Lipoprotein metabolism : Importance of magnesium

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Summary

The purpose of this report is to present new data on the effect of Mg deficiency on lipoproteins, to bring into view the influence of different experimental conditions, and to discuss the possible consequence of modifications in lipoproteins on cellular metabolism and on cardiovasular system.
Severe Mg deficiency in the rat increases VLDL and LDL, decreases HDL and changes the fatty acid profile of plasma lipids and lipoproteins. VLDL size and apoproteins are modified. Less intense hypertriglyceridemia suggests an adaptation of animals to the effect of Mg deficiency in long term experiment. Moderate Mg deficiency induces lipid modifications of the same type than those previously described with more severe deficient diet. Other results using the same experimental model show modifications of membrane fluidity, increased susceptibility to thrombosis and changes in blood pressure.

Résumé

Le but de ce rapport est d'apporter des données nouvelles sur l'effet de la carence en magnésium sur les lipoprotéines, de discuter l'influence de différentes conditions expérimentales et d'envisager les conséquences possibles des modifications du métabolisme des lipoprotéines sur le métabolisme cellulaire et le système cardiovasculaire.

La carence sévère en magnésium chez le rat augmente le taux de VLDL et LDL, diminue le taux de HDL et modifie le profil des acides gras des lipides plasmatiques et des lipoprotéines. Les VLDL ont une taille augmentée et une composition anormale en apoprotéines. Cependant la régression de l'hypertriglycéridémie indique une certaine adaptation des animaux lors de carence de longue durée. Le déficit modéré s'accompagne de modifications du métabolisme lipidique de même type que celles observées lors de carence plus sévère. Ces mêmes animaux présentent des anomalies membranaires (modification de la fluidité), des anomalies plaquettaires (augmentation de la thrombose) et des modifications de la pression sanguine.

Zusammenfassung

Es werden neue Befunde präsentiert, die den Einfluß eines Magnesiummangels auf den Lipoprotein-Stoffwechsel aufzeigen; weiter wird der Einfluß unterschiedlicher experimenteller Bedingungen aufgezeigt, und der mögliche Einfluß modifizierter Lipoproteine auf den Zellstoffwechsel und das kardiovaskuläre System diskutiert.

Bei Ratten sind im schweren Mg-Mangel VLDL und LDL erhöht, HDL erniedrigt, und das Fettsäureprofil der Plasmalipide und der Lipoproteine ist verändert. Die Größe von VLDL ist modifiziert, ebenso die Apoproteine. Eine schwächer ausgeprägte Hypertriglyzerinämie läßt vermuten, daß sich die Tiere im Langzeitversuch an den Mg-Mangel adaptieren. — Mäßiger Mg-Mangel induziert Lipidveränderungen gleichen Typs wie bei schwerem Mangel. Andere Ergebnisse, die unter gleichen experimentellen Bedingungen gewonnen wurden, zeigen Veränderungen der Membranfluidität, erhöhte Thromboseneigung und Blutdruckveränderungen.

Introduction

Dietary magnesium (Mg) deficiency tends to produce cardiovascular damage in experimental and domestic animals [62, 63]. More then 50 years ago, *Sjollema* [67] reported a disease in grazing cattle of the Netherlands characterized by hypomagnesemia, hyperexcitation and degenerative changes of vascular wall with deposition of calcium salts [34, 44]. Experimental Mg deficiency also produces similar type of vascular lesions [38]. Disturbances in lipid metabolism in Mg deficiency has also been known as early as 1933 when *Kruse, Orent* and *MacCollum* demonstrated the presence of high total cholesterol as a typical change of blood lipids during Mg deprivation [28]. While Mg deficiency of short duration does not modify total plasma cholesterol level, long term deficiency increases serum cholesterol concentration. Increased dietary Mg has been shown to result in decreased amount of lipid deposit within the vessels of young rats fed an atherogenic diet and increased fecal fat excretion due to its soap forming capacity. However, the mechanism by which Mg reduces vascular sudanophilia remained obscure [25, 26, 40–42, 71–73] since total plasma cholesterol was not significantly affected by Mg supplementation and the effect of Mg on lipoprotein was unknown [64].
In view of the association of Mg deficiency and cardiovascular disease and the possible role for Mg in lipid metabolism, we came to the hypothesis that modification of lipoprotein could be produced by Mg deficiency. Repeated observations from our laboratory have indicated a significant impact of Mg deficiency in rats on lipid metabolism. The literature on these observations has been reviewed previously [48–51, 55]. The purpose of this report is — to present new data on the effect of Mg deficiency on lipoproteins —, to bring into view the influence of different experimental conditions that could be the

origin of certain confusions in the literature and to discuss the possible consequences of modifications in lipoprotein metabolism on cellular metabolism and on cardiovascular system.

I. Mg deficiency and lipoprotein

Plasma lipids

Elevated concentration of plasma lipids has been associated with dietary Mg deficiency in rats. Subsequent experimentations were aimed to investigate the mechanism which may be involved in the etiology of hyperlipidemia. All experiments were conducted in weanling rats randomly divided into Mg-deficient and control pair fed groups receiving 0.035 and 1 g/kg of Mg, respectively. Synthetic diets containing (g/kg) casein (200), carbohydrate (705), lipid (50), mineral and vitamin mixture were used as previously described [56]. Rats were fed this experimental diet for 8 days. The experimental conditions should be explicitely recognized. The diet was rich in sucrose, contained no cholesterol and the lipid was corn oil, a well studied unsaturated fat. The rats were young and were only on the experimental diet for a relatively short time. The most pronounced effect of Mg deficient diet was on plasma triglycerides while the level of total cholesterol remained unchanged. The proportion of cholesterol that was esterified was decreased. Fatty acid pattern of total plasma lipids in Mg deficient rats was quite different from those of control rats [55]. This alteration includes increased levels of oleic and linoleic acids and lower levels of stearic and arachidonic acid. Plasma lipids were further fractionated into phospholipids, triglycerides and cholesterol esters and their fatty acid profiles were compared. Deficient rats had higher levels of linoleic acid in plasma triglycerides and plasma phospholipids, and lower levels of arachidonic acid in plasma triglycerides whereas no significant variation occurred in the arachidonic content of phospholipids.

Mechanism of hyperlipidemia

Different methods including the use of Triton WR 1339 were applied to demonstrate that the rate of triglyceride secretion was not modified in Mg deficient rats compared to control rats [53]. Triton inhibits the peripheral utilization of plasma triglyceride thus providing a reasonable estimate of the rate of hepatic triglyceride secretion since in rats fed a low fat diet, intestinal lipoproteins and chylomicrons contribute only 10–20% to the output of triglyceride into plasma. Experiments on triglyceride clearance following Intralipid administration indicated that hypertriglyceridemia is the consequence of a decreased clearance of lipids [54]. Hydrolysis of triglycerides by lipoprotein lipase (LPL) is a key step in the removal of circulating triglycerides. Mg deficient rats were found to have a decreased molar ratio of insulin to glucagon [37] and a reduced insulin response after intravenous glucose challenge [20]. A slight reduction in heparin released LPL has been recently demonstrated [21]. Unfortunately it is difficult to relate this defect to changes in the in vivo activity of LPL and more studies are needed to determine to what extent the abnormalities in LPL activity contribute to hyperlipemia. A marked reduction in plasma activity of lecithin-cholesterol acyltransferase (LCAT) may explain the decrease in esterification of cholesterol and may contribute to the impair transport and disposal of triglycerides [22].

The concomitant increase of 18 : 2 and the decrease of 20 : 4 in total plasma lipids is a suggestive evidence for a possible block in the synthesis of 20 : 4 from 18 : 2 [56]. In addition, the possibility exists that hypertriglyceridemia can influence fatty acid profile. *Cunnane* has reported a negative correlation between the percentage of arachidonic acid in triglycerides and the total amount of triglycerides in plasma. This author suggested the existence of a class of triglycerides which contains a higher proportion of 20 : 4 than does the rest which responds slowly to dietary influences on total triglyceride mass [7].

Lipoproteins

Hyperlipidemia observed in deficient animals is the consequence of a decrease in the removal of circulating triglycerides [53] and is accompanied by changes in the fatty acid composition of plasma lipids [55]. In order to clearly define this difference, we have measured the level and composition of very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) in the serum of normal and deficient rats. Serum VLDL, LDL and HDL were separated by ultracentrifugation at d = 1.006, 1.063 and 1.21 g/ml. HDL cholesterol obtained by precipitation method were in good agreement with the ultracentrifugation method [56].

The concentration of VLDL and LDL in serum of Mg deficient rats is about 4 times higher, but the concentration of HDL is less than in the controls. The difference in triglycerides occurs mainly in VLDL which is the chief source of serum triglycerides. The level of LDL triglycerides in deficient rats is also higher than normal. Cholesterol levels increases in the VLDL and LDL fractions, but decreased in the HDL fraction. It is well known that the fatty acid composition of lipoproteins shows a notable alteration as the density of the lipoprotein increases [61].

Tab. 1: Fatty acid composition of lipoproteins in the serum of control or Mg deficient rats

Lipoproteins		Fatty acids					
		16:0	16:1	18:0	18:1	18:2	20:4
VLDL	Control	26.3±0.5	3.4±0.4	5.3±0.5	26.3±1.7	29.1±1.4	7.3±0.7
	Mg deficient	22.9±1.0	2.3±0.4	4.3±0.2	25.8±0.9	38.0±1.5*	5.9±0.5
LDL	Control	23.5±0.4	3.2±0.4	6.8±0.5	23.4±1.2	26.9±0.7	14.3±1.5
	Mg deficient	25.9±0.8	2.1±0.3	5.0±0.2	23.2±0.6	36.2±1.2*	6.8±0.2*
HDL	Control	26.1±1.1	1.6±0.2	12.2±0.3	9.8±0.8	24.6±0.4	24.6±1.9
	Mg deficient	28.1±0.6	1.7±0.2	9.5±0.3**	12.0±0.3**	30.7±0.4**	17.0±0.5**

Results are expressed in mol % of the major fatty acids. Means ± SEM for 6 animals within each group. Significantly different *P < 0.05 **P < 0.01

Tab. 2: Lipid and protein composition of rat VLDL

	Protein	Triglycerides	Cholesterol	Phospholipids
Control	17.8±1.8	60.7±0.5	5.8±0.2	15.7±0.8
Mg deficient	12.9±0.5*	65.0±0.8*	6.1±0.2	16.0±0.4

Values are given as percentage by weight. Means ± SEM of 6 samples for each group. *P < 0.05

Arachidonic acid is more concentrated in HDL than in VLDL while linoleic acid is higher in VLDL than in HDL but stearic acid is higher in HDL.

Further investigation on these lipoprotein fractions (Table 1) shows marked changes in the general characteristic of fatty acid composition in the deficient rats notably the lower content of 20 : 4 and the elevated 18 : 2 content in LDL and HDL. These results are consistent with earlier report on the fatty acid profile of total plasma lipids. VLDL from control and deficient rats contain the same proportion of cholesterol and phospholipids but less protein and more triglycerides (Table 2) in the deficient group. The increased ratio of triglycerides to protein indicates that particle size is modified [45]. The modification of lipid and fatty acid composition and the augmentation of lipoprotein size are relative to the decreased catabolism of VLDL in Mg deficient animals. Work in progress shows an increase in VLDL apo B in magnesium deficient rats.

II. Experimental conditions and conflicting interpretations

From careful evaluation of literature, it is clear that Mg deficiency is associated with various aspects of lipid metabolism [62, 63, 48–51, 54]. Nutritional designs of experiments are important since the results depend on the type and amount of carbohydrate, fat, vitamin and mineral status. The influence of dietary components on hyperlipidemia associated with Mg deficiency has been previously reviewed [50]. However, feeding pattern, the age of the experimental animal, the duration of the deficiency, and dietary content of Mg may also account for some of the discrepancies in the results. Our aim is then to bring forward the influence of these elements in rats receiving the diet already described [56].

Feeding pattern

It was soon recognized that some of the observed effects of severe deficiency might be the result of reduced appetite and consequently, general malnutrition. To separate these effects from those specific to Mg deficiency, controlled feeding of a diet adequate in Mg became recognized as an essential part of well planned experiments.

The interpretation of many long term experiments is difficult even when pair feeding is employed. Restricted control animals eat all their food within a short period of time and then fast for the rest of the day whereas deficient animals consume the same amount of food throughout the period of 24 hrs. It is well known that feeding pattern modifies the metabolism of lipids and an automatic feeding apparatus is used in our laboratory in order to obtain the same pattern of feeding in both control and Mg deficient groups [29].

Post prandial period vs fasting

The post-prandial phase is non-static phase where activation of all the processes of formation, catabolism and interchange of the different lipoprotein fractions takes place [15]. The results regarding lipoprotein metabolism during this period are therefore often variable, depending on the processes of intestinal absorption. It is therefore necessary to obtain the same pattern of feeding in control and deficient animals for this type of investigation [29].

Lipid and lipoproteins differ in control and Mg deficient rats not only at fasting but also in the post prandial phase (Fig. 1). In deficient animals, the highest modification are obtained under feeding conditions while fasting (16 hours) reduces the high plasma triglyceride level and strongly increases the level of HDL/total cholesterol ratio. In the control rats, fasting compared to feeding period results in significant decrease in plasma triglycerides but does not modify HDL/total cholesterol ratio. The effect of Mg deficiency on fatty acid profile of total plasma lipid is also more noticeable

under feeding than fasting. These results indicate that the effects of Mg deprivation on lipid metabolism are of particular importance in the post prandial period.

Age of animals

Young rats fed a severe magnesium deficient diet rapidly develop hyperlipemia. The age of the animals during the deficient period influences the characteristic of the lipemia as shown in male rats of 28–30 g in weight [6]. Adult rats seem to be less susceptible to alteration of lipid metabolism associated with Mg deficiency. With a high carbohydrate diet, starch did not bring about increased plasma triglycerides in adult deficient rats as did the sucrose diet. Also in this situation, the response was weaker than that observed in young rats [48].

Short and long term experiments

The duration of deficiency also influences the response. Recent studies on the evolution of lipid metabolism in weanling rats fed the sucrose diet previously described [56] (Fig. 2) indicate that hypertriglyceridemia is less intense at week 4 than at week 1 in deficient animals. Feeding the magnesium deficient diet for several weeks was accompanied by reduced food intake, spontaneous tetanic convulsions and mortality. The two majors factors responsible for the maintenance of plasma triglyceride levels are the secretion of triglycerides from the liver and the removal rate of circulating triglycerides by extrahepatic tissues. The adaptation of animals may be the consequence of a decreased secretion and/or increased uptake of triglycerides by extrahepatic tissues.

Moderate deficiency

Our previous experiments were performed with diet severely deficient in Mg. The effect of less severe Mg deficiency on lipid metabolism was recently investigated. Weanling rats were fed a purified diet [56] containing either a normal or a suboptimal quantity of Mg (0.080 g/kg). The deficiency of two weeks (Fig. 3) results in the same type of modifications [56] previously observed with severe defi-

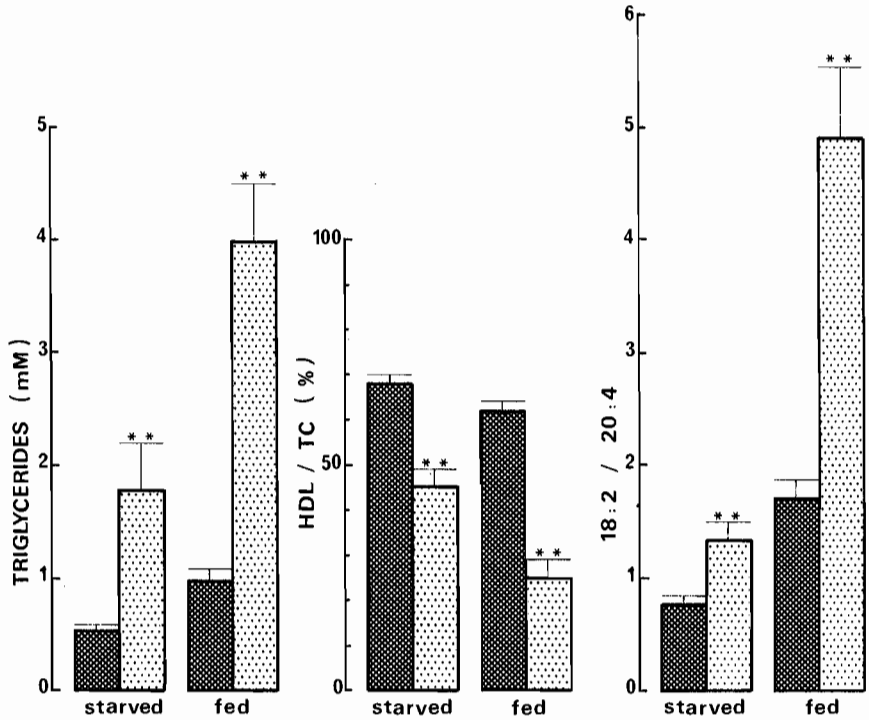

Fig. 1: Influence of the feeding condition on serum lipids (See Fig. 2 for symbols). HDL cholesterol/total cholesterol ratio. Values for 18 : 2 and 20 : 4 are expressed in mol % of the major fatty acids. Means ± SEM for 8–10 rats within each group. Significantly different ** P < 0.01

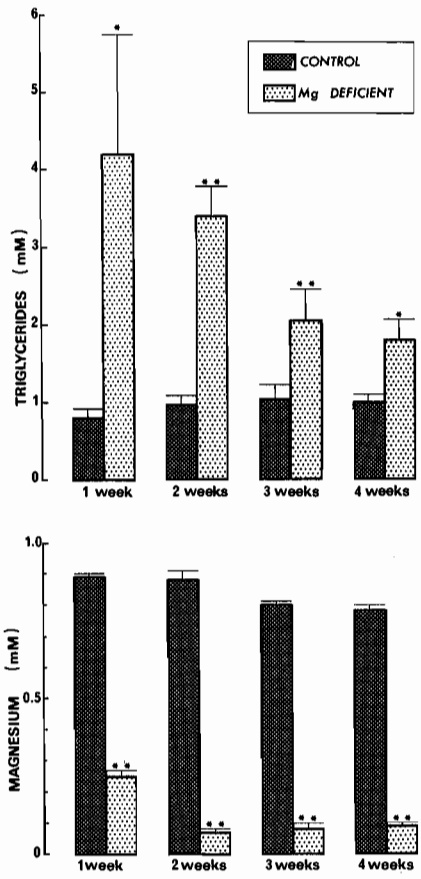

Fig. 2: Influence of the duration of the experiment on serum lipids. Means ± SEM for 8–10 rats within each group. Significantly different * P < 0.05; ** P < 0.01

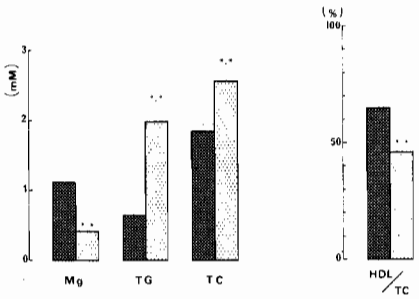

Fig. 3: Effect of moderate deficiency on serum lipids in rats fed the purified diet for 2 weeks (See Fig. 2 for symbols). TG : triglycerides; TC : total cholesterol; HDL : cholesterol; HDL/TC ratio. Means ± SEM for 8–10 rats within each group. Significantly different * P < 0.05; ** P < 0.01

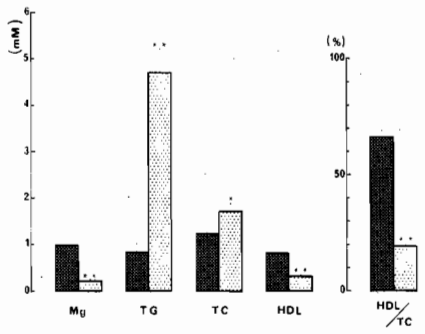

Fig. 4: Effect of moderate deficiency on serum lipids in rats fed the purified diet for 6 weeks (See Fig. 2 and Fig. 3)

cient diet (increase in triglyceride and decrease in HDL/total cholesterol ratio). Moderate and chronic deficiency of 6 weeks (Fig. 4) results in hypertriglyceridemia, elevation of total cholesterol and decrease in HDL/total cholesterol ratio. Fatty acid profile of total plasma lipids shows significant decrease in 18 : 0 and increase in 18 : 2 while 20 : 4 is not significantly affected. Thus, chronic deficiency demonstrates persistent hypertriglyceridemia, and fatty acid modifications at lower intensity but with increased total cholesterol when compared to severe deficiency of short duration.

The effects of magnesium on tissue levels of lipids and fatty acids have been evaluated in rats fed a magnesium-deficient diet for 14 weeks [9]. Serum cholesterol was significantly higher in magnesium deficient rat. The main change in tissue fatty acid composition was the higher serum docosahexaenoic acid (22-6n-3) in magnesium deficient rats than in controls.

Serum triglycerides and 18 : 2/20 : 4 ratio were not significantly affected. *Cunnane* suggests that the effect on serum 18 : 2/20 : 4 is time dependent. Mg deficiency of long duration requires a severe feeding restriction of the controls. It has been previously demonstrated that pair-fed rats have a higher proportional content of 18 : 2 and a lower proportional content of 20 : 4 in serum than ad libitum rats [8] and pair feeding the controls may have masked an effect of magnesium depletion on the 18 : 2/20 : 4 ratio, an indicator of 18 : 2 desaturation.

These experiments indicate modifications of lipoprotein metabolism in severe or moderate Mg deficiency. Alteration in plasma lipids already described in other species (monkey, rabbit, pig, dogs) also implicate modifications in lipoprotein metabolism [43, 72, 41, 28], but similar studies have not been done in these species.

III. Cellular and cardiovascular implications

Membranes

The membrane lipid composition of cells may be influenced by lipoprotein lipid composition as a consequence of the exchange between cholesterol and phospholipid of lipoprotein and the corresponding lipids in cellular plasma membranes [46]. A shift in the dynamic equilibrium between membrane and lipoprotein lipids through abnormalities in lipoprotein lipid pattern would therefore be expected to lead to changes in membrane lipid composition and so indirectly to membrane and cellular dysfunction. The effects of Mg deficiency in rat on the properties of erythrocyte membrane have been recently reported. The fluidity of the membrane [66] was monitored by fluorescence polarization using the probe 1-6-diphenyl-hexatriene (DPH). The ghosts from deficient rats had an increased fluidity as compared to control [70]. Liposomes from erythrocyte membrane lipids of Mg deficent rats also show significant increase in fluidity (*Tongyai* et al., unpublished results). This suggests that abnormalities in the composition of plasma lipoproteins are associated with corresponding changes in the erythrocyte membrane lipid composition [66]. If membrane fluidity of other cells is similarly affected, this may influence a number of cellular processes many of them are known to be disturbed in Mg deficiency [24]. This possibility has been recently mentioned for lymphocytes [23].

Platelet and thrombosis

Since the incidence of thrombotic complications is increased in certain types of hyperlipoproteinemia, we have investigated platelet function in Mg deficient rats showing hyperlipemia [56]. The effect of Mg deficiency on platelet agregation and on adrenalin induced thrombosis in rats has been recently reported. Mg deficient rats show increased susceptibility of platelets to thrombin-induced aggregation and injection of adrenalin at a dosage not affecting control rats, induce the formation of huge thrombi in the left atrium of deficient animals [54].

It is of particular interest to mention that although Mg deficient rats received a diet low in lipids (5% corn oil), they show hyperlipemia and thrombosis comparable to the response observed in rats fed a thrombogenic diet rich in saturated fat for several weeks [57].

Atherosclerosis

The role played by the lipoproteins in the process of atherogenesis has long been known [15]. The increase in LDL and VLDL is widely recognized as one of the main risk factors in atherosclerotic cardiovascular diseases. HDL is not only negatively correlated with ischemic heart disease but seems to act as protective factor as well [18].

In species resistant to atherosclerosis, Mg deficiency alone does not induce vascular lipid infiltration unless associated with aggravating factors such as saturated fat, dietary cholesterol or predisposing lesion of the vascular wall [48].

Blood pressure

Extensive studies in man and experimental animals suggest that Mg affects the contractile state of vascular smooth muscle and blood pressure [1, 4, 14, 36]. The effect of Mg on blood pressure has been recently investigated in progressive stages of deficiency using our experimental model producing hyperlipemia. A period of hypotension was observed in the early stage of Mg deficiency and disappears later on [30]. Moderate Mg deficiency for two weeks simultaneously induces hyperlipemia and a significant increase in cardiovascular response to norepinephrine [31]. We do not know if any correlation exist between changes in plasma lipids and vascular responsiveness in these animals. Alterations in calcium handling and metabolism of smooth muscle cells in Mg deficiency are more probable causes [1].

Clinical data

While acute Mg deficiency has been shown to induce hyperlipemia, thrombosis and vascular lesions in experimental animals, the hypothesis that Mg depletion affects lipid metabolism in man is less convincing. Studies in human are scarce and generally indirect involving relationships between plasma Mg and lipids rather than dietary Mg. Certain studies failed to demonstrate any relationship between Mg and lipid metabolism [32, 33, 39, 68, 69] while other suggest this association [3, 27, 5, 47, 10, 17]. Several clinical disorders include alteration in lipid metabolism, Mg depletion and vascular complications [54]. Considerable evidence has shown that Mg metabolism in the diabetic state is markedly altered from normal [13, 35]. The role of Mg in lipid metabolism might then be implicatcol in the dyslipidemia of diabetic patients [54]. Chronic alcoholism is one of the most frequent causes of human Mg deficiency [12]. Although a great deal of evidence has established the direct role of ethanol in disturbances of lipid metabolism [2], malnutrition and other factors associated with alcohol abuse could play additional roles [52]. In view of the frequent occurence of Mg deficiency in alcoholic patients, and the association of Mg deficiency with hypertriglyceridemia and decreased esterification of cholesterol, more attention should be directed to the possible role of Mg in dyslipidemia. Diuretics commonly used in treatment of hypertension and for prevention of cardiovascular disease give adverse actions which include K and Mg excretion and alterations in lipid and carbohydrate metabolism [65, 60, 58, 59]. Mg loss provoked by diuretics may be, to some extent, responsible for the atherogenic alterations in lipid and carbohydrate metabolism and Mg supplementation of patients treated with diuretics has been shown to reduce triglycerides and increase HDL cholesterol [10]. Future investigations should clarify the role of Mg on lipid metabolism and the possible implications in cardiovascular pathology.

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