MAGNESIUM SUPPLEMENTATION AND MICROELEMENT HOMEOSTASIS

Klára Szentmihályi[a], Zoltán May[a], Ibolya Kocsis[b], Krisztina Süle,[a,c] Anna Blázovics[c]

Keywords: magnesium supplementation, rat, essential elements, metabolic alteration, hyperlipidemia.

Magnesium participates in numerous enzymatic reactions in the human body and it has an essential role in maintaining the antioxidant system. Our purpose was to investigate the effect of magnesium on element content in blood. Male Wistar rats were divided into four groups. The animals in group ND were fed with normal diet, the animals in group ND+Mg. were fed with normal diet and treated with magnesium polygalacturonate (200 mg Mg/kg body weight ad libitum daily). The animals in group FD were fed with fat rich diet containing cholesterol (2.0% w/w), sunflower oil (20% w/w) and cholic acid (0.5% w/w) added to the control diet. The animals of group FD+Mg were fed with fat-rich diet and magnesium polygalacturonate. The rats were kept on the diets for 9 days. The element concentration (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Ni, P, Pb, S, Si, Sn, Sr, Ti, V, Zn) of blood samples was determined with an ICP-OES after digestion with a mixture of nitric acid and hydrogen peroxide. The results show that the concentration of several elements changed significantly in both magnesium-treated groups, nevertheless the alteration was different in the control and hyperlipidemic groups. It has been concluded that high amount of magnesium supplementation alters the metal ion homeostasis in short time experiment. Although some favourable effects were found in the hyperlipidemic group by magnesium polygalacturonate treatment, it is worth to note that supplementation with magnesium should be carried out carefully especially in metabolic diseases like fatty liver.

* Corresponding Authors
Fax: +36-1-438-1139
E-Mail: szentmihalyi.klara@ttk.mta.hu
[a] Institute of Materials and Environmental Chemistry Research Centre for Natural Sciences of the HAS, H-1025 Budapest, POBox 17, Hungary
[b] Central Laboratory, Semmelweis University, H-1086 Budapest, Hungary
[c] Department of Pharmacognosy, Semmelweis University, H-1086 Budapest, Hungary

Introduction

Magnesium is essential for the function of living organisms. It has structural roles in bones, cell membranes and chromosomes. Active transport of several ions like potassium and calcium through cell membranes needs magnesium. It is also required for muscle contraction, for the function of heart and nerve cells, and for metabolism of carbohydrates and fats to produce energy in magnesium-dependent biochemical reactions. All in all, magnesium plays a key role in more than 300 enzymatic reactions in the body [1,2].

Energy-consuming metabolic processes require magnesium in form of a complex with adenosine triphosphate (MgATP) which stores energy in phosphate bonds. Magnesium is essential in the synthesis and metabolism of carbohydrates, lipids, proteins, and nucleic acids, for example, for synthesizing DNA and RNA in mitochondria[2,3]. Magnesium is also required for the synthesis and maintenance of the antioxidant defence system, including enzymes and antioxidant molecules[4,5].

Low magnesium intake is a risk factor in the development of several diseases, e.g. diabetes, ischemic heart disease, severe retinopathy[6,7,8]. Magnesium supplementation may have protective effect against the development of different diseases, and may increase magnesium concentration and insulin sensitivity[9,10].

Hypomagnesemia is one of the common features in obesity, and in liver diseases such as fatty liver and alcoholic or other cirrhosis. In these diseases, decreased magnesium level in serum, erythrocytes, lymphocytes, liver tissues, heart muscle, skeletal muscle and bone is a well-known symptom[11,12,13].

The absorption of magnesium depends on several factors. In general, 30-40% of the daily dietary magnesium intake is absorbed in the small intestine. In case of low magnesium intake, absorption rate may be as high as 75%, while in case of high intake it may be reduced to 25%. Presence of calcium and other divalent cations, as well as phosphorus, fat and lactose, also influences the rate of magnesium absorption[14].

Low magnesium absorption in jejunum and ileum is the cause of low magnesium status in several diseases. Increased urinary magnesium excretion also contributes to latent magnesium deficiency, the first sign of which is low level of serum Mg (hypomagnesemia)[15,16].

Induced alimentary hyperlipidemia and consecutive development of fatty liver is a suitable experimental model for studying liver damage and metal element homeostasis, so in the present work this model was used to study the effect of magnesium supplementation in form of Mg polygalacturonate on metal element homeostasis in hyperlipidemic rats.

Experimental

Materials

Basic magnesium polygalacturonate from pectin was produced by In Vitro Kft, according to a patent and the permission of OÉTI[1,17,18]. The magnesium polygalacturonate, was characterized using elemental analysis, infrared spectroscopy, thermogravimetry etc.[18]. Magnesium content of the product was 7.2% w/w.

Table 1. Weight (g) of different organs and body of rats (n=10)

	ND		ND-Mg		FD		FD-Mg		Significance between the four groups at $P<0.05$ by ANOVA
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Liver (g)	11.71	1.44	11.76	1.03	14.38	1.06	13.84	1.16	Sign.
Heart (g)**	0.89	0.12	0.93	0.15	0.90	0.08	0.78	0.10	Sign.
Lung (g)	1.28	0.37	1.55	0.49	1.13	0.09	1.13	0.11	Sign.
Spleen (g)	0.70	0.13	0.61	0.11	0.66	0.14	0.61	0.13	Not sign.
Thymus (g)**	0.46	0.15	0.52	0.12	0.50	0.09	0.94	0.07	Sign.
Kidney (g)	0.99	0.10	1.05	0.13	1.00	0.08	0.94	0.10	Not sign.
Body weight (g)**	314.5	18.36	322.4	12.38	294.0	11.2	269.9	14.6	Sign.

** significant difference between hyperlipidemic (FD) and hyperlipidemic+Mg-treated (FD-Mg) group at $P<0.05$ (ANOVA

Table 2. Routin parameters in sera of rats (n=10)

	ND		ND-Mg		FD		FD-Mg		Significance between the four groups at $P<0.05$ by ANOVA
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
UA (μmol/L)	114.6	20.4	115.6	34.0	131.0	31.4	113.6	18.5	Not sign.
GGT (U/L)	0.125	0.820	0.600	1.260	0	1.050	1.20	1.750	Not sign.
Triglicerid (mmol/L)**	1.22	0.42	1.47	0.44	0.57	0.28	0.86	0.30	Sign.
GPT (U/L)	65.43	18.60	62.00	22.83	76.20	19.75	54.67	21.00	Not sign.
GLUC (mmol/L)	7.63	0.98	8.44	0.75	8.57	0.66	7.87	0.68	Sign.
T-bilirubin (mmol/L)	1.25	0.63	1.22	0.67	1.11	0.60	0.89	0.93	Not sign.
Albumin (g/L)*	28.25	1.06	31.00	1.05	32.50	1.51	32.50	1.09	Sign.
BUN (mmol/L)	5.01	0.68	5.57	0.72	4.09	0.66	4.26	0.72	Sign.
ALP (U/L)**	584.9	102.0	561.8	50.1	1458	43.2	1217	166	Sign.
Total protein (g/L)	50.75	2.75	52.38	1.41	54.10	2.42	55.70	2.21	Sign.
Creatinin (μmol/L)	35.38	1.51	36.70	3.12	43.10	2.06	41.00	3.02	Sign.
GOT (U/L)	123.4	45.9	133.4	22.1	145.8	43.2	126.5	38.5	Not sign.
Cholesterol (mmol/L)*	1.67	0.38	2.11	0.34	5.48	1.83	4.49	1.38	Sign.
Amylase (U/L)**	2520	512	2771	175	4094	1082	3234	284	Sign.

* significant difference between control (ND) and control+Mg-treated (ND-Mg) group at $P<0.05$ (ANOVA)

** significant difference between hyperlipidemic (FD) and hyperlipidemic+Mg-treated (FD-Mg) group at $P<0.05$ (ANOVA)

Animal experiment

Male Wistar rats (n=40; 150-200 g bw) were divided into four groups. The animals in group ND were fed with normal diet from BIOFARM FARM PROMT Kft (BFP, Gödöllő, Hungary). The animals in group ND-Mg were fed with normal diet and were treated with magnesium polygalacturonate (200 mg Mg/kg body weight ad libitum). Rats in group FD were fed with a fat-rich diet containing cholesterol (2.0%), sunflower oil (20%) and cholic acid (0.5%), added to the normal feed, and animals of the FD-Mg group had fat-rich diet and magnesium polygalacturonate.

The rats were kept on these diets for 9 days. Following the exposure period, the rats were anaesthetized with Nembutal (35 mg/kg) and were exsanguinated from the abdominal vein. The liver and other organs were removed and weighted.

Hyperlipidemia was verifyed by elevated serum lipid parameters. The whole experiment was performed with the permission of the Animal Health and Food Control Station (MÁB 1.81.4/2006

Routin laboratory parameters

Routine laboratory parameters obtained using a Hitachi 717 analyser were alkaline phosphatase (ALP), amylase, glucose, total bilirubin (T-bilirubin), albumin, total protein, blood urea nitrogen (BUN), serum triglycerides (TG), cholesterol (CHOL), uric acid (UA), creatinine, gamma glutamyl transferase (GGT), glutamate-oxaloacetate transaminase (GOT), and glutamate-pyruvate aminotransferase (GPT.

Measurement of element concentrations

Element concentration (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Ni, P, Pb, S, Si, Sn, Sr, Ti, V, Zn) of the plasma optical emission spectrometry (type of instrument: Spectro Genesis ICP-OES, Kleve, Germany). After digestion of 3 g of total blood samples with a mixture of nitric acid and hydrogen peroxide (10 + 5 mL) and dilution with double distilled water to 25 mL, concentration of elements was determined. [19]

Table 3. Element content (μg/g) in total blood of rats (n=10)

	ND		ND-Mg		FD		FD-Mg		Significance between the four groups at P<0.05 by ANOVA
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Al**	11.73	6.76	12.63	5.10	14.00	4.25	23.25	7.48	Sign.
As	1.96	0.47	2.10	0.34	1.94	0.32	2.15	0.38	Not sign.
B**	28.89	16.38	21.52	6.19	25.30	8.96	59.48	17.48	Sign.
Ba**	0.0690	0.019	0.0638	0.02	0.0744	0.0298	0.161	0.118	Sign.
Ca*	144.7	17.08	53.78	4.78	60.30	8.31	57.63	15.39	Sign.
Cd*,**	0.0061	0.0004	0.0139	0.0019	0.0121	0.0051	0.0052	0.0015	Sign.
Co	0.011	0.008	0.007	0.0007	0.016	0.016	0.015	0.007	Not sign.
Cr*	0.0641	0.0706	0.120	0.018	0.144	0.089	0.093	0.036	Sign.
Cu*,**	0.704	0.104	0.830	0.093	0.915	0.177	0.777	0.087	Sign.
Fe*	521.6	53.5	422.8	133.9	473.2	73.7	488.2	172.7	Not sign.
K*,**	1873	99	1587	123	1716	278	2005	301	Sign.
Li	2.01	0.32	2.07	0.25	2.00	0.33	1.66	0.54	Sign.
Mg	44.03	4.03	43.50	3.63	42.04	6.97	43.84	5.69	Not sign.
Mn	0.0116	0.0081	0.0094	0.0121	0.0065	0.0048	0.0476	0.1175	Sign.
Ni*	0.142	0.061	0.035	0.011	0.121	0.186	0.125	0.215	Not sign.
P*	596.8	72.4	477.8	55.4	481.9	88.4	458.1	99.1	Sign.
Pb*	0.259	0.085	1.16	1.08	0.447	0.340	0.225	0.109	Sign.
S	1498	347	1728	242	1638	235	1614	336	Not sign.
Si*,**	78.12	37.60	127.9	25.89	140.4	25.60	42.97	33.63	Sign.
Sn	0.181	0.129	0.211	0.125	0.212	0.111	0.135	0.123	Not sign.
Sr*	0.074	0.0168	0.0476	0.0079	0.073	0.0179	0.0906	0.0793	Not sign.
Ti	0.302	0.244	0.174	0.113	0.217	0.08	0.184	0.120	Not sign.
V	0.0298	0.0102	0.026	0.0062	0.0237	0.007	0.0228	0.0024	Not sign.
Zn	4.14	0.48	4.95	0.58	5.15	1.00	4.78	0.69	Sign.

* significant difference between control (ND) and control+Mg-treated (ND-Mg) group at P<0.05 (ANOVA)

** significant difference between hyperlipidemic (FD) and hyperlipidemic+Mg-treated (FD-Mg) group at P<0.05 (ANOVA).

Statistical analysis

Means and standard deviations (SD) were calculated from the results. For comparison of the means of groups, one way analysis of variance (ANOVA) was used by GraphPAD software version 1.14 (1990). Significance was accepted if P<0.05.

Results

Magnesium supplementation affected the body weight and weight of different organs of the rats (Table 1). The measured weights were significantly different in the four groups (ANOVA, P<0.005) except spleen and kidney. The weights in groups ND and ND-Mg were similar, while in the hyperlipidemic groups significant changes were observed for heart, thymus and bodyweight. Nevertheless the difference was larger between the normal and fat-rich diet groups than between the ND and ND-Mg or FD and FD-Mg.

Routine laboratory parameters showed significant changes in triglyceride, glucose, albumin, BUN, total protein, creatinine, GOT, cholesterol, ALP and amylase content among the four groups (Table 2). Magnesium supplementation hardly changed the lab parameters of rats in the normal diet groups, significant elevation was only seen in albumin and cholesterol levels, while in the FD-Mg group the level of triglyceride, ALP and amylase changed significantly vs. FD. Similarly to the organ weights, the difference was larger between ND and FD groups than between the Mg-treated and untreated rats fed the same diet.

In this short time experiment, high magnesium intake changed metal ion homeostasis whilst magnesium concentration in the rast' blood did not change significantly. The element concentration changes in the whole blood were significant among the four groups for Al, B, Ba, Ca, Cd, Cr, Cu, K, Li, Mn, P, Pb, Si and Zn. Significant changes in the concentration of Ca, Cd, Cr, Cu, Fe, K, Ni, P, Pb, Si and Sr were also observed between group ND and ND-Mg, while the concentration of Al, B, Ba, Cd, Cu, K and Si was significantly different between FD and FD-Mg. (Table 3).

Discussion

For magnesium supplementation, several magnesium products are available in the market. Magnesium polygalacturonate was selected for this experiment because of its favourable absorption properties proven by human studies[20]. The most important benefit of magnesium polygalacturonate is that the carrier is of natural origin. According to acute oral toxicological investigation, these complexes are nontoxic even at high concentration (LD_{50} > 5000 mg/kg body weight)[1]. By oral application of the complexes, no side effects have been observed so far[20]. Since there was no magnesium deficiency in rats, this natural origin may be the reasons that magnesium concentration in total blood did not increase. The

concentration of several elements changed significantly in both magnesium-treated groups, nevertheless the alterations were different in the control and hyperlipidemic groups.

Our results showed that a short period of high amount magnesium supplementation can change metal ion homeostasis. Although some favourable alterations were evoked in the lab parameters of the hyperlipidemic group by magnesium treatment, it is worth to note that supplementation with relatively high amount of magnesium should be carried out carefully especially in metabolic diseases like hyperlipidemia because of the alteration of metal ion homeostasis. Similar results were found in rats on supplementation with magnesium malate[21,22]. The reason may be that both magnesium compounds are of natural origin, and the uptake occurs similarly by intestinal absorption and via active transport[23].

References

[1]Lakatos, B., Szentmihályi, K., Sándor, Z., Vinkler, P., *Gyógyszerészet,* **1997**, *41*, 534.

[2]Fazekas, T., Selmeczi, B., Stefanovits, P., *Magnesium in biological systems*. Akadémiai Kiadó, **1994.**

[3]Siegel, A., Siegel, H. *Metal Ions Biol. Syst.,* **1990**, *26*, 1.

[4]Minnich, V., Smith, M.B., Brauner, M., J. *J. Clin. Invest*., **1971,** *50*, 507.

[5]Kuzniar, A., Mitura, P., Kurys, P., Szymonik-Lesiuk, S., *BioMetals,* **2004**,*16*, 349.

[6]Djurhuus, M. S., Skott, P., Hother-Nielson, O. *Diabet. Med*., **1995**, *12*, 664.

[7]Fagan, T. E., Cefaratti, C., Romani, A., *Am. J. Physiol. Endocrin. Metab.,* **2004,** *286*, E184.

[8]Lopez-Ridaura, R., Willett, W. C., Rimm, E. B., *Diab. Care,* **2004,** *27*, 134.

[9]Rodriguez-Moran, M., Guerrero-Romero, F., *Diab. Care*, **2000,** *26* 1147.

[10] Song, Y. Q., Manson, J. E., Buring, J. E., Simin, Liu**.,** *Diab. Care*, **2004,** *27*, 59.

[11]Sheehan, J. P., Sisam, J. P., Schumacher, O. P., *Clin. Res*., **1985,** *33*, A315.

[12]Tosiello, L., *Arch. Intern. Med*., **1996,** *156*, 1143.

[13]Kazaks, A. G., Stern, J. S., *California Agricult.*, **2007,** *61*, 119.

[14]Brannan, P. G., Vergne-Marinin, P., Pak, C. Y. C., Hull, A. R., Fordtran, J. S., *J. Clin. Invest*., **1976,** *57*, 1412.

[15]Keenoy, B. M., Moorkens, G., Vertommen, J., Noe, M., Nève, J., De Leeuw, I., *J. Am. Coll. Nutr.,* **2000**, *19*, 374.

[16]Hans, C. P., Sialy, R., Bansal, D. B., *Curr. Sci*., **2002,** *83*, 1456.

[17]Kröel-Dulay, N., Sándor, Z., Dengelné-Szentmihályi, K., Lakatos, B., Vinkler, P., Szabó, K., Szatmári, E., Deutsches Patent No. 195 20 743, **1995.**

[18]Szentmihályi, K., Lakatos, B., Sándor, Z., Hajdú, M., Vinkler, P. *Magnesium. Magnesium and interaction of magnesium with trace elements,* **1998**, 241**.**

[19]Szentmihályi, K., Blázovics, A., Kocsis, I., Fehér, E., Lakatos, B., Vinkler, P., *Acta Alim.*, **2000,** *29*, 359.

[20]Lakatos B., *Magnesium in Biological Systems, Environmental and Biomedical Aspect,* Akadémiai Kiadó, **1994**, 291.

[21]Bérci, I., May, Z., Fodor, J., Rapavi, E., Kocsis, I., Blázovics, A., Jalsovszky, I., Szentmihályi, K., *Trace elements in the food chain, Deficiency or excess of trace element in the environment as a risk of health,* Bakai Beata Press, **2009,** 36.

[22]Virág, V., May, Z., Kocsis, I., Blázovics, A., Szentmihályi, K., *Orvosi Hetilap*, **2011**, *152*, 1075.

[23]de Baaij, J. H. F., Hoenderop, J. G. J., Bindels, R. J.M., *Clin. Kidney J*., **2012**, *5*, i15.

Received: 15.10.2012.
Accepted: 17.10.2012.