer lipids, and defective membrane function could be the primary lesion underlying the cellular disturbances that occur in magnesium deficient animals (6).

The mechanisms behind the hypertriglyceridemia of Mg-deficient rats have been addressed in several studies. Hyperlipemia can be produced either by excessive production and release of lipids into the circulation, by their impaired removal from the circulation, or by a combination of both. Previous experiments indicate that decreased clearance of circulating triglycerides is a major mechanism contributing to hyperlipemia in Mg-deficient rats (7). Because lipoprotein lipase is the rate-limiting enzyme in the removal process, reduction of its activity can lead to hypertriglyceridemia (8). The present experiment was designed to test the hypothesis that hypertriglyceridemia associated with Mg deficiency can be causally related to alterations in LPL activity.

MATERIALS AND METHODS

Weanling male Wistar rats weighing about 60 g were randomly divided into magnesium-deficient and control groups. They were housed four to a cage and pair-fed with the appropriate diets for eight days using an automatic feeding apparatus. The initial food intake of 6 g/day rose to 8 g/day per rat. Distilled water was provided *ad libitum.* The synthetic diets contained (g/kg) casein 200, sucrose 705, corn oil 50, mineral mixture 35, and vitamin mixture 10, as described previously (1). Their magnesium contents determined by analysis were 30 mg/kg (deficient) and 960 mg/kg (control).

The experiments were performed between 9–10 a.m. on non-fasting rats. In some studies, the rats were food deprived 12 hr before experimentation. Plasma or other tissue samples were obtained on rats anesthetized with sodium pentobarbital (40 mg/kg of body weight).

Lipoprotein isolation and analysis. Lipoprotein isolation and analysis were carried out on unfrozen plasma from non-fasting rats. NaN_3 (0.02%), merthiolate (0.005%) and Na_2-EDTA (0.04%) were added. Samples were overlayered with 0.15 M NaCl/0.01% EDTA (d=1.006 g/mL) and chylomicrons were recovered following two centrifugations for 30 min at 12000 × *g*. Lipoproteins were separated into various density classes by sequential preparative ultracentrifugation. Very low density lipoproteins (VLDL) (d < 1.006 g/mL), low density lipoproteins (LDL) (d=1.006–1.050 g/mL) and high density lipoproteins (HDL) (d=1.050–1.21 g/mL) were isolated after the density had been adjusted with solid KBr. Ultracentrifugation was performed at 20°C in a Beckman model L5-50B ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) with a Ti-50 rotor.

Post-heparin plasma. Blood samples were withdrawn from the subclavian venous plexus of fed or fasted rats into tubes containing heparin. The plasma was separated in a refrigerated centrifuge and was used for the determination of Mg and triglycerides. Blood was taken again 10 min after intravenous administration of heparin (Produits Roche, Neuilly, France) at 25 U per 100 g of body weight.

Tissue lipoprotein lipase activity. Epididymal fat pads in fed rats, and the diaphragm and heart in fasted rats were excised immediately. Tissues were rinsed in 0.15 M NaCl solution at 4°C, blotted on filter paper, weighed and frozen on dry ice.

Measurement of post-heparin lipase activity in plasma. Lipolytic activity was measured with an emulsion of glycerol tri[1-^{14}C]oleate as substrate (9,10). The stock substrate contained 25 μCi (926.10^3 Bq) glycerol tri-[1-^{14}C]oleate, 69 mg unlabelled triolein, 3.33 mg soybean phosphatidylcholine and 5 mL glycerol. The mixture was

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Abbreviations: HDL, high density lipoprotein; HL, hepatic lipase; IHD, ischemic heart disease; LDL, low density lipoprotein; LPL, lipoprotein lipase; PHLA, post-heparin lipolytic activity; VLDL, very low density lipoprotein.

emulsified on ice for 5 min using a polytron (IKA, Staufen, Germany). The emulsion was stable for six weeks. The assay mixture contained 150 μL of the enzyme solution and 100 μL of diluted substrate (40 μL of emulsified stock substrate, 40 μL of 5% bovine serum albumin solubilized in 0.62 M Tris/0.16 M NaCl and adjusted to pH 8.6, and 20 μL of fresh human serum). After incubation at 37°C for 20 min, the labelled fatty acid products released were extracted and radioactivity was counted. Enzyme activity (1 mU) was defined as the amount of enzyme required to catalyze the release of 1 nmol of fatty acid per min at 37°C.

Measurement of tissue lipoprotein lipase (LPL) activity. Acetone/diethyl ether powder was prepared, and the crude lipoprotein lipase was extracted with 25 mM NH_4OH/NH_4Cl buffer (pH 8.5). The enzyme preparation was then homogenized with a polytron, centrifuged for 30 min at 4000 × g at 4°C and the supernatant collected. Measurements were made on 50–150 μL of supernatant.

Characterization of post-heparin lipase activity in plasma. In some assays to measure the effect of 1 M NaCl and the serum dependence of the lipolytic activity, serum was omitted from the incubation medium and was replaced by 0.16 M NaCl. The incubation was carried out with 100 μL of enzyme plus 50 μL of 5 M NaCl and 100 μL of substrate.

Heparin-Sepharose affinity chromatography was done on CNBr Sepharose 4B containing covalently linked crude heparin. The 1 × 5 cm column was equilibrated with 0.005 M barbital buffer containing 0.4 M NaCl (pH 7.4), and chromatography was carried out at 4°C. Two mL of diluted post-heparin plasma 0.5 M NaCl (v/v) was applied to the column, and the column was washed with 10 mL 0.25 M NaCl buffer and 30 mL 0.5 M NaCl. Successive 2-mL fractions were eluted with NaCl solutions of increasing molarity [10 mL of 0.7 and 1.5 M NaCl in 0.005 M barbital buffer (pH 7.4)]. Measurements were made on 150 μL enzyme fractions.

Lipase activity was also measured by use of an antiserum against purified LPL from rat adipose tissue, raised in goats as previously described (11). This antiserum has been shown to partially inhibit whole post-heparin plasma and it did not inhibit hepatic triacylglycerol lipase separated from rat post-heparin plasma by affinity chromatography. Post-heparin plasma (75 μL) diluted 1:10 was preincubated for 5 min at 37°C with 75 μL antiserum. The mixture was then centrifuged and incubated with substrate. Lipolytic activity was measured before and after incubation with the antiserum.

Other analytical methods. Magnesium was determined with a Perkin-Elmer series 400 atomic absorption spectrophotometer. Plasma was analyzed directly after dilution in lanthanum chloride solution containing 1 g La/L. Plasma triglycerides (Biotrol, Paris, France), cholesterol (BioMérieux, Charbonnières-les-Bains, France) and phospholipids (BioMérieux, Charbonnières-les-Bains, France) in plasma were determined by enzymatic procedures (12–14).

Calculations and statistical analysis. Values are given as means ± SEM. Data were analyzed by Student's t-test or Mann-Whitney U test and regression analysis.

RESULTS

The mean final body weights of the magnesium-deficient and control rats were 85 and 91 g, respectively, at the end of the experimental period and clinical symptoms of Mg deficiency were observed. An obvious turbidity and hyperlipemia was visible in the plasma from deficient animals when rats were not fasted before sacrifice; this was due mainly to hypertriglyceridemia. Triglyceride, cholesterol and phospholipid distribution in lipoprotein fractions are shown in Table 1. With Mg deficiency, triglycerides were significantly increased in chylomicrons and in the VLDL, LDL and HDL fractions. Both phospholipids and cholesterol were significantly increased in the VLDL and LDL fractions, but significantly lower in the HDL fraction.

Plasma Mg levels were significantly lower in the Mg-depleted group. PHLA in non-fasting deficient rats was significantly lower than in control rats (Table 2) and was inversely correlated with plasma triglyceride concentration (Fig. 1). The plasma post-heparin activity of Mg-deficient rats was also lower than that of the control rats

TABLE 1

Effect of Dietary Mg on Lipid Composition of Plasma Lipoproteins on Non Fasted Rats[a]

	Triglycerides (mg/100 mL)	Cholesterol (mg/100 mL)	Phospholipids (mg/100 mL)
Chylomicrons			
Control	17 ± 3	—	—
Mg deficient	102 ± 25**	—	—
VLDL			
Control	43.8 ± 5.9	7.2 ± 1	11.6 ± 1.8
Mg deficient	151.7 ± 15.8***	19.1 ± 3.3**	40.1 ± 7.5**
LDL			
Control	9.5 ± 1.1	7.4 ± 0.6	7.9 ± 0.4
Mg deficient	26.5 ± 1.7***	10.8 ± 0.5***	15.7 ± 0.9***
HDL			
Control	4.7 ± 0.4	29.0 ± 1.3	34.4 ± 2.4
Mg deficient	6.5 ± 0.4**	14.7 ± 0.7***	27.5 ± 2.7*

[a]Means ± S.E.M. of eight determinations per group. Statistical analysis was by Student t-test. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

TABLE 2

Effect of Dietary Mg on Post-Heparin Plasma Lipolytic Activity in Non Fasted Rats[a]

	Mg (mM)	PHLA total (nmol/min/mL)	+ NaCl[b] (nmol/min/mL)
Control	0.77 ± 0.02 (7)	803 ± 24 (6)	82 ± 2.3 (5)
Mg-deficient	0.14 ± 0.01 (7)	278 ± 42 (7)	55 ± 3.0 (6)
	$P < 0.001$	$P < 0.001$	$P < 0.001$

[a]Numbers in parentheses indicate number of determinations for each assay. Values are given as means ± SEM. Statistical analysis was by Student t-test.

[b]Determination of enzyme activity under standard conditions described in Material and Methods, + NaCl, assay in the presence of 1 M NaCl without addition of serum.

TABLE 3

Characterization of Post-Heparin Plasma Lipolytic activity in Control and Mg-Deficient Rats[a]

	Total affinity (nmol/min/mL)	+ antiserum to LPL (nmol/min/mL)	LPL after affinity chromatography (nmol/min/mL)
Fed control (3)	679.7 ± 50.6	66 ± 6	143 ± 15
Fed Mg-deficient (3)	362.0 ± 23.6	38 ± 3	58 ± 13
	P < 0.05	P < 0.05	P < 0.05

[a]Numbers in parentheses indicate number of determinations for each assay. Values are given as means ± SEM. Statistical analysis was by Mann-Whitney U test.

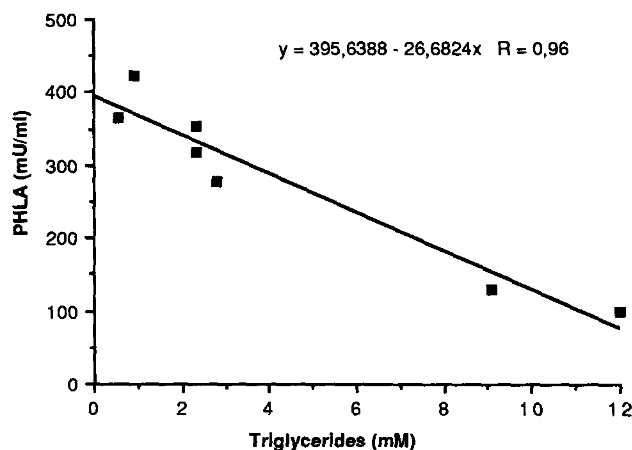

FIG. 1. Relationship between plasma triglyceride concentrations and post-heparin lipolytic activity in Mg-deficient animals.

when lipolytic activity was determined at 1.0 mol NaCl in the absence of human serum (Table 2).

For further characterization of PHLA (Table 3), the LPL fraction was separated on a heparin Sepharose affinity column. The fraction, which was inhibited by antilipoprotein lipase serum, was significantly reduced in Mg-deficient rats. When post-heparin plasma was incubated with antiserum, the lipolytic fraction of Mg-deficient rats was also lower than that of control rats. Thus, low PHLA was shown to be mainly due to a decline in LPL, but hepatic lipase (HL) was also significantly decreased. When rats were food deprived before sacrifice, the effect of Mg deficiency on plasma triglyceride concentration was also noticeable and PHLA was significantly lower than in control rats (Table 4). The adipose tissue LPL activity was the same in non-fasting control and Mg-deficient rats, and the heart and diaphram enzyme activity was not different in the two experimental fasted groups (Table 5).

TABLE 4

Effect of Dietary Mg on Post-Heparin Plasma Lipolytic Activity in Fasted Rats[a]

	Mg (mM)	TG (mM)	PHLA (nmol/min/mL)
Control (8)	0.80 ± 0.03	0.24 ± 0.02	575.5 ± 17.2
Mg-deficient (7)	0.27 ± 0.02	3.41 ± 0.86	312.4 ± 20.4
	P < 0.001	P < 0.01	P < 0.001

[a]Numbers in parentheses indicate number of determinations for each assay. Values are given as means ± S.E.M. Statistical analysis was by Student *t*-test.

TABLE 5

Effect of Dietary Mg on Lipoprotein Lipase Activity in Rat Organs[a]

	Control (nmol/min/g)	Mg-deficient (nmol/min/g)	
Epididymal fat (fed)	227.6 ± 30.6 (10)	252.0 ± 28.6 (11)	NS
Heart (fasted)	20.9 ± 0.7 (6)	23.2 ± 3.0 (6)	NS
Diaphragm (fasted)	206.0 ± 19.2 (6)	230.2 ± 28.8 (6)	NS

[a]Numbers in parentheses indicate number of determinations for each assay. Values are given as means ± S.E.M. Statistical analysis was by Student *t*-test. NS, not significant.

DISCUSSION

The hyperlipemic effect of Mg deficiency was observed in post prandial conditions or following fasting. The post prandial phase is a non-static phase where activation of the processes of formation, catabolism and interchange of the different lipoprotein fractions takes place. The results are often variable since they depend, at least partially, on the stage of intestinal absorption. An automatic feeding apparatus was therefore used in the present experiment in order to obtain the same pattern of feeding in both control and Mg-deficient rats. This was not the case in the previous experiment (1). Moreover, as the hyperlipemic effect of Mg deficiency was still observed when rats were food deprived 12 hr before sacrifice, this effect was not the consequence of a modification in intestinal absorption.

Chylomicrons, VLDL and LDL levels were higher in Mg-deficient rats, but HDL levels were lower than in controls. Triglycerides were elevated and this was primarily due to an increase in chylomicron and VLDL concentrations. Cholesterol levels increased in the VLDL and LDL fractions. Since plasma cholesterol levels tend to decrease as a result of the decrease in HDL levels, total cholesterol

levels were not modified. In fact, the contribution of VLDL and LDL cholesterol to the total plasma cholesterol is very small in the rat (15). In contrast to the slight modification in total cholesterol in severe magnesium deficiency of short duration, other experiments indicate a significant increase in total cholesterol during moderate magnesium deficiency of long duration (16).

Several defects can produce hyperlipemia. These defects can consist of either an overproduction of a lipoprotein or a decrease in catabolism. By studying intralipid clearance, we were able to show that hyperlipemia developed because triglyceride removal was impaired (7). Moreover Mg-deficient rats exhibited delayed clearance of ^{14}C-labelled VLDL compared to control rats, whereas the catabolism of [^{125}I]LDL was not affected (17).

The present experiment provides what is believed to be the first direct evidence that Mg deficiency decreases PHLA. After an intravenous injection of heparin, triglyceride hydrolase activity appears in plasma (8,18). LPL is not the only lipase released by heparin into the bloodstream, HL is also released. Both enzymes are located in the capillary bed where lipolytic activity is expressed. LPL is stimulated by apolipoprotein CII and inhibited by NaCl 1.0 M, whereas HL seems not to require any specific serum factor and is activated by high salt concentrations. The lipolytic degradation of circulating triglyceride-rich lipoproteins involves loss of triglyceride and phospholipid from the particles. The two enzymes are believed to participate in this process. LPL is associated primarily with adipose tissue, and skeletal muscle shows a particular affinity for chylomicrons and VLDL. The function of the other enzyme, HL, is less well documented (19) and has a higher affinity for smaller particles. Several studies have suggested that its actions may be directed primarily at the metabolism of small VLDL, LDL and HDL. The separation of enzymes was efficiently obtained by heparin-Sepharose chromatography, a useful procedure when it is imperative to exclude the possible effects on enzymatic activity of other components in the post-heparin plasma samples (20). Inhibition of LPL by specific antibodies was another way to assay for HL and to quantitate LPL by subtracting the resistant lipolytic activity from total post-heparin lipolytic activity. Suppression of LPL by NaCl was another approach.

Two factors may be responsible for defective lipolysis of plasma triglycerides in Mg-deficient rats—a reduction in the availability of LPL or an abnormality in the lipoproteins themselves, rendering them a poor substrate for the enzyme. The apolipoproteins of VLDL isolated from rats fed deficient diets differ significantly from those of rats fed adequate diets. The VLDL in Mg-deficient rats appear to have reduced proportions of apo E but increased apo C. The role of this altered VLDL composition on defective clearance has previously been discussed (10). In these studies, it could not be determined how the changes in apolipoprotein patterns were related to hyperlipoproteinemia. The question remained whether the alterations in pattern cause hyperlipoproteinemia or whether these alterations were the results of the metabolic changes that produces hypertriglyceridemia (17).

In the present study, the assays for post-heparin lipolytic activity were carried out in the presence of human serum, that is providing any lipoprotein lipase cofactor protein requirement. Plasma triglyceride concentration correlates inversely with post-heparin plasma lipolytic activity. The experiment suggests that the alterations in the lipoprotein profile are the result of a reduction in the availability of LPL and not of an abnormality in the lipoproteins themselves.

The low lipolytic activity provides an appropriate explanation for both elevated levels of VLDL fraction and chylomicrons and for the reduction of HDL, because LPL activity is one determinant of plasma HDL levels. The accumulation of lipids in the blood occurs when their rate of entry into the blood exceeds their rate of removal. Sucrose, as compared to starch, markedly increases the hyperlipidemic effect of magnesium deficiency (1). The hypertriglyceridemic effect of sucrose is associated with a greater conversion of fructose to fatty acids in liver into plasma relative to dietary glucose or starch. Impairment of triglyceride clearance following a reduction in PHLA in Mg-deficient rats may explain the synergistic hypertriglyceridemia in Mg deficiency and sucrose feeding.

Since magnesium deficiency was associated with a dramatic decrease in LPL, it seemed worthwhile to determine the LPL activity in adipose tissue and muscle. After a carbohydrate-rich meal, enzyme activity increases rapidly in adipose tissue and declines in muscle. During a fast, the lipolysis of triglycerides stored in adipose tissue accelerates. This change is accompanied by decreasing levels of LPL in adipose tissue, but by stable or even increasing levels of lipase in muscle (cardiac, skeletal) (21,22). Therefore, LPL activity of adipose tissue was measured in post prandial conditions, whereas LPL activity of muscles was measured following food deprivation. Assuming that LPL activity in epididymal adipose tissue reflects that in other localizations (23), and activity in the heart and diaphragm is representative of that of muscles (24), an interesting aspect of the decrease in plasma LPL in Mg-deficient rats is that it occurs without decreases in muscle and adipose tissue LPL.

LPL is synthesized as an inactive precursor in the endoplasmic reticulum. This inactive form is transported to the Golgi apparatus where terminal processing and activation occur (18). Active LPL is released from the cells and moves to the vascular lumen where it is bound at the endothelial surface by glycosaminoglycans. In tissues such as adipose tissue and muscle, LPL exists in both functional and non-functional forms. The functional form appears to be largely associated with the endothelial surface of tissue capillaries and is readily released by heparin. The non-functional form of LPL, which is not readily releasable by heparin, appears to be associated with the tissue parenchymal cells (20). Two techniques, acetone/diethyl ether powder preparations and heparin eluates, have been described for assay of enzyme activity in adipose tissue and muscle. It is generally considered that the acetone/diethylether preparation represents the total tissue activity, *i.e.*, both the intracellular and the extracellular pools, including the physiologically active fraction at the endothelial surfaces. Heparin eluates, however, largely reflect extracellularly located enzyme (20). It would be of interest to determine if there is a defect within the tissues from Mg-deficient rats in maintaining the heparin-releasable pool of enzyme activity. With the acetone/diethylether preparation in whole tissue only, this selective decrease may not be discernable.

The mechanism responsible for the effect of Mg deficiency on LPL activity is unclear. The role of Mg in secretion and mechanisms of endothelial binding of LPL is unknown, and there is still only a limited knowledge of the heparin releasable pool of enzyme activity and its important relationship to the *in vivo* function of the enzyme. There are several explanations for the low post PHLA in Mg-deficient rats. One possibility is that Mg deficiency could impair secretion and that Mg is necessary for the transport of the active enzyme, and relocation to the endothelium. Other possibilities could be that the enzyme is degraded by the endothelial cells or that Mg deficiency increases the removal of the enzyme from the circulation, thus explaining why low activity is found in post-heparin plasma. Severe acute Mg deficiency induces hypertriglyceridemia and a decrease in PHLA. The time course development and the reversibility of these modifications by Mg refeeding should be examined.

Since the effect of marginal Mg deficiency of long duration is more relevant to human nutrition (25), the influence of moderate Mg deficiency and whether long term adaptation may occur which would affect the response remain also to be determined. While acute Mg deficiency has been shown to induce hyperlipemia and vascular lesions in experimental animals (2), the possibility that chronic Mg deficiency may be one among several factors that favor vascular disease in humans, is often disregarded (2). However, several observations indicate that patients with ischemic heart disease (IHD) are often Mg-deficient and that Mg deficiency may, together with several other factors, be involved in the development of IHD. Recently, Rasmussen *et al.* (26) found that oral Mg supplementation reduced plasma concentrations of triglycerides, VLDL and apo B in patients with IHD, suggesting that LPL activity might be reduced by Mg deficiency and increased by oral supplementation. Future investigations should clarify the influence of magnesium on LPL activity and lipid metabolism.

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