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Plasma esterase activities in rats fed magnesium-deficient diets

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In a study with rats it was determined whether dietary magnesium concentration affects plasma esterase activities. The feeding of a diet with 0.01% (w/w) instead of 0.04% magnesium reduced plasma magnesium concentration by 50%. Plasma total esterase, arylesterase and butyrylcholinesterase activities were significantly decreased in the magnesium-deficient rats. In rats fed a diet containing 0.02% magnesium, plasma magnesium concentration was lowered by 30%, and group mean plasma total esterase activity was decreased, but not the activities of arylesterase and butyrylcholinesterase.

Keywords: dietary magnesium, esterases, magnesium deficiency, plasma, rat

Introduction

Rat plasma contains various esterases that can catalyze the hydrolysis of artificial fatty acid esters of aromatic alcohols (Augustinsson 1961). Various reports have begun to suggest that some of these esterases are involved in triglyceride and cholesterol metabolism (Kutty 1980, Patel *et al.* 1990, Van Lith *et al.* 1992, Van Lith & Beynen 1992). The activity of plasma lecithin-cholesterol acyltransferase is markedly diminished in magnesium-deficient rats (Gueux *et al.* 1984). Possibly, magnesium status also affects plasma esterase activities. Thus, we have examined in rats the effect of dietary magnesium concentration on plasma activities of total esterase (EC 3.1.1), arylesterase (EC 3.1.1.2), butyrylcholinesterase (EC 3.1.1.8) and carboxylesterase ES-1 (EC 3.1.1.1).

Materials and Methods

Animals and housing

Female rats of an outbred specified-pathogen-free Wistar colony (Hsd/Cpb:WU) were purchased at the age of 3 weeks. The rats had been fed *ad libitum* a commercial, pelleted, non-purified diet (RMH-B®, Hope Farms BV, Woerden, The Netherlands) and had free access to tap water. For 10 days (pre-experimental period) the rats were fed a purified, powdered diet containing 1.02% (w/w) sodium carbonate, 70.93% glucose, 0.2% phosphorus (5.0 g $NaH_2PO_4 \cdot 2H_2O$ 100 g^{-1} diet) and 0.04% magnesium (apart from the sodium carbonate, glucose and phosphorus content, the composition of this diet was identical to that of the 0.04% magnesium experimental diet described below). During the pre-experimental period the rats were housed in groups in wire-topped polycarbonate cages with a layer of sawdust as bedding. During the experimental period the rats were housed individually in metabolic cages as described (Hoek *et al.* 1988). The cages were located in a room with controlled lighting (light: 0700–1900 h), temperature (22–24 °C) and relative humidity (40–60%).

Experimental diets

The rats had free access to food and demineralized water. Diets were stored at 4 °C until feeding. On day 0 of the experimental period, the rats were divided into three dietary groups of eight animals each. The groups were stratified for body weight.

The experimental diets contained 0.4% phosphorus and either 0.01, 0.02 or 0.04% magnesium. The 0.04 magnesium diet consisted of the following components (g 100 g^{-1} diet): casein, 15.1; corn oil, 2.5; coconut fat, 2.5; glucose, 70.26; cellulose, 3.0; $CaCO_3$, 1.24; $NaH_2PO_4 \cdot 2H_2O$, 1.51; Na_2CO_3, 0.68; $MgCO_3$, 0.14; KCl, 0.10; $KHCO_3$, 0.77; mineral premix, 1.0; vitamin premix, 1.2. The composition of the mineral and vitamin premixes has been described (Van Lith *et al.* 1992). To formulate the other experimental diets, the amount of $MgCO_3$ was decreased to 0.069 or 0.034 g 100 g^{-1} diet, and the amount of glucose increased to maintain the sum of diet components at 100 g.

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Blood sampling and chemical analyses

At the beginning (day 0) and end of the experiment (day 17 or 28), heparinized blood samples of the non-starved rats were taken by orbital puncture while they were under light diethyl ether anesthesia. Immediately after the blood collection, the anesthetized rats were killed by cervical dislocation. Plasma was collected by low-speed centrifugation and kept at −20°C until analysis.

Total magnesium in plasma was determined by atomic absorption spectroscopy (Hoek *et al.* 1988). Total esterase activity in plasma was measured with *p*-nitrophenylbutyrate as substrate as described (Van Lith *et al.* 1992). The method of measuring and reporting ES-1 activities has been described in detail (Van Lith *et al.* 1991). Plasma butyrylcholinesterase activity was determined by the method of Ellman *et al.* (1961) using butyrylthiocholine as substrate. Arylesterase activity was assayed using phenylacetate as substrate with subsequent chromogenic determination of the phenol released (Lorentz *et al.* 1979).

Statistical analyses

Results are presented as means ± SEM. The Kolmogorov–Smirnov one-sample test indicated that all data were normally distributed. Student's one-sample *t*-test for paired data was used to evaluate the significance of changes that occurred during the experimental period within dietary groups. To compare group means, two-tailed Student's *t*-test with pooled (for equal variances) or separate (for unequal variances) variance estimates was used. The equality of variances was tested using a two-tailed *F*-test. The level of significance was pre-set at $P < 0.05$. Statistical analyses were carried out using a SPSS-PC computer program (SPSS 1990).

Results

Daily inspection of the rats revealed that after about 14 days those fed the 0.01% magnesium diet developed signs of illness such as depressed food intake, increased water intake, hyperthermia of the ears, scabs on the forehead and an unkept coat. We decided to kill these rats on day 17. Four randomly chosen animals of the 0.04% magnesium group served as controls (Table 1). The remaining animals were sampled and killed on day 28 (Table 2).

Body weight and weight gain were somewhat reduced by the 0.01% magnesium diet (Table 1). Plasma magnesium concentration was reduced by about 50%. Plasma total esterase, arylesterase and butyrylcholinesterase activities were significantly lowered by magnesium deficiency. Plasma ES-1 activity was not influenced by the amount of magnesium in the diet.

After 28 days on the diets, 0.02 versus 0.04% magnesium in the diet had no effect on body weight (Table 2). Plasma magnesium concentration was reduced by about 30%. Rats fed the 0.02% magnesium diet had lower group mean plasma total esterase activities, but arylesterase, butrylcholinesterase and ES-1 activities were not influenced by the amount of magnesium in the diet.

Table 1. Effect of 0.04 versus 0.01% (w/w) magnesium in the diet on body weight, plasma magnesium concentration and plasma esterase activities of rats after feeding the diets for 17 days

Measure	Dietary magnesium	
	0.04%	0.01%
Body weight (g)		
day 0	69 ± 4	70 ± 3
day 17	129 ± 5	120 ± 4
gain	61 ± 3	50 ± 3[b]
Plasma magnesium (mg l^{-1})		
day 17	17.8 ± 1.8	8.2 ± 1.1[b]
Plasma esterase activity		
total esterase (μmol min^{-1} ml^{-1})		
day 0	2.78 ± 0.28	3.10 ± 0.18
day 17	2.54 ± 0.29	1.90 ± 0.12[b]
change	−0.23 ± 0.30	−1.21 ± 0.13[a,b]
arylesterase (μmol min^{-1} ml^{-1})		
day 0	108 ± 9	106 ± 7
day 17	153 ± 10	105 ± 11[b]
change	+45 ± 17	−1 ± 7[b]
butyrylcholinesterase (nmol min^{-1} ml^{-1})		
day 0	45 ± 5	43 ± 2
day 17	57 ± 4	37 ± 4[b]
change	+11 ± 3[a]	−6 ± 2[a,b]
carboxylesterase ES-1 (%, relative to ES-1 standard)		
day 0	51 ± 21	46 ± 5
day 17	58 ± 25	47 ± 5
change	+7 ± 6	+1 ± 4

Values are means ± SEM for four (0.04% magnesium diet) or eight animals (0.01% magnesium diet) per group.
[a] Change significantly different from zero ($P < 0.05$; two-tailed paired Student's *t*-test).
[b] Significantly different from group fed the 0.04% magnesium diet ($P < 0.05$; two-tailed Student's *t*-test).

Discussion

A decrease in dietary magnesium concentration from 0.04 to 0.01% significantly lowered plasma total esterase, arylesterase and butrylcholinesterase activities but not that of carboxylesterase ES-1. However, feeding the 0.02% magnesium diet for 28 days did not produce a fall of the activities of arylesterase and butrylcholinesterase. There are various explanations for this discrepancy. The mag-

Table 2. Effect of 0.04 versus 0.02% (w/w) magnesium in the diet on body weight, plasma magnesium concentration and plasma esterase activities of rats after feeding the diets for 28 days

Measure	Dietary magnesium	
	0.04%	0.02%
Body weight (g)		
day 0	72 ± 3	70 ± 2
day 28	159 ± 7	155 ± 5
gain	87 ±5	85 ± 4
Plasma magnesium ($mg\ l^{-1}$)		
day 28	16.6 ± 0.5	12.1 ± 0.5[b]
Plasma esterase activity		
total esterase ($\mu mol\ min^{-1}\ ml^{-1}$)		
day 0	2.99 ± 0.33	2.95 ± 0.16
day 28	3.30 ± 0.42	2.91 ± 0.21
change	+0.31 ± 0.10[a]	−0.04 ± 0.15
arylesterase ($\mu mol\ min^{-1}\ ml^{-1}$)		
day 0	108 ± 13	116 ± 5
day 28	152 ± 15	158 ± 7
change	+44 ± 12[a]	+42 ± 7[a]
butyrylcholinesterase ($nmol\ min^{-1}\ ml^{-1}$)		
day 0	46 ± 4	42 ± 3
day 28	84 ± 20	71 ± 6
change	+38 ± 18	+29 ± 6[a]
carboxylesterase ES-1 (%, relative to ES-1 standard)		
day 0	31 ± 9	50 ± 6
day 28	30 ± 11	46 ± 4
change	−1 ± 3	−4 ± 5

Values are means ± SEM for four (0.04% magnesium diet) or eight animals (0.02% magnesium diet) per group.
[a] Change significantly different from zero ($P < 0.05$; two-tailed paired Student's t-test).
[b] Significantly different from group fed the 0.04% magnesium diet ($P < 0.05$; two-tailed Student's t-test).

nesium effect on the esterase enzymes may occur in rather severe magnesium deficiency only. It is also possible that the observed effect of magnesium deficiency was an indirect effect caused by the disease state. Alternatively, the decrease in arylesterase and butrylcholinesterase activities seen after 17 days on the 0.01% magnesium diet may have been transient and thus did not show after 28 days. Feeding the 0.02% magnesium diet for 28 days did lower group mean plasma total esterase activities. Apparently, this effect resided in esterase enzymes other than the ones measured.

As to the mechanism accounting for the possible direct effect of severe magnesium deficiency on plasma esterase activities, we can only speculate. Hypoproteinemia due to reduced hepatic protein synthesis is an early symptom of magnesium deficiency (Schwartz *et al.* 1970). By analogy, impairment of esterase synthesis could occur in magnesium-deficient rats. In rats fed a purified diet containing 0.01% magnesium, there was enhanced loss of albumin in urine as a result of kidney calcification (Van Camp *et al.* 1990). Perhaps, plasma esterases are also excreted in urine by magnesium-deficient rats.

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