The hypercholesterolaemic effect of magnesium deficiency following cholesterol feeding in the rat *)

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Zusammenfassung

Der Einfluß des Mg-Gehaltes im Futter auf den Lipidstoffwechsel wurde bei jungen Ratten untersucht, die eine gereinigte, Saccharose-haltige Diät mit und ohne Zusatz von Cholesterin erhielten. Alimentärer Mg-Mangel verursachte bei den Ratten, die zusätzlich Cholesterin erhielten, eine deutliche Erhöhung der Serumtriglyceride und des Cholesterins bei niedrigen HDL-Spiegeln. Der steile Abfall des Verhältnisses HDL-Cholesterin zu Gesamtcholesterin unterstützt die Annahme, daß Mg-Mangel eine atherogene Wirkung besitzt.

Summary

The effect of dietary magnesium on lipid metabolism was examined in weanling rats fed a purified diet containing sucrose with or without supplementary cholesterol. Dietary magnesium deficiency in rats fed diets with supplementary cholesterol produced a marked elevation in serum triglyceride and cholesterol concentrations with a low level of high density lipoprotein cholesterol. The sharp fall in the high density lipoprotein cholesterol: total cholesterol ratio supports the concept of an atherogenic effect of magnesium deficiency.

Résumé

L'influence du déficit magnésique sur le métabolisme lipidique a été étudiée chez des rats au sevrage recevant un régime purifié contenant du saccharose supplémenté ou non en cholestérol. La carence en magnésium chez des rats recevant le régime supplémenté en cholestérol entraîne, au niveau du sérum sanguin, une élévation significative des triglycérides et du cholestérol total, associée à une baisse du cholestérol des lipoprotéines de haute densité. La baisse brutale du rapport cholestérol des lipoprotéines de haute densité: cholestérol total est en accord avec l'effet athérogène du déficit magnésique.

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The role of magnesium deficiency in the pathogenesis of vascular diseases has been reported previously [11, 8]. Severe magnesium deficiency in weanling rats fed a high carbohydrate diet containing sucrose produces a marked hypertriglyceridemia modifications in triglyceride and cholesterol distribution in lipoprotein fractions but no change in total plasma cholesterol [9]. When cholesterol feeding was combined with magnesium deficiency, the plasma cholesterol level was significantly increased [4]. Since hypercholesterolemia is known to be a positive risk factor in the development of atherosclerosis [3] the influence of magnesium deficiency in rats fed a cholesterol supplemented diet seemed to provide a worthwhile subject for further study.

Materiel and Methods

Experiments were performed with weanling male Sherman rats weighing about 50 g, reared in our laboratory. Rats were housed individually and subjected to alternating 12 hours periods of light and darkness. They were randomly divided into four experimental groups and given the following diets: control diet; control + cholesterol composed of 1 % cholesterol and 0.3 % cholic acid added to the control diet at the expense of sucrose; magnesium deficient; magnesium deficient + cholesterol composed of cholesterol and cholic acid were added to the magnesium deficient diet at the expense of sucrose. The basic composition of magnesium deficient diet is given in table 1. The magnesium content determined by analysis was 0.035 g/kg for the deficient group and adjusted to 1.000 g for the control group by adding magnesium oxide. Distilled water was available **ad libitum**. Rats were sacrificied at the end of the dark period and blood was collected by aortic puncture after Nembutal® anaesthesia (40 mg/kg).

Analysis

Serum was obtained by centrifugation and frozen immediately for subsequent analysis. Magnesium was estimated by atomic absorption spectrophotometry. Triglycerides [14] were measured by glycerol determination [15] after enzymatic hydrolysis. Free and total cholesterol were estimated separately [10]. Serum high density lipo-

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protein (HDL) cholesterol was measured by a precipitation method with heparin-$MgCl_2$ (Merck®): 0.050 ml of serum were mixed with 1 ml of heparin-magnesium solution, shaken for 1 min and allowed to stand at room temperature for 15 min. The mixture was centrifuged at 3000 rpm for 15 min. The supernate was forced through a 0.22 µm filter placed in a Sartorius® membrane filter holder. Cholesterol in the supernatant is determined as HDL cholesterol. The data were subjected to analysis of variance or Student's t test [12].

Tab. 1: Composition of the purified diet.

Ingredients [1])	%
Casein	20.0
Sucrose	70.5
Corn oil	5.0
Mineral mix [2])	3.5
Vitamin mix [3])	1.0

[1]) Dietary constituents were obtained from the indicated sources. Casein from Louis François, Paris; minerals from Prolabo, Paris; vitamin mix including choline bitartrate from UAR, Epinay-sur-Orge.

[2]) Per kg mixture: calcium phosphate, dibasic 500.0 g; sodium chloride 74.0 g; potassium citrate monohydrate 220.0 g; potassium sulfate 52.0 g; manganous carbonate 3.5 g; ferric citrate 6.0 g; zinc carbonate 1.6 g; cupric carbonate 0.3 g; potassium iodate 0.01 g; sodium selenite 0.01 g; chromium potassium sulfate 0.55 g.

[3]) Per kg mixture: thiamine HCl 2.0 g; riboblavin 1.5 g; pyridoxine HCl 1.0 g; nicotinic acid 10.0 g; D-calcium panthotenate 7.0 g; folic acid 500 mg; d-biotin 30 mg; cyanocobalamin 5 mg; vitamin A 1.980.000 UI (Retinyl palmitate), vitamin E 17.000 UI (dl-α-tocopherylacetate), vitamin D_3 600.000 UI (cholecalciferol); menaquinone 5 g; choline bitartrate 136 g.

Results

The influence of dietary magnesium on plasma Mg, triglycerides and cholesterol in rats fed the experimental diets is shown in table 2. Serum magnesium levels were unaffected by dietary cholesterol but were significantly lowered in the magnesium depleted group. Serum triglycerides cholesterol total, free and esterified were significantly affected by dietary cholesterol and magnesium. In control rats, the cholesterol diet produced a mild hyperlipidemia with a significant increase in free, esterified and total cholesterol. No change in the HDL cholesterol fraction was observed and the increase in serum triglycerides was not statistically significant. Magnesium deficiency without cholesterol induced a significant increase in triglycerides, no change in total cholesterol and a significant decrease in HDL cholesterol. A significant increase in free cholesterol was accompanied by a slight decrease in esterified cholesterol. Rats fed a magnesium deficient diet containing cholesterol showed particularly high triglycerides and total cholesterol levels with a significant decrease in HDL cholesterol. Both free and esterified cholesterol were significantly increased. Table 3 shows the effect of experimental diets on esterified cholesterol/total cholesterol ratio. Magnesium deficiency induced a significant decrease in the esterified density lipoprotein (VLDL) and relative decrease in the concentration of HDL.

Magnesium deficiency results in a marked shift to lower density lipoproteins. There was a decrease in HDL cholesterol in agreement with previous results in rats on a high carbohydrate diet [9]. When serum lipoproteins were isolated by ultracentrifugation, cholesterol levels were increased in the VLDL and low density lipoprotein (LDL) and significantly lowered in the HDL fraction. However, magnesium deficiency without cholesterol feeding does not increase total serum cholesterol.

Compared to control rats fed cholesterol, magnesium deficient rats develop hypercholesterolemia following cholesterol feeding [4]. The shift to lipoproteins of lower density resulted in a dramatic decrease in the percentage of plasma cholesterol transported by HDL lipoprotein. Whereas in control rats, HDL is the principal cholesterol-transporting lipoprotein. The proportion of serum cholesterol carried by HDL is only 8,4 ± 1.1 % in magnesium deficient rats fed cholesterol.

Total serum cholesterol was not altered in rats on a magnesium deficient diet without cholesterol and the decrease in esterified cholesterol/total cholesterol ratio has been previously reported [9]. Lecithin cholesterol acyltransferase (LCAT), an extracellular enzyme that is secreted by the liver, circulates in the plasma and catalyses cholesterylester formation. The decrease in esterified cholesterol/total cholesterol ratio could result from a hepatic disturbance linked to magnesium deficiency with a drop in LCAT activity [8].

With a high cholesterol diet, acyl-CoA cholesterol acyltransferase (ACAT) might be important in controlling the esterified cholesterol transport from the gut. The secretion rates of unesterified and esterified cholesterol in triglyceride-rich lipoproteins may be regulated by ACAT in the intes-

Tab. 2: Influence of dietary magnesium on serum magnesium, triglycerides and cholesterol following cholesterol feeding in the rat.

	Magnesium	Triglycerides	Total Cholesterol	Free Cholesterol	Esterified Cholesterol	HDL Cholesterol
Control	0.99 ± 0.03[a1)]	1.54 ± 0.23[a]	1.55 ± 0.07[a]	0.30 ± 0.03[a]	1.25 ± 0.04[a]	0.96 ± 0.04[a]
Control, Cholesterol	0.95 ± 0.03[a]	2.22 ± 0.54[ab]	3.93 ± 0.62[b]	0.87 ± 0.13[b]	3.06 ± 0.64[b]	1.10 ± 0.06[a]
Mg deficient	0.20 ± 0.03[b]	2.83 ± 0.22[b]	1.75 ± 0.09[a]	0.63 ± 0.07[b]	1.12 ± 0.07[a]	0.56 ± 0.05[b]
Mg deficient, cholesterol	0.16 ± 0.01[b]	3.91 ± 0.40[c]	7.30 ± 0.92[c]	2.23 ± 0.30[c]	5.08 ± 0.64[c]	0.61 ± 0.04[b]
Anova [2)]	Mg	Mg, C	Mg, C Mg x C	Mg, C Mg x C	Mg, C	Mg

[1)] Means ± SEM from 6 rats, values not sharing a common letter superscript are significantly different $P < 0.05$.
[2)] Anova: analyses of variance; Mg: magnesium effect significant; C: cholesterol effect significant.

tinal mucosa [1]. A normal ACAT activity in magnesium deficient rats could explain a subnormal esterified cholesterol/total cholesterol ratio in animals fed high cholesterol diets whereas a decreased LCAT activity could account for the low ratio observed in magnesium deficient animals without exogenous cholesterol in the diet. In the present results, the dramatic decrease in the HDL/total cholesterol ratio in hypercholesterolemic magnesium-deficient rats should be stressed. In the rat resistant to atherosclerosis most plasma cholesterol resides in high density lipoprotein, whereas in man who is susceptible to atherosclerosis LDL has cholesterol/total cholesterol ratio in rats fed a sucrose diet (64.3 ± 2.9 vs 80.0 ± 1.4 %). Cholesterol feeding in magnesium deficient group appears to modify this ratio as shown by statistical analysis (Table 3). Cholesterol and magnesium deficiency both decreased the HDL cholesterol/total ratio. This ratio was particularly low (8.4 ± 1.1 %) in magnesium-deficient rats fed cholesterol compared to control rats (62.2 ± 3.0 %) [Table 3].

Tab. 3: Influence of dietary magnesium on serum HDL cholesterol as percent of total cholesterol (HDLC/TC), serum esterified cholesterol as percent of total cholesterol (EC/TC) following cholesterol feeding in the rat [1)].

	HDLC/TC	EC/TC
Control	62.2 ± 3.0[a]	80.0 ± 1.4[a]
Control, Cholesterol	28.2 ± 4.7[b]	76.8 ± 2.4[a]
Mg deficient	32.8 ± 3.6[b]	64.3 ± 2.9[b]
Mg deficient, cholesterol	8.4 ± 1.1[c]	69.5 ± 1.3[b]
Anova	Mg, C	Mg, Mg x C

[1)] See table 2.

Discussion

Cholesterol feeding has been shown to increase the dietary requirement for magnesium [13]. In the present experiment, signs of magnesium deficiency appeared in both depleted groups at the same time and magnesium levels were unaffected by dietary cholesterol. The absence of effect of dietary cholesterol on blood serum magnesium level may be related to the short duration of the experimental period in the present experiment. Hypertriglyceridemia in magnesium deficient rats fed a sucrose-rich diet is in agreement with previous findings [9]. Hyperlipemia can be produced either by an increased production of lipids from the intestine and the liver or by a decreased removal of serum lipids from the blood. A defective removal from the blood might be suggested in the present experiment (magnesium deficient + cholesterol diet) and the possibility exists of a decrease activity of plasma and adipose tissue lipoprotein lipase activity in magnesium deficient rats [8].

In controls, the principal plasma lipoproteins are the HDL with very low concentrations of the lower density lipoproteins. Cholesterol feeding results in alterations in plasma lipoproteins. The HDL fraction carried a lower proportion of serum cholesterol than controls. In cholesterol fed animals 28.2 ± 4.7 % of total cholesterol was carried by HDL; the control values were 62.2 ± 3 %. These results are in agreement with the characteristic changes in plasma lipoproteins induced by cholesterol feeding in the rat [5, 6]. It has been previously shown that the serum lipoprotein pattern of rats fed a high cholesterol diet differs markedly from those of control rats in that an intermediate lipoprotein class (IDL) appears in

addition to an increase in the concentration of very low been shown to carry the major portion of plasma cholesterol. In magnesium deficient rats following cholesterol feeding, lower density lipoproteins were enriched in cholesteryl ester and carried a larger proportion of serum cholesterol. Accompanying these changes was a reduction in plasma HDL. Based on the current hypothesis on the role of HDL lipoproteins [2], changes in cholesterol distribution would also change the net accumulation of cholesterol by arterial wall and hence the risk of atherosclerosis [7].

These results partly explain the influence of dietary magnesium on the development of atherosclerosis in rats fed various diets including fat and cholesterol [4] and may be of importance in man in agreement with previous reports on the relationship between dietary magnesium and epidemiology of coronary heart disease [11].

Acknowledgments:

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