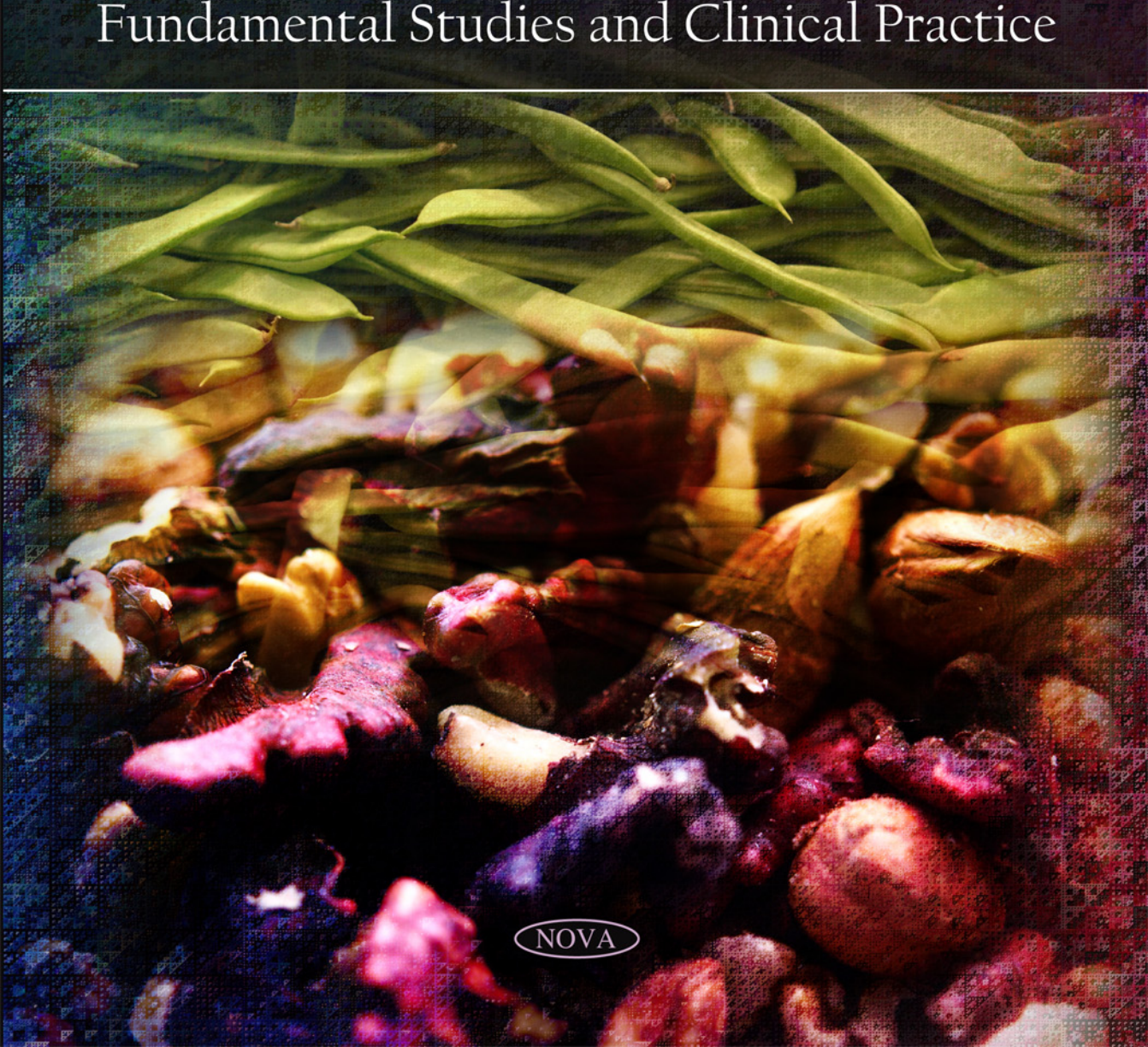
Ivan Y. Torshin | Olga Gromova

BIOCHEMISTRY RESEARCH TRENDS SERIES

MAGNESIUM AND PYRIDOXINE

Fundamental Studies and Clinical Practice



NOVA

Biochemistry Research Trends Series

MAGNESIUM AND PYRIDOXINE: FUNDAMENTAL STUDIES AND CLINICAL PRACTICE

Biochemistry Research Trends Series

Glycolysis: Regulation, Processes and Diseases
Paul N. Lithaw (Editor)
2009. ISBN: 978-1-60741-103-1

HDL and LDL Cholesterol: Physiology and Clinical Significance
Irwin S. Pagano and Nathan B. Strait (Editors)
2009. ISBN: 978-1-60741-767-5

Magnesium and Pyridoxine: Fundamental Studies and Clinical Practice
Ivan Y. Torshin and Olgar Gromova
2009. ISBN: 978-1-60741-704-0

Biochemistry Research Trends Series

MAGNESIUM AND PYRIDOXINE: FUNDAMENTAL STUDIES AND CLINICAL PRACTICE

IVAN Y. TORSHIN
AND
OLGA A. GROMOVA

Nova Science Publishers, Inc.
New York

LIBRARY OF CONGRESS CATALOGING-IN-PUBLICATION DATA

Torshin, Ivan Y.
Magnesium and pyridoxine : fundamental studies and clinical practice / Ivan Y. Torshin and Olgar Gromova.
p. ; cm.
Includes bibliographical references and index.
ISBN 9781607417040
1. Magnesium in the body. 2. Magnesium--Physiological effect. 3. Magnesium deficiency diseases. 4. Vitamin B6. I. Gromova, Olgar. II. Title.
[DNLM: 1. Magnesium--metabolism. 2. Magnesium--pharmacology. 3. Magnesium Deficiency--physiopathology. 4. Pyridoxine--physiology. QU 130 T698m 2009]
QP535.M4T67 2009
612.3'924--dc22
2009017715

Published by Nova Science Publishers, Inc. ✣ New York

CONTENTS

FOREWORD

This book is intended for doctors and medical students. It provides a wealth of data on clinical research, molecular biology and biochemistry of magnesium. The book also aims to correct a number of misconceptions concerning biological roles of magnesium. Synergic interactions of magnesium with pyridoxine as well as with minerals and with drugs are detailed. The book can be recommended to doctors of different specialties (neurologists, cardiologists, physicians, pediatricians, obstetricians and gynaecologists, pathologists, nutritionists and others) which can fruitfully use the information presented in the book in their clinical practice. The book will also be helpful to medical students studying experimental and clinical pharmacology.

The authors gratefully acknowledge the support of the Russian Fund of Fundamental Research

INTRODUCTION

> "The intricate connection between the living organisms and the chemistry of the Earth's crust … indicates that the solution of the life's mystery can not be obtained by only studying the organisms. We have to go to the [biochemical] source of life - to the properties of the chemical elements that comprise the Earth's crust".
>
> V.I. Vernadskiy,
>
> Biogeochemical essays, 1949

Normal levels of magnesium in the body are now recognized as a fundamental parameter that has direct health implications. The essential value magnesium has for the functioning of all the 12 organ systems and during all stages of human development is no longer doubted. According to MEDLINE database, tens of thousands of scientific papers on clinical, biochemical, cellular, and molecular significance of magnesium were published during last decades. The amount of the research papers that high indicates that physiological roles of magnesium and of its deficiency in human health do not represent a mere academic debate but is, rather, an important matter of individual and public health. Specificity of the symptoms of magnesium deficiency, coupled with modern laboratory diagnostics of the trace element status, provided an important nosologic niche for this condition. Since 1995, WHO classified magnesium deficiency as a distinct pathological condition (ICD-10 diagnosis E61.3).

The technique of "ecological zoning" originally formulated by VI Vernadskiy, NI Vavilov, AP Vinogradov, V. Kowalski (Vernadskiy, 1934; Vernadskiy, 1994) was instrumental for the epidemiologic characterization of magnesium deficiency. A number of important studies were conducted on a geographical distribution of the magnesium deficiency in water and soil (Voss, 1962; Moskalev, 1985; Borisenko, 1986; Murray, 1990; Altura, 1998; Rubenowitz, 2000; Spasov, 2000; Yagodin, 2001; Suslikov, 2003; Iezhitsa, 2008 *etc*). These studies have statistically confirmed the correlation between living in the geographic regions characterized by low magnesium content, occurrence of the magnesium deficiency and higher incidence of the diseases among the population.

Today, however, the low magnesium content in water and soil of certain geographic regions isn't the major concern for the public health. Modern people, especially urban dwellers, are not so dependent on the produce grown in the region they live. The food basket of a modern urban resident contains products from geographically different regions (including those thousands kilometers away from the end consumer) - yet the problem of magnesium

deficiency is, nevertheless, actual. The major risk factors for the magnesium deficiency are no longer the soil and water content of magnesium but, rather, chronic stress and unbalanced diet (overindulgence in the junk foods, prevalence of meats and carbohydrates over vegetables *etc*). In a way, the deficit of magnesium is one of the diseases of the contemporary Western civilization. The technological "revolution" in food production, which began with a 1930s-1950s of the last century, aimed at mass production of ever increasing quantities of food stuffs and not so much at their nutritional value. This was paralleled by the profound lack of nutritional literacy among majority of the populations throughout the world and even among the specialists.

Both the poor nutritional quality of the massively produced foods and the wide-spread nutritional illiteracy significantly influence the integral parameter of the health of a nation: both longevity and the quality of life. In countries with the highest life expectancy (WHO data on 2002) such as Japan (men, 78 years; women, 85 years), Cyprus (men, 78 years; women, 82 years), Greece, Italy, *etc*, the respected populations show considerable differences in the traditions of nutrition in comparison to the junk diets common in the rest of the West. In the Mediterranean region, for example, these differences include systematic consumption of olive oil (characterized by a high content of squalene and polyunsaturated fatty acids), of other vegetable oils that improve lipid profile (grape-stone, pumpkin, walnut, corn, soybean oils, canola), low consumption of animal fat, and, especially, presence in the diet of a considerable amount of numbers magnesium-containing products: fresh herbs, fresh fruits and vegetables, seafood, fish, unrefined grains, and bread made from coarse flour of organic grains. This kind of diet is known as "Mediterranean diet" or "modified Mediterranean diet" and it was proven to be efficient in reduction of the mortality and for the prevention of the major diseases such as CVD (Singh, 2002; Trichopoulou, 2005; Dontas, 2007; Trichopoulou 2007; Fitó, 2007 *etc*). Throughout the entire world, there is a trend to go back to roots, to the best traditions of the nutritionally sound diet systems which increase longevity by providing easier assimilated macronutrients and by not leaving out the essential micronutrients.

The deficit of magnesium is often coupled with low level or even deficit of pyridoxine (ICD-10 diagnosis E53.1). Symptoms of pyridoxine deficiency are reminiscent of the clinical picture of magnesium deficiency in a number of ways (Chapters 4, 8). Identification of the population-wide low or border-line levels of the group B vitamins (in particular, of folates, pyridoxine, cyancobalamine) in several countries (Finland, Germany, France, USA) stimulated development and implementation of certain measures to reduce populational risks of hyperhomocysteinemia and atherosclerosis. Many countries (Finland, Japan, France, Germany, Switzerland, Canada, Poland and others) have entered long-term government programs for the treatment of magnesium deficiency and the deficiencies of selenium, iodine *etc*. These programs include population screening for clinical and laboratory signs of magnesium deficiency with subsequent implementation of the compensatory and educational measures including increase in the nutritional literacy of the populations, introduction of Mg-containing table salt (sea salt or specific mineral compositions), use of water enriched in magnesium ions, and, finally, use of pharmaceutical Mg-based preparations for the treatment of advanced cases of the magnesium deficiency.

As a result of the nutriological programs of different countries, a number of large-scale epidemiological studies were conducted. For example, according to the Health Ministry of Finland, prevention and correction of the population-wide micronutrient deficiencies (selenium, folates, magnesium *etc*) resulted in halving down the risk of myocardial infarction

in people 40-60 years of age. There are data on the importance of correcting the deficit of magnesium to reduce the cancer incidence. These figures depend not only on the consumption of magnesium, but also correlate with long-term use of diets rich in fresh fruit and vegetables (WHO, 1999-2003; American Society of Clinical Oncology, 1976-2006). Government programmes for the correction of magnesium and pyridoxine deficiency in pregnant resulted in reduction in the severity of pre-eclampsia, premature births, low-weight births, perinatal damage of the central nervous system as well as of the infant mortality (Japanese Association of Gynecology and Obstetrics, 2004; Kosheleva, 2006). These programs often include early preventive use of the safe methods of magnesium and pyridoxine correction: special diet and drinking regimen along with *per os* usage of the safe doses of magnesium preparates of the second generation (magnesium lactate, magnesium citrate, magnesium pidolate, magnesium asparaginate and others).

Despite the impressive resuls at the level of public health programs, one still can find an observable amount of skepticism among the medical specialists concerning the effectiveness of treatment with magnesium preparations. It should be said that, virtually always, *the skepticism of the sort is based on a few negative examples which are cited all too often and thus weed out the numerous positive ones*. Let's consider usage of magnesium preparations (even in such archaic forms as magnesium sulfate) in cardiovascular medicine. For example, a few particular studies of a particular trial group called Magnesium in Coronaries (MAGIC) alleged absence of effects of a magnesium treatment and were published in a highly acclaimed journal (see ref. MAGIC trial investigators, 2002). In this study, short-term mortality in 6213 patients with ST-elevation myocardial infarction was evaluated. Magnesium treatment studied included 2g intravenous magnesium sulfate administered over 15 min, followed by a 17 g infusion of magnesium sulfate over 24 h vs placebo (injection of the physiosolution). At day 30, similar numbers of patients in both treatment and placebo groups had died: 475 (15.3%) magnesium group and 472 (15.2%) placebo group (OR=1.0, $P<0.1$).

The above-mentioned study can be often cited as a "strong proof" of "inefficiency of magnesium therapy". However, if the magnesium treatment of the crash course type mentioned in (MAGIC trial, 2002) had no observable effect on mortality during 30 days, it does not mean at all that there won't be any other positive cardiovascular effects on a wider time scale. Aside from remarkable drawbacks of this MAGIC study, such as very short-term observation, absence of stratification of an ultra-large group and other violations of the data analysis (see Torshin, 2007), usage of an inorganic form of magnesium, doubtful Mg concentrations and questionable regimen *etc*, it is just one study that is mentioned too often in professional media – apparently, at the expense of dozens of other studies. These other studies, including several meta-analyses, point to an entire mesh of actual proofs that indicate, both directly and indirectly, the value of adequate levels of magnesium for the human health. These proofs come from studies focused on quite different aspects, from geographic to biochemical and clinical, and indicate a variety of the positive effects of the magnesium treatment. The latter statement holds true even in the case of such crude forms of magnesium treatment as intravenous magnesium sulfate. Below, we cite some of these studies along with the tags indicating the focus of study.

GEOGRAPHIC

The relationship between the levels of magnesium in drinking water and the cardiovascular mortality was shown long ago (Vernadskiy, 1934; Voss, 1962). A relatively recent Swedish study of 1679 patients indicated that the risk of death from myocardial infarction was lower in the quartile with high magnesium levels (>0.83 mmol/L) than in the quartile with lower magnesium (<0.75 mmol/L). The odds ratio for death from acute myocardial infarction in relation to water magnesium was 0.64 (95% CI = 0.42-0.97) for the highest quartile relative to the lower ones. In other words, magnesium in drinking water is associated with lower mortality from acute myocardial infarction (Rubenowitz, 2000).

HISTOLOGICAL

Pathoanatomical studies indicate that myocardial tissues of IHD patients who died of cardiovascular reasons usually contain no more than a half the amount magnesium found in patients who died from other causes (Chakraborti, 2002).

BIOCHEMICAL AND CLINICAL

A study of 323 patients with symptomatic peripheral artery disease indicated that, compared with patients in the highest tertile of Mg serum levels (>0.84 mmol/L), patients with Mg serum values <0.76 mmol/L (lowest tertile) exhibited a 3.3-fold increased adjusted risk (95% CI 1.3-7.9; P=0.01) for neurological events (Amighi, 2003). A study of mortality after coronary artery bypass graft surgery in a cohort of 957 patients indicated that serum magnesium level (<0.8 mmol/L) increased 2-fold (hazard ratio 2.0, 95% CI 1.2-3.4) the risk of death or myocardial infarction at 1-year followup (Booth, 2003). Framingham Offspring Study of 3,327 eligible subjects indicated that lower potassium ($p = 0.002$) and lower magnesium ($p = 0.01$) levels were associated with higher prevalence rates of ventricular arrhythmias (Tsuji, 1994).

CLINICAL

Several meta-analyses indicated positive results of the adequate magnesium treatment regimens on the clinical outcomes. Seven trials collectively indicated 55% reduction in mortality ($p<0.001$) when using intravenous magnesium in suspected acute myocardial infarction (Teo, 1991). Meta-analysis of magnesium therapy for the acute management of rapid atrial fibrillation indicated that magnesium was effective in achieving rate control (OR 1.96, 95% CI 1.24 to 3.08) and rhythm control (OR, 1.60, 95% CI 1.07 to 2.39). An overall response was achieved in 86% and 56% of patients in the magnesium and control groups, respectively (OR 4.61, 95% CI 2.67 to 7.96, Onalan, 2007). A meta-analysis of 20 randomized trials, enrolling a total of 2490 patients, indicated that magnesium administration

decreased the proportion of patients developing postoperative atrial fibrillation (odds ratio 0.54, 95% CI 0.38-0.75) (Miller, 2005).

It should be noted that the design and interpretation of randomized clinical trials and of meta-analyses should take into account differences in the study design as well as biologically plausible hypotheses of the treatment effect (Woods, 2002). By neglecting the study design and the biology, the researchers conducting the study leave themselves at the mercy of statistical flukes which will only lead to false negatives or false positives. For example, meta-analysis of 12 randomized controlled trials of intravenous Mg^{2+} in acute myocardial infarction gave a null effect of Mg-treatment (odds ratio 1.02, 95% CI 0.96 to 1.08). However, when the authors accounted for study heterogeneity ($P < .0001$) and the bias introduced by a single large study (in which Mg was generally given too late and after fibrinolytic treatment), the adjusted model gives a pooled odds ratio 0.61 (95% CI 0.43 to 0.87, $P = 0.006$). This transition from a negative finding to a positive one indicates that the first attempt of analysis produced a false positive. Thus, inadequate study design, neglect of the study heterogeneity or inadequate assessment of the study heterogeneity during meta-analysis can result in a seeming contradiction with the animal studies which clearly show that timing of Mg^{2+} administration before or after reperfusion is critical for myocardial protection (Woods, 2002). More details on the intricacies of meta-analysis is available in (Torshin, 2007).

Apart from the problems introduced when researchers confuse studies of different design and clinical setting, the problem with many of the papers published under the rubric of evidence-based medicine is that they often lack biological justifications of the findings. These biological justifications, which became apparent in the era of the molecular biology and post-genomic biomedicine, are the molecular mechanisms of the action of magnesium.

It can be said that insufficient intake of the dietary magnesium, often coupled with deficit of pyridoxine, is one of the major nutritional problems of the modern human. The present book presents a systematic review of the epidemiological, clinical, biochemical and molecular evidence pertaining to the biological effects of magnesium and the detrimental impact the magnesium deficiency has on the human health. The discussion of the general roles of magnesium ions in human physiology (Chapters 1, 2) is followed by a detailed analysis of the clinical manifestations of the magnesium deficiency (Chapters 3, 4) and the methods of its correction (Chapters 5, 6). Then, we consider toxicology of magnesium (Chapter 7), physiological roles of pyridoxine and its derivatives (Chapter 8) along with a few methods for determination of the levels of magnesium and pyridoxine in biological substrates (Chapter 9).

Special attention is given to the molecular mechanisms that mediate physiological effects of magnesium. This is especially important in post-genomic era, when genome-wide studies of the genomes, transcriptomes and proteomes hold a promise of comprehensive understanding of the human physiology at the molecular level with subsequent application of these data in personalized medicine. In the human genome, there are approximately 29,000 genes of which 14,000 are annotated. According to the analysis of the annotated portion of the human genome, there are at least 500 genes that encode Mg-binding proteins (enzymes, ion transporters *etc*). Systematic analyses of these proteins (using the methods described in Torshin 2007, Torshin 2009) allow us to outline the complex molecular nature of magnesium impact on human physiology.

Understanding the actual complexity of the physiologic effects of magnesium is essential in order to avoid oversimplification of the problem of magnesium deficit. In recent years, alas, there is a trend towards primitive interpretations of this wide-spread condition, the

magnesium deficiency. An oversimplified clinical interpretation often results in higher rates of misdiagnosis and mistreatment of the patients. Moreover, irrational commercialization of the problem of the chronic nutritional deficiencies of magnesium and of other minerals has flooded the market with many untested magnesium preparations the positive and negative effects of which are difficult to predict without having an appropriate expertise. This issue concerns, in particular, the *widespread use of magnesium oxide and inorganic magnesium salts despite abundant pharmacokinetic evidence that these forms of magnesium do not only have extremely low bioavailability (<5%) but, at the same time, are also characterized by considerable toxicity.* The laxatives and antacid drugs based on inorganic magnesium (MgO, $Mg(OH)_2$ *etc*) can not be used for correction of magnesium deficit because of the low bioavailability and due to the prominent laxative effect.

Thus, the goal of this book is to present an overview, more or less systematic, of the most important directions in the study of biological and clinical roles of magnesium in the human body. According to epidemiological data and studies in evidence-based medicine, the clinical effects of magnesium supplementation and magnesium deficiency are related to the common conditions such as chronic stress, chronic fatigue, hypertension and vascular disease, cancers, diabetes, diseases of dependence *etc*. We also detail the issue of clinical pharmacology of magnesium.

Chapter 1

1. THE BIOLOGICAL ROLES OF MAGNESIUM

1.1. BACKGROUND

In chemistry, magnesium (Mg) is an element of the group II of the Mendeleev's periodic table. Magnesium's atomic number is 12 and atomic weight is 24.31 $g \cdot mol^{-1}$. In free state, it is a light-weight metal (density of only 1.74 $g \cdot cm^{-3}$, compare to that of iron, 7.87 $g \cdot cm^{-3}$) which brightly burns even in the air. Magnesium was initially discovered in 1808 by H. Davy who managed to obtain a small amount of the metal in the process of electrolysis of magnesia (MgO). In 1828, the famous French chemist A. Bussy obtained the metal by reducing molten magnesium chloride with metal sodium. Davy called the metal as "magnesium", perhaps after the Greek city of Magnesia which from antiquity produced certain ores of magnesium and manganese that were known to early alchemists.

Among the other elements, magnesium is the 8th most frequent element: the Earth's crust contains an average 1.87% of magnesium. Magnesium salts are particularly abundant in the sea water which, on average, has concentration of magnesium of 1.35 g/L (3rd place after chlorine and sodium). The total amount of magnesium in the oceans is estimated to be $2 \cdot 10^{15}$ tons.

Among the metal cations which occur in biological systems, magnesium is not not only widespread (4th place after sodium, potassium, and calcium), but also has a very wide range of essential biological meanings: magnesium is a cofactor of hundreds of enzymes with very different functions (glycolytic, biosynthetic, and, especially, of the enzymes that catalyze transfer of phosphate groups). Mg is required for the fatty acid and vitamin metabolism. It is the central metal ion in the porphyrin ring of the plant chlorophyll (figure 1-1).

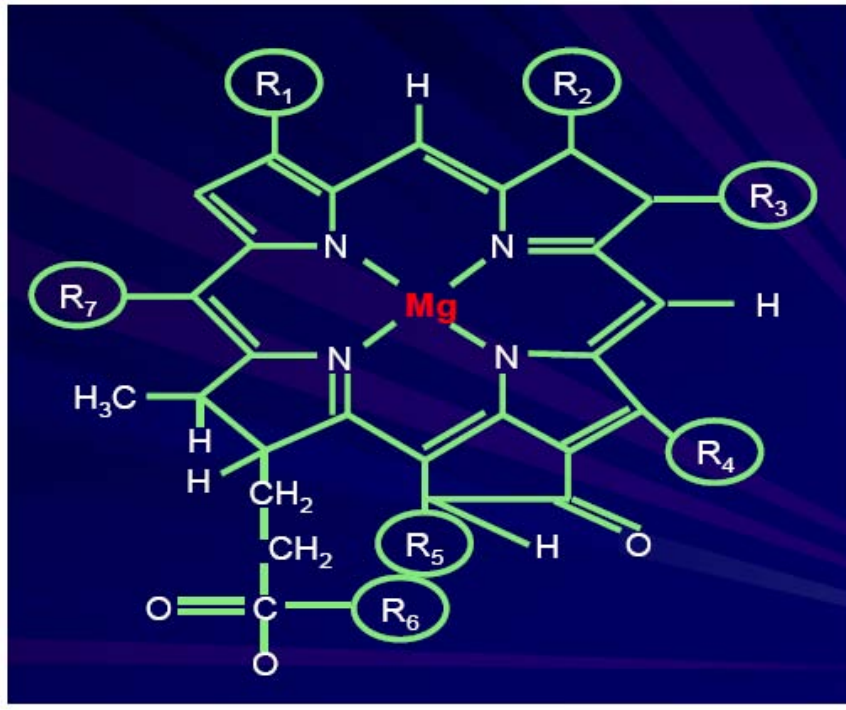
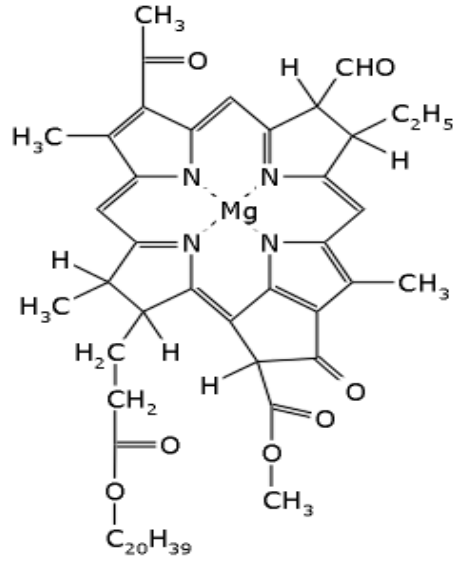


Figure 1-1. Magnesium in the porphyrins. a) coordination of magnesium coordinated within the chlorophyll, b) coordination of magnesium ion within the bacteriochlorophyll.



In certain microorganisms, chlorophyll appears in the form of bacteriochlorophyll. The latter is essential substance for the biosynthetic pathways of the bacteria and is required for the survival of functional microflora of the human bowel. While it can be said that in plants and in bacteria the magnesium's major role is to form the coordination centre within the

porphyrin ring, in humans the roles of magnesium are much more sophisticated and impact very many different branches of the metabolism. We briefly consider these biochemical processes further in this chapter and in the Chapter 4.

1.2. EPIDEMIOLOGY

Nutrition of contemporary humans is often characterized by moderate to severe distortions of the mineral composition of the diet, with predominance of NaCl and deficit of the salts of K, Mg and Ca (figure 1-2). According to (Engstrom, 1983), in USA alone 16-42% of the general population consume less than 2/3 of the recommended amount of magnesium. Similar situation is known to exist in Europe, Russia, China and other countries. For instance, a study of ~16000 Germans indicated suboptimal level of Mg consumption in 34% of the general population (Schimatschek, 2001), the respective figures for K and Ca were 29% and 23%. It should be noted that 14.8% of this population sample suffered considerable hypomagnesemia, had a prominent clinical picture of the deficiency and, apparently, required pharmacological correction of magnesium. At the same time, an abnormally high consumption of NaCl was found in 46% of the population (Schimatschek, 2001).

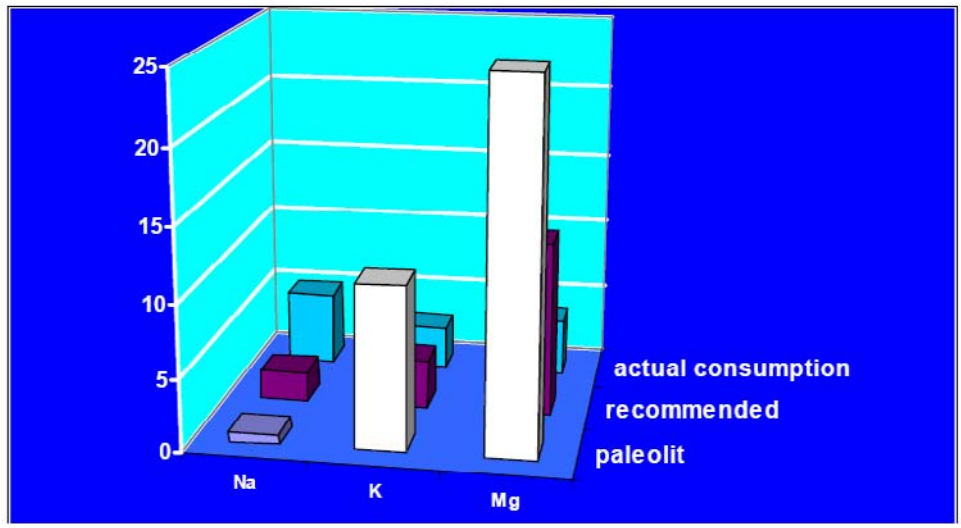


Figure 1-2. Distortions in the mineral consumption (Na, K and Mg).

A number of epidemiological studies performed in different geographical regions pointed out at the inverse relationship that exists between the magnesium content of the drinking water and the frequency of coronary heart disease (CHD). The strongest was association between insufficient consumption of magnesium and the sudden death of CHD patients (Eisenberg, 1992). Many pathoanatomical studies have shown that myocardial tissues of the CHD patients who died of cardiovascular reasons usually contains no more than a half the amount magnesium found in patients who died from the other causes (for instance, Bloom, 1986; Chakraborti, 2002). Cardiomyocytes at the infarction foci are characterized by abnormally high content of sodium and calcium while the level of potassium and magnesium

are lowered. It is also known that using large doses of magnesium can limit the size of the infarction foci (Chapter 4).

Once again, today's most common causes of the magnesium deficit throughout the world lie in the changes in the agricultural technology, food processing and changes in the lifestyle. Hysterical advertisement through the mass media converts humans into a sort of *"consuming animal", which did not only lost any perspective of its place in the universe but also brutally neglects its own health*. From the nutritional point of view, the common diet of the most of the Western countries is literally reduced *ad absurdum*: this modern "food" almost entirely excludes valuable micronutrients and includes unstudied or outright toxic compounds (so called "food additives", "colors", "stabilizers" *etc*).

Unhealthy diet provides a fertile ground for the diseases of dependence. As the result, there is a vicious circle: magnesium deficiency stimulates the formation of the addictive habits and the diseases of dependence (Marshak, 2003) while alcohol, drugs and smoking considerably accelerate elimination of the magnesium from the body. It should also be noted that the improperly crafted courses of dieting, extremely common nowadays, contribute to a higher excretion of magnesium almost as significantly as alcohol, smoking and drugs.

Improper use of fertilizers augments magnesium deficiency in the cultural soil (Yagodin, 2001). The qualitative change in the composition of food, increase in the proportion of animal products, decrease in the vegetable consumption, high consumption of protein and fat foods increase the need for magnesium while extra food processing and refining leads to profound loss of many minerals including magnesium (figure 1-3).

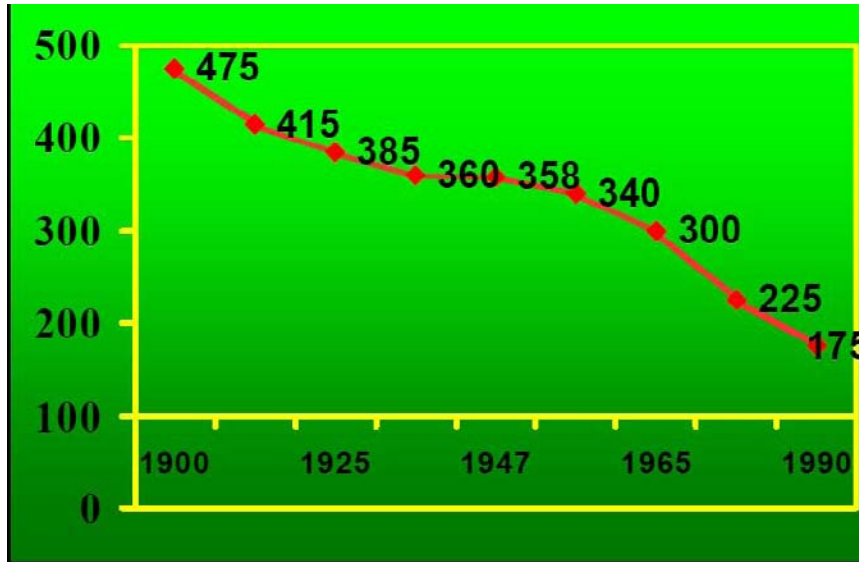


Figure 1-3. The gradual decline in nutritional consumption of magnesium (mg/day) in the twentieth century (Yagodin, 2001).

The deformed modern diet includes excessive salting of food. An acute rise in the incidence of cardiovascular disease which was observed during 20th century remarkably coincides with the fact that the table salt became widely available and very cheap (Price, 1937). Epidemiology of protracted salt-dependency and, consequently, of the increased incidence of arterial hypertension is clearly visible on the example of the residents of Japan

and Bahamas. Before the governmental nutrition programs (which included reduced salt consumption) were implemented in these regions, the incidence of hypertension was higher 1.8-2 times than that worldwide.

Epidemiology also indicated gender differences in magnesium homeostasis. The diseases related to excessive salt consumption (hypertension, kidney disease, hyperaldosteronism etc) occur more frequently ammong women than men. The salt-consuming women loose the magnesium much more intensely than men. Women have higher concentrations of magnesium deposited in tissues of the body (table 1-1) and are more susceptible to a magnesium deficiency (figure 1-4), especially taking into account the important role magnesium has in pregnancy and support of the placental function (Torshin, Gromova, 2009). Accordingly, excessive salt is undesirable for women's health and reproductive potential.

Table 1-1. The concentration of magnesium in the hair (Caroli et al, 1992)

Mg content (μg/g of dry weight)			
Average		Median	
Males	Females	Males	Females
22,11	31,07	19,20	25,53

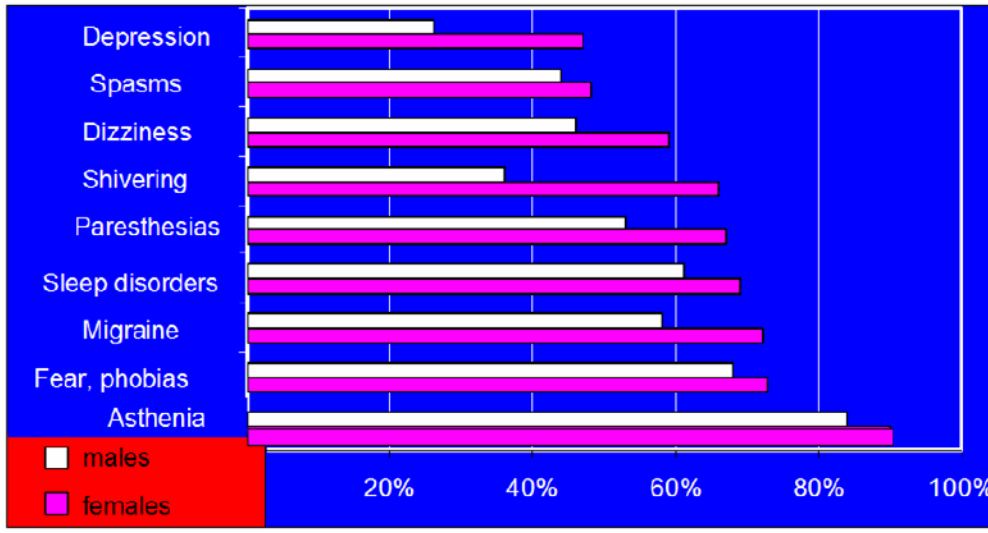


Figure 1-4. Magnesium deficiency is more frequent in women (Fehlinger, 1991).

In addition to the gender differences, there are clearly expressed climatic and geographical differences in the hair concentrations of magnesium. Underlying these differences are the nutrition culture of particular regions as well as the magnesium content of the drinking water. For example, the residents of Japan and New Zealand, who regularly consume products high in magnesium (fish, seafood, seaweed) are characterized, on average, by the highest magnesium content (table 1-2). Nevertheless, epidemiological studies also indicate that in any country there is a considerable proportion of the general population (on the order of 20%-40%) of people suffering from a magnesium deficiency. These are people who experience state of hunger quantitative and qualitative hunger, those living in a chronic

state of nervous, physical and emotional tension, those suffering from depression, diseases of dependence (smoking, alcoholism, drug addiction), infectious diseases, hypertension, bronchial asthma, osteoporosis, diabetes and iatrogenic diseases.

Table 1-2. The concentration of magnesium in the hair of healthy controls from various countries (μg/g of dry weight)

Countries	Italy (Cardi et al., 1992)	UKK (Ward et al., 1992)	USA (Mineral Lab, 1987)	Japan (Kamakura, 1983)	New Zealand (Ward et al., 1987)	Bulgaria (Ward et al., 1987)
Mg (content range)	0,32-137,5	30,37-81,65	0,06-160,0	14,0-567,0	73,45-149,3	25,32-128,9
Average value	68,91	56,01	80,03	290,50	111,37	77,11

1.3. BIOLOGICAL ROLES OF MAGNESIUM

Tens of thousands of papers dealing with biological roles of magnesium were published starting from 1950s. There are over 70,000 relevant references in MEDLINE database alone. The trends in publications during the last 2-3 decades are of especial interest (figure 1-5). Analysis of the abstract databases shows that most of publications on magnesium deal, in some or other way, with physiological roles of magnesium (figure 1-5a). Despite that the number of publications *per* year dealing with magnesium physiology is greater than the number of clinical studies published in the same year (figure 1-5b), two distinct trends can be observed.

First, during the last decades the interest of the researchers to physiological roles of magnesium steadily declines. The peak of interest to the classical physiological studies of magnesium (animal research, for the most part) was in mid-1970s. Second, the interest to the clinical applications of the magnesium preparations steadily grows. Apparently, the interest shifts from the evidence based on experimentation on cells in culture and animal studies to the evidence obtained in the framework of the *clinical studies*.

However, a very important aspect of the physiological effects of magnesium (namely, the *molecular mechanisms of the magnesium action*) is often left out of view both in the physiological and in the clinical studies. In this section, we discuss both the results of the physiological studies of the magnesium effects and some of the molecular mechanisms that mediate the physiological effects.

Formally, magnesium belongs to the macroelements (minerals): the total magnesium content of an adult is ~0.027% which amounts to ~25g (21-29g) in an adult. Other macroelements, besides magnesium, include sulfur, calcium, potassium, sodium, chlorine and phosphorus. As the data in the table 1-3 suggest, magnesium has, actually, an intermediary position between the macroelements and the trace elements.

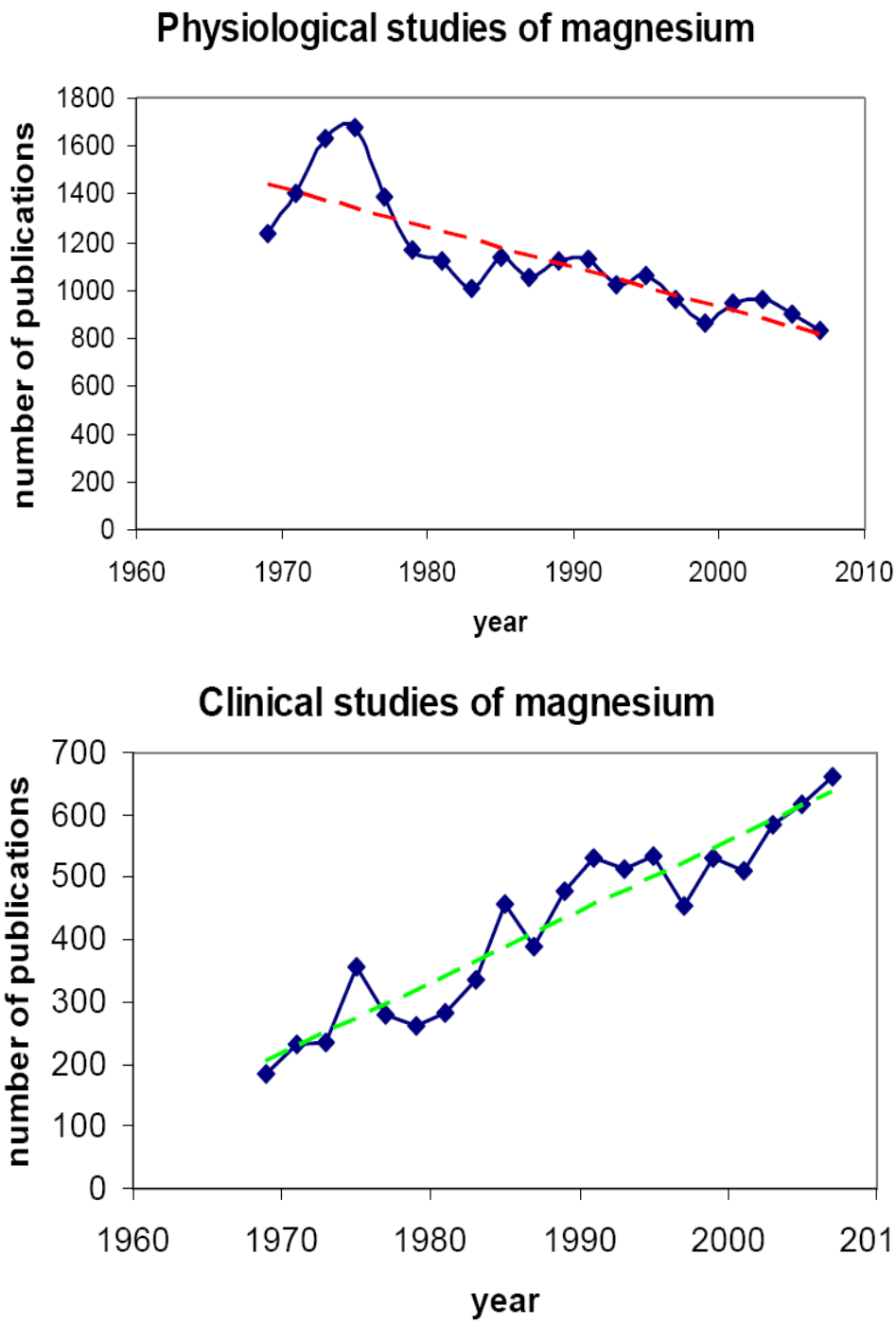


Figure 1-5. Magnesium publications by year (PubMed/MEDLINE data). a) Studies dealing with role of magnesium in human and animal physiology were found using "physiology" as the keyword and excluding the keywords for the clinical studies. b) Clinical studies involving magnesium were found in PubMed database using keywords "intervention", "prevention", "therapeutic use", "treatment".

Table 1-3. Average content of mineral elements in mammalians. Hydrogen, oxygen, carbon and nitrogen comprise over 90% of the body mass and are not included in the table

% of the body mass	Elements	Element group
1-9	Ca	Macroelements
0.1-0.9	P K Na S Cl	
0.01-0.09	Mg	
0.001-0.009	Fe Zn F Sr Mo Cu	Microelements (trace elements)
0.0001-0.0009	Br Si Cs J Mn Al Pb Cd B Rb	
0.00001-0.00009 0.000001-0.000009	Se Co V Cr As Ni Li Ba Ti Ag Sn Be Ga Ge Hg Sc Zr Bi Sb U Th Rh	Ultra-microelements

Only ~2% of the total magnesium can be found in biological fluids (1% of magnesium in the intercellular space, 0.5% - in erythrocytes and 0.3% - in plasma) with the other 98% being concentrated in bones, skeletal muscle and soft tissues (figure 1-6). More than half of the total magnesium (60%) concentrated in dentin and teeth enamel, the bone and the tissues characterized by high metabolic activity and high concentration of mitochondria (namely, brain, heart, muscle, kidney, liver and placenta). Placenta is the leading tissue which appears to have the highest levels of magnesium among all other tissues (Spatling, 1988). In the brain, magnesium has a higher concentration in grey matter in the frontal cortex in comparison to the white matter and olfactory bulbs (Gromova, 2004).

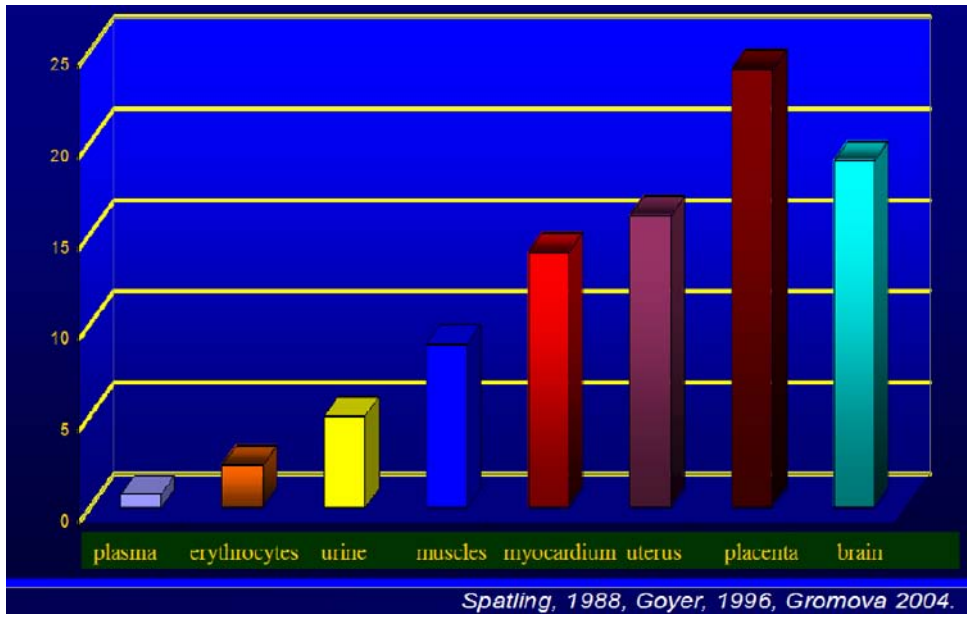


Figure 1-6. The levels of magnesium in various biosubstrates (plasma and urine - mmol/L; tissues and erythrocytes - mmol /kg).

One of the most interesting results that biomedical studies lead to is that the content of magnesium in the body can not be viewed in isolation from homeostasis of other elements. For example, magnesium deficiency often occurs in the context of a deficit K, S and Zn, at least in Japanese (Shimbo et al, 1999). The serum and erythrocyte levels of magnesium are linked with the contents of Cr, Co, Cu, Fe and Ni and this should be taken into account when these elements accumulate in excess either due to professional or environmental conditions (Yilmaz, 1999). In insulin-independent diabetes, magnesium deficiency is accompanied by imbalance of Cr, excess Cu and deficit of Zn (Zargar et al., 1998). With age, the severity and the incidence of the magnesium deficiency grows considerably. At the age 70-99 years, the magnesium deficiency occurs in more than 80% of the elderly. Animal studies indicated that accumulation of the magnesium in the bone depot decreases with age (De Blasio, 2007).

Our study of the levels of minerals in 650 children 2-12 years old with attention deficit hyperactivity disorder (ADHD) indicated characteristic deficiencies and abundances of the trace elements that were associated with magnesium deficiency (figure 1-8). The children who had excess lead always had also magnesium deficiency. Normally, the Pb:Mg ratio should be 1:250. The higher proportions (1:100) indicate elimination of magnesium stimulated by the lead, while the ratios higher than 1:25 indicate a threat of the lead intoxication. And contrariwise, the ability of the magnesium preparations to expel lead is known in the evidence-based medicine (Antonenkov, 1999).

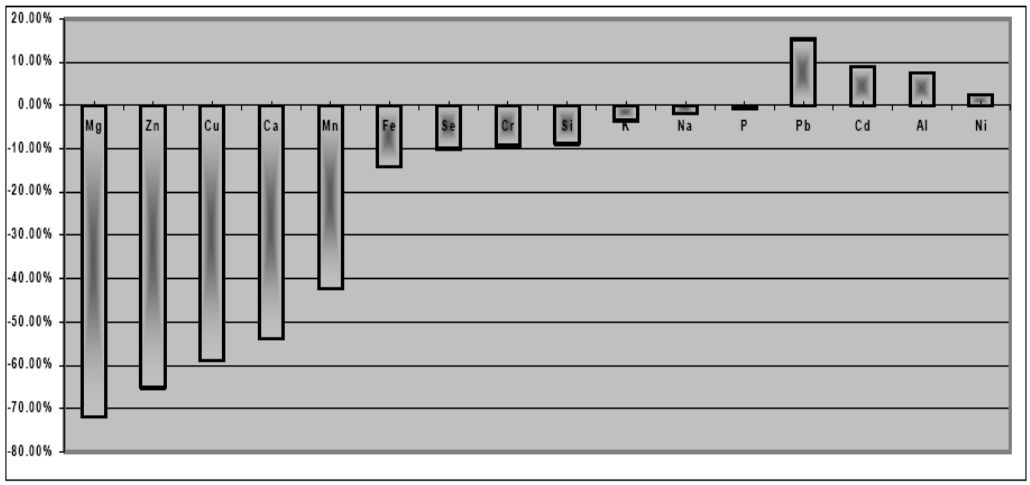


Figure 1-8. Disbalance of the trace elements (hair shaft) in 3-8y old children with ADHD (Fedotova, Gromova 2005).

Absorption of dietary magnesium occurs mainly in small intestine (duodenum and jejunum). On average, up to 35% magnesium from foodstuffs is absorbed. Kidneys are the primary regulator which maintains constant level of magnesium in the body and, normally, ~30% of the magnesium derived from food is excreted with urine. A small amount of magnesium is excreted with sweat. When magnesium is depleted in the body, excretion of magnesium is reduced or stops altogether. There are several basic facts concerning bioavailability of magnesium which have important pharmacologic and therapeutic implications:

- Especially favorable effect on the absorption of magnesium have milk (especially goat milk) and casein;
- Of the magnesium salts, the organic salts such as glycinate, lactate, pidolat, citrate, gluconate, acetate *etc* have far better adsorption than inorganic salts (chloride, sulphate), magnesium oxide or magnesium hydroxide;
- Bioavailability of magnesium is increased in complexes with amino acids;
- Adsorption of magnesium can reduce calcium levels as these two cations share a common system of cation transport in the small intestine;
- Excess of phosphorus inhibits Mg absorption, increases Mg loss with urine;
- High content of phytanic acid and fatty acids in the diet can result in maladsorption of magnesium;
- Iron can reduce the absorption of magnesium in the intestine;
- Sodium inhibits interstitial absorption of magnesium;
- Aluminum and beryllium increase the withdrawal of magnesium from the body;
- In sportsmen and in people attending Turkish and other bath too frequently, the loss of magnesium with sweat can become quite noticeable.

In biological fluids and inside the cells, magnesium is found in the form of hydrated divalent ions, in complex with ATP, in complex with RNA and also in complexes with hundreds of different proteins. The concentration of the hydrated ions inside the cells is about 2.5-3 times higher than in extracellular fluids. The highest concentration of magnesium is found in mitochondria in the form of the Mg-ATP complex (figure 1-7).

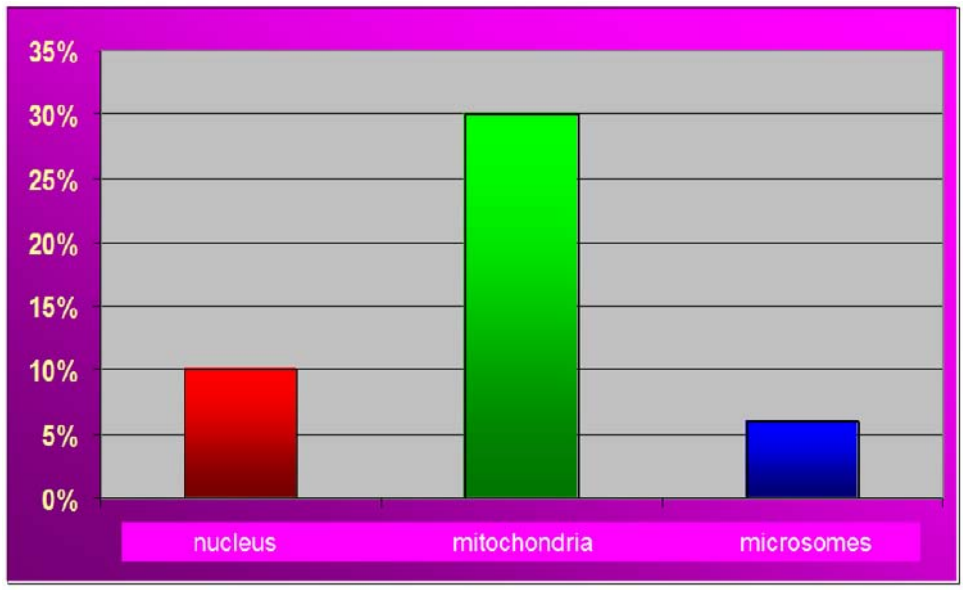


Figure 1-7. Distribution of Mg in the cell (Curtis, 1985).

Most of the physiological effects of magnesium on the cells and tissues of the body are due to highly specific interactions with the specific proteins. At present, over 500 Mg-binding proteins are known. These proteins correspond to the same number of the genes encoded in the human chromosomal DNA. Although these genes are scattered throughout all of the 24

chromosome types, at least 50% of the genes that code Mg-binding proteins are located in just about 20 cytogenetic bands which comprise less than 20% of all the genome (table 1-4). In other words, some of the chromosomal locations are enriched in the genes that code Mg-binding proteins. The latter observation has a number of implications for genetic disorders that afflict the normal magnesium homeostasis, especially genetic disorders caused by large-scale chromosomal aberrations.

Table 1-4. The human genome loci enriched in Mg-related genes

Cytogenetic band	Genes, totally	Annotated genes	Mg-related
19q13	917	587	24
11q13	321	210	19
6p21	385	233	17
19p13	644	437	17
16p13	316	173	16
17q21	346	232	15
22q13	247	149	12
3p21	225	157	11
1p34	204	125	10
16p11	207	85	10
20q13	281	181	10
1p36	494	260	9
14q24	149	83	9
14q32	391	105	9
1q32	199	117	8
4q21	92	50	8
9q34	243	153	8
10q22	120	60	8
11p15	439	253	8
12q13	327	231	8
15q21	100	56	8
17p13	270	176	8
Total	6917	4113	252

From molecular point of view, biochemical and physiological effects of magnesium, as well as clinical manifestations of the magnesium deficiency, can be explained in terms of the altered function of these 500 proteins. Throughout this book, we present a number of examples illustrating the results of systematic analyses of the molecular mechanisms of the biological action of magnesium in the case of different pathologies. Although the amount of the protein-bound magnesium does not exceed 0.1% of the total amount, magnesium serves as an essential cofactor for these hundreds of proteins.

It is *important to keep in mind that the ATP-Mg^{2+} complexes (the major form of Mg inside the cells) are often more stable than the complexes of Mg^{2+} with proteins or the hydrated Mg^{2+} ions.* Physical chemistry suggests that the partial charges on the oxygen atom of the phosphate group are greater than those in the proteins where magnesium is bound mostly by carboxylates (figure 1-9). Indeed, our estimates of the binding energies of Mg^{2+} to

proteins and to ATP using the ECMMS molecular mechanics program (Torshin, 2005) indicated that binding energy of magnesium to ATP almost always +0.2kcal/mol greater than the energy of magnesium binding to most of the known Mg-binding site in the proteins.

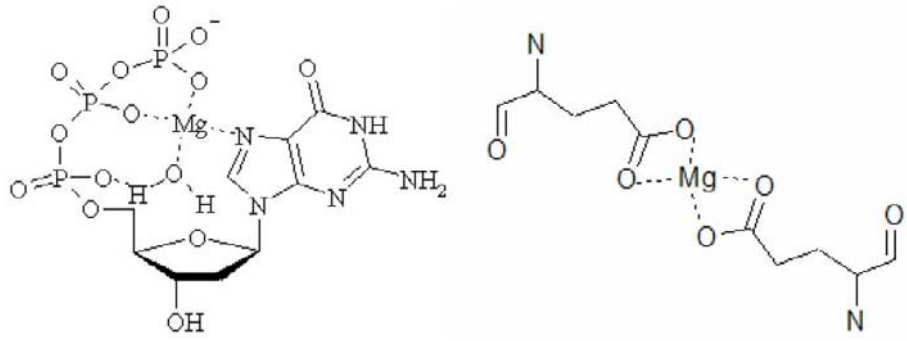


Figure 1-9. Magnesium binding to ATP (left) and to the specific binding sites in proteins (right).

Accordingly, *under conditions of magnesium deficiency, the intracellular pool of hydrated Mg^{2+} and the pool of protein-bound Mg^{2+} will suffer most* thus impairing the biological activities of all of the Mg-binding proteins (and not so much interactions of Mg with ATP). Biochemical studies of the cells in culture and of the individual Mg^{2+}-binding proteins indicate that Mg^{2+} is required for the proper function of molecular cascades that are involved, in particular, in the following biochemical processes:

- Energy metabolism (in particular, glycolysis and oxidation of fatty acids)
- Electrolyte exchange
- Group B vitamins' metabolism
- Hydrolysis of ATP
- Protein synthesis
- Synthesis of the secondary messenger cAMP
- The synthesis of nitric oxide in the endothelial vessels

Magnesium is important for the proper function of all of the 12 major organ systems (figure 1-10), especially for muscular, skeletal, reproductive, digestive, urinary, neural and integumentary (connective tissue) systems. Evidence-based medicine indicates a wide spectrum of effects of magnesium on various aspects of human homeostasis (Box 1). Contrariwise, magnesium deficiency will adversely impact the functioning of all the organ systems, especially the nervous, reproductive systesm and the connective tissue. The connective tissue displasias appear as a long-term result of the magnesium deficiency and this will have an adverse impact on all the other organ systems. We consider adverse effects of magnesium deficiency on each of the organ systems in the Chapter 4. Here, let's see how a deficit of magnesium can impact just one organ system (the neural system), at the molecular level and at the level of clinical manifestations.

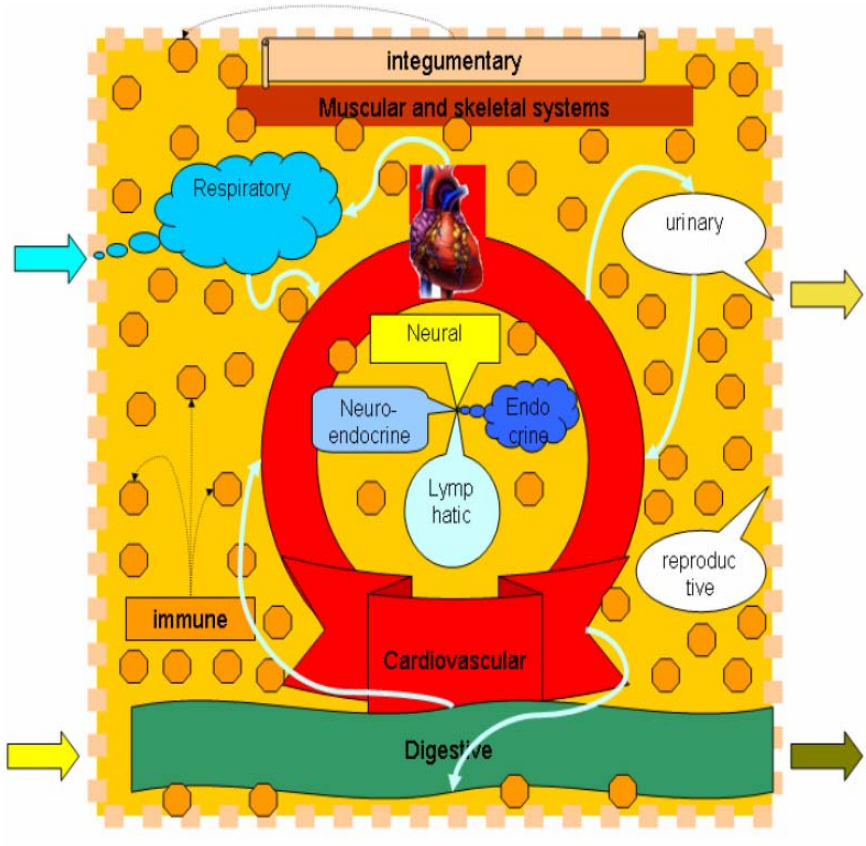


Figure 1-10. The organ systems of the body interlocked in homeostasis (Torshin, 2007).

Abnormalities in the function of the neural system are one of the early indications of magnesium deficiency. Deficiency of magnesium in the neural and muscular tissues results in seizures, dizziness, headaches, fatigue, fear and anxiety or, on the contrary, apathy and depression. One of the characteristic effects of the magnesium deficiency on the neural system also includes reduced ability to concentrate and somewhat impaired memory operation. Indeed, magnesium is a physiological regulator of excitability of the neural cells (it is essential for cell's depolarization), so a shortage of magnesium leads to hyperexcitability of the neurons.

The most likely molecular mechanism that links magnesium and neuronal excitability is inhibition of the activity of NMDA-receptors (glutamate receptors). Activation of NMDA receptors is essential for the rapid synaptic transmission in the brain which comes as a result of altering the flow of sodium/potassium through the membrane (Shiekhattar, 1992). Excessive stimulation of NMDA receptors could lead to epileptic-type convulsions. By blocking the NMDA receptors, magnesium lowers excitability of the neural pathways (figure 1-11). Model of the three-dimensional structure of the neurotransmitter-binding domain of NMDA receptor is shown in the figure 1-12.

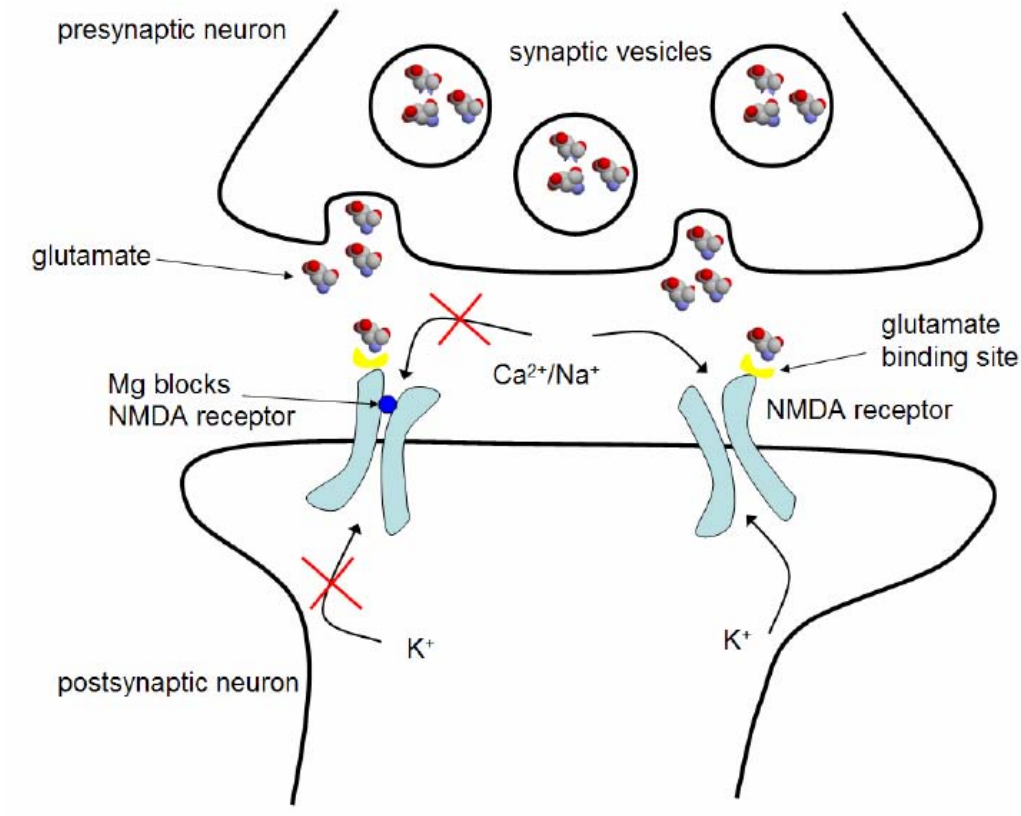


Figure 1-11. Blocking of NMDA receptors by magnesium lowers neuron excitability.

Box 1. The clinical effects of magnesium preparates

Each of the effect is annotated with levels of reliability as follows: A, high reliability, based on systematic reviews; B, moderate reliability, several independent randomized studies; C, limited reliability, based on the results for a few cases; D no evidence, only an opinion of experts.

Narcotic (intravenous, dose-dependent A)
Sedative (*per os*, dose-dependent A)
Analgesic (intravenous, dose-dependent A
Anticonvulsive (*per os*, intravenous, dose-dependent A)
Tonic (*per os*, dose-dependent B)
Spasmolytic (*per os*, intravenous, dose-dependent A)
Anti-hypertensive (*per os*, intravenous, dose-dependent A)
Anti-ischemic (*per os*, intravenous, dose-dependent B)
Antacid (*per os*, dose-dependent AB)
Bile normalizing (*per os*, dose-dependent AB)

Box 1. (Continued)

Laxative (*per os*, AB)
Duretic (*per os*, A)
Anti-arrhythmic (proven in patients with magnesium deficiency B)
Anticoagulant (inhibitory effect on platelet aggregation, AB)
Lipid-lowering (A)
Adaptation of the body to cold (D),
Anti-osteoporotic (AB)
Tocolytic (AB)

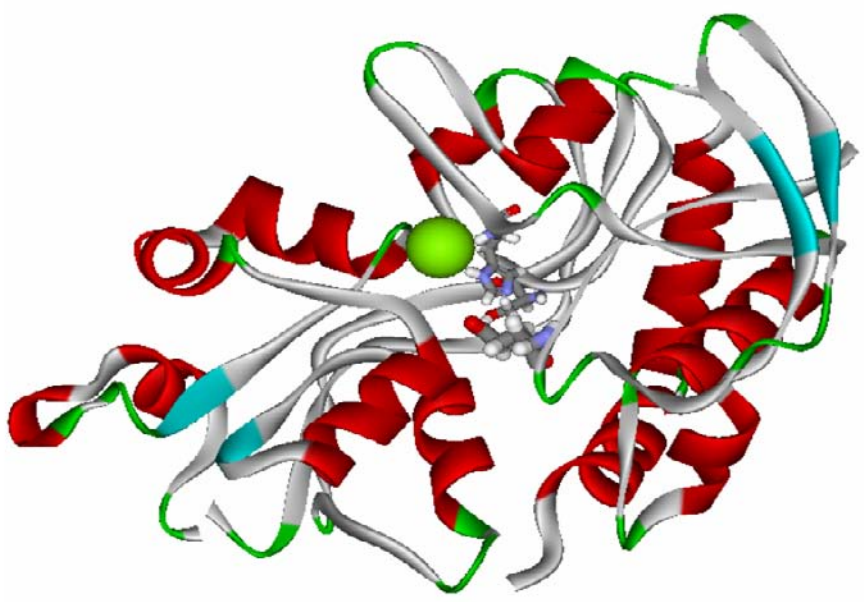


Figure 1-12. Model of the three-dimensional structure of the neurotransmitter-binding domain of the N-methyl-D-aspartate (NMDA) receptor, on the example of the receptor 2B, gene GRIN2B. Putative binding sites of the magnesium ion (sphere) and of the glutamate (wireframe model) are shown.

Magnesium deficit also affects the monoamine balance in the brain, catecholamines and serotonin before all (Kantak, 1988). Magnesium reduces secretion of corticoliberin and, accordingly, of cortisol, hence lowering activity of the hypothalamus-pituitary axis. This occurs largely through activation of the catechol-O-methyltransferase (COMT) which requires magnesium as an essential cofactor (figure 1-13).

The inverse correlation between the magnesium and the neuronal excitability (through the NMDA mechanism) as well as the inverse correlation between the magnesium and the levels of catecholamines (the COMT enzyme) are reflected in the clinical manifestations of the magnesium deficiency. For example, a deficit of magnesium is found in up to 70% of children with attention deficit hyperactive disorder (Gromova, Burtsev 1998; Gromova, 2005). ADHD – disease which implies an excessive excitation of the neural-muscular pathways.

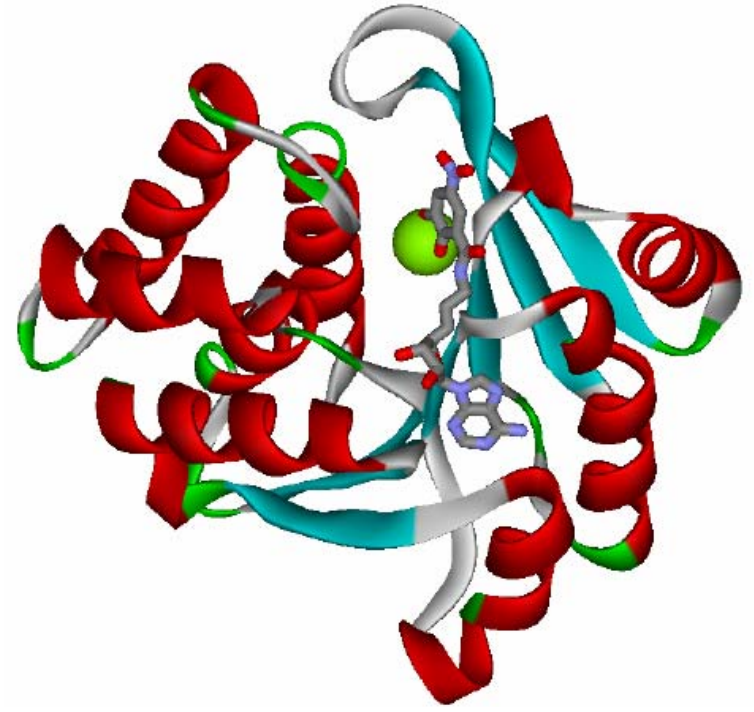


Figure 1-13. The spatial structure of catechol-O-methyltransferase. Magnesium ion (sphere) is shown along with the substrate analogue bound in the active site of the enzyme (PDB file 1JR4).

Mg deficit can also negatively affect the ability of adequate response to stress. The type "A" subjects (aggressive behavior) are more sensitive to stress and produce more catecholamines than other personality types (Henrotte, 1986). A magnesium deficiency will only aggravate the negative consequences produced by the stress.

The above example indicated the intrinsic relationship that exists between the magnesium status and the operation of the neural system at the molecular level. Magnesium status, which influences operation of the neural and other organ systems, depends on genetic factors as well as on factors of external environment. In general, the known *genetic (monogenic) disorders* that result in severe magnesium-wasting are relatively rare (1 in 50,000 in a population). However, nucleotide polymorphisms in the genes implicated in magnesium homeostasis (see Appendix IV) can moderately influence the levels of magnesium.The role of *environmental factors* such as food, water, stress, overpopulation, alcoholism, drug addiction and toxic elements (lead, nickel, aluminum, beryllium, *etc*) is considerably more important in the etiology of the magnesium deficiency.

Hypomagnesemia is also often detected in patients with diabetes type II, arterial hypertension, coronary heart disease, asthma bronchiale and diminished magnesium correlates with aggravation of these diseases. With age, the depth and frequency of Mg deficit usually increases and in populations after 70 years magnesium deficiency occurs in up to 80% of the surveyed. Both the genetic and the environmental factors contribute to absorption/elimination of magnesium as well as to the etiology of numerous human diseases (Chapter 4).

Thus, the normal levels of magnesium in the tissues of the body is one of the basic parameters that indirectly affects the human health. The abnormalities in the magnesium homeostasis have a prominent role in the etiology of various diseases and conditions affecting, before all, the neural, skeletal, integumental, digestive and reproductive organ systems. The evidence-based medicine proven a number of important pharmacological effects of the magnesium preparations (figure 1-14) which we consider in greater detail further in this book.

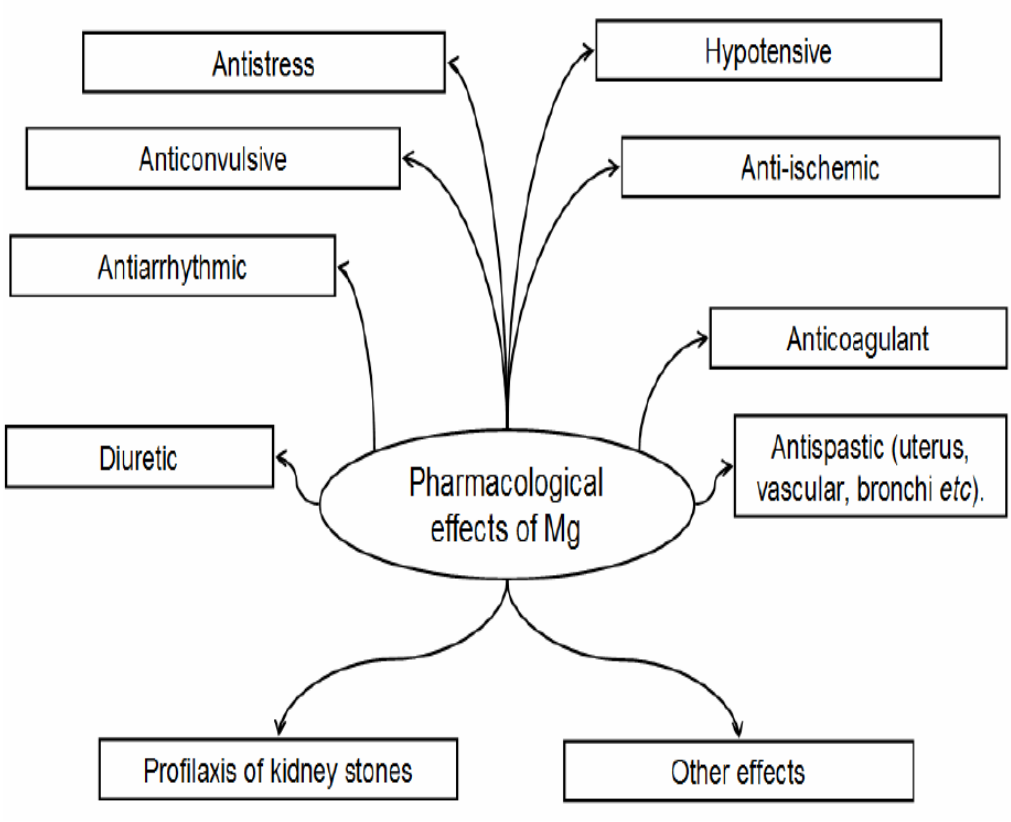


Figure 1-14. Pharmacological effects of the magnesium preparations.

Chapter 2

2. ABSORPTION, ELIMINATION AND THE DAILY REQUIREMENT OF MAGNESIUM

Absorption of magnesium in the small intestine involves only about 30% of the total Mg which arrives with food and the remaining 70% are excreted with feces. Parathyroid hormone, vitamin B6 (pyridoxine) and vitamin B1 increase absorption of magnesium in the digestive tract. Considerable proportion of the total magnesium that arrives in digestive tract is endogenous magnesium secreted by the intestine itself (278 mg), stomach (117-127 mg), liver (14-21mg), salivary glands (0.1-11 mg), pancreas (6-7 mg) *etc*. Normally, there is a balance between the processes of the absorption and elimination and this allows to maintain the magnesium quota of the organism (i.e., 24..28g).

Absorption of magnesium occurs mostly in the small intestine, especially duodenum (Aikava, 1971). A favorable effect on the absorption of magnesium has milk casein. On the contrary, a meal high in calcium reduces the absorption of magnesium; and excess of dietary phosphorus also inhibits absorption of magnesium and increases endogenous losses (Weisinger, 1998). Oxalic acid, tannin and phytates abundant in the strong tea form with magnesium insoluble complexes thus making it difficult to assimilate magnesium in the intestine.

Elimination of magnesium occurs mainly through the digestive tract. However, urinary system also plays its role: on average, 30% of magnesium which arrived with food is eliminated with urine. Kidneys are, apparently, the primary regulator maintaining magnesium levels in the body. A healthy person excretes with urine about 100 mg of magnesium per day. With the increased intake of magnesium with food or water, the excess cations are quickly disposed of through kidneys while with the depletion of magnesium excretion is reduced or terminated altogether. Loss of magnesium quickly progresses in patients with tubulopathies which violate the resorbtion of magnesium in the kidneys. Children with tubulopathy always display significant reduction of plasma levels of magnesium up to severe hypomagnesemia (Morger, 1999). It is important to remember that stress increases with loss of magnesium with urine: adrenaline and cortisone, elevated during stress, increase the elimination of magnesium through the kidneys.

It should also be remembered that a small amount of magnesium is eliminated with sweat (at the level of 1.5-2 mg per day). Normally, this value can be neglected. However, in the case of people who regularly attend Turkish bath or sauna, athletes, those living in too warm

climes, and those performing heavy physical labour, losses of magnesium can become quite noticeable and can reach 15% of the total intake. This requires adequate compensation through consumption of magnesium products, vitamin supplements, drinking water and/or special diets enriched in magnesium. Concentration of magnesium in the hair is ~1-10 mg per 100g and only a small amount of magnesium (about 7.5 micrograms per day) is lost with hair.

The magnesium requirements: Minerals (magnesium included) cannot be synthesized in the organism and have to be supplied from external sources. Daily physiological need for a magnesium in adults is ~400 mg/day, maximum 800 mg/day. These numbers are calculated on the base of 5 mg of magnesium requirement *per* kilogram of body weight *per* day. Some people are at a greater need for magnesium because of the significant losses:

a children (5-10 mg/kg daily);
b pregnant or nursing women (10-15 mg/kg/day);
c athletes (10-15 mg/kg/day);
d b) patients with magnesium deficiency (5-15 mg/kg/day).

Alas, the food standards commonly adopted in Europe, Russia, China and USA, do not provide sufficient magnesium to the body and this fact can, in part, explain the wide-spread prevalence of the deficiency of this element. Products rich in magnesium are often high caloric (nuts, chocolate, black, pulses, khalwa *etc*) and are excluded in the special diets aiming at weight loss. Information on the recommended daily requirements of magnesium in Europe & USA is given in the tables 2-1 and 2-2.

Table 2-1. Recommended daily allowances of magnesium (RDA were calculated for magnesium salts). After "PDR for Nutritional Supplements. Medical Economics", Thomson Healthcare, 2004

Age	Daily requirement	Age	Daily requirement
Infants		Males	
0 – 6 months	30 mg/day	19 – 30 years	400 mg/day
7 – 12 months	75 mg/day	31-50 years	420 mg/day
		51 – 70 years	420 mg/day
		> 70 years	420 mg/day
Children		Females	
1 – 3 года	80 mg/day	19 – 30 years	310 mg/day
4 – 8 years	130 mg/day	31-50 years	320 mg/day
		51 – 70 years	320 mg/day
		> 70 years	320 mg/day
Boys		Pregnant	
9 – 13 years	240 mg/day	14 – 18 years	400 mg/day
14 – 18 years	410 mg/day	19 – 30 years	350 mg/day
		31 – 50 years	380 mg/day
Girls		Lactating	
9 – 13 years	240 mg/day	14 – 18 years	360 mg/day
14 – 18 years	360 mg/day	19 – 30 years	310 mg/day
		31 – 50 years	320 mg/day

Note: The upper limit of additional daily consumption of magnesium: children 1-3 years old - 65 mg; 4-8 years - 110 mg; pregnant 14-50 years - 350 mg; lactation period - 350 mg.

Table 2-2. The daily allowances of Mg at a magnesium deficiency (Gilman, 2006)

Category	Age(years)	Mg, mMol
Children	0-0,5	1,67
	0,5-1	2,5
	1-3	3,33
	4-6	5
	7-10	7,08
Males	11-14	11,25
	15-18	16,67
	19-24	14,58
	25-50	14,58
	50+	11,67
Females	11-14	12,5
	15-18	11,67
	19-24	11,67
	25-50	11,67
	50+	11,67
Pregnant		13,33
Lactating		14,79

It should be stressed that the intake of the maximum daily doses of magnesium preparations implies one-time dosage or several such dosages in a short therapeutic course (provided regular clinical observation and laboratory monitoring of the level of magnesium in blood plasma, erythrocytes, urine). The usage of these maximum doses presupposes that the patient was diagnosed with magnesium deficiency and do not have oligoanuria, chronic renal insufficiency, thrombophilia or thrombocytopenia.

To facilitate the unit conversion of the data presented in the tables, we can use the following rules of a thumb: 1 mmol of magnesium corresponds to 280mg of magnesium pidolate (the solution for drinking, Magne-B6 preparation) and this corresponds to 24,3mg of magnesium ions. In the case of magnesium lactate, 1 mmol of magnesium corresponds to ~240mg of magnesium lactate and this covers the dose of ~24,1mg of Mg^{2+}.

Chapter 3

3. THE DEFICIENCY OF MAGNESIUM

When nutrition is balanced and sufficient, the body receives ~350 mg of magnesium (Mg^{2+}) a day. Deficit of magnesium in the modern food, combined with increased magnesium expenditure because of stress, does not allow to replenish the level of magnesium thus leading to magnesium deficiency. Magnesium deficiency is often accompanied by deficiencies of calcium, zinc, iodine and selenium.

3.1. ETIOLOGY OF MAGNESIUM DEFICIENCY

We briefly mentioned in the Chapter 1 the causes of the deficit of magnesium. One of the most complete classifications of the causes leading to magnesium deficiency is available in the monograph of Spasov AA (2000), the tables 3-1 and 3-2 provide a brief summary.

Table 3-1. Factors causing deficit of magnesium in the human body (Spasov, 2000)

<table>
<tr><th>The state of nutrition</th><th>The state of organism</th><th>Environmental Factors</th></tr>
<tr><td>1. Disorders of digestive system</td><td rowspan="3">1. Physiologic condition
-- Children age
-- Pregnancy
-- Lactation
-- State of health</td><td rowspan="3">Stressful factors
-- Temperature (too high or too low)
-- Intense rhythm of life
-- Traumas and injuries
-- Emotional stress</td></tr>
<tr><td>2. The fiber content of the food products</td></tr>
<tr><td>3. Regular use of food concentrates</td></tr>
<tr><td rowspan="3">4. Substances that impede absorption
-- Vitamin D (natural sources, food additives)
-- Ca, P imbalance (surplus or the lack of)
-- Imbalance of Na, K, Cl
-- Imbalance in trace elements
-- Lack of vitamins B1, B6,
-- Excess intake of fats
-- Excess of carbohydrates
-- Imbalance in protein intake
-- Excess of phytanic acids</td><td>2. Genetic factors affecting
-- Absorption and excretion,
-- Homeostasis
-- Kidney function</td><td rowspan="2">2. Infection</td></tr>
<tr><td>3. Gender differences (women are more likely to have magnesium deficiency)</td></tr>
<tr><td>4. Hormonal status
-- Parathormone, calcitonin
-- Catecholamines
-- Corticoids</td><td>3. Medication (aminoglicosides, pentamidine, cisplatin, etc.)</td></tr>
</table>

Table 3.1. (Continued).

The state of nutrition	The state of organism	Environmental Factors
	5. Individual psychology: excessive emotionality (the influence of catecholamines and corticoids)	4. Surgical intervention
	6. Mental and physical activity (the influence of catecholamines)	
5. Diets (liquid diet, protein diet, parenteral nutrition products)	7. Diseases: alcoholism, IHD	5. Starvation

In clinical practice, to ease the detailed analysis of the patient's anamnesis, it is more convenient to group the particular causes leading to the formation of magnesium deficiency, as it is done in the table 3-2.

Table 3-2. The causes of magnesium deficiency

Group	Particular cause
Lowered consumption	1. Reduction of the magnesium content in "civilized" food 2. Special dieting courses 3. Alcoholism 4. Parenteral nutrition
Reduced enteric adsorption	5. Diarrhea 6. Maladsoprtion syndrome 7. Inflammatory enteropathias 8. Condition after bowel resection 9. The high consumption of calcium 10. A protein-rich meal 11. High levels of fat in food 12. Alcohol intake 13. Frequent consumption of coffee (caffeine-containing drinks)
Increased demand	14. Pregnancy and lactation 15. Increased sweating 16. Childhood (period of intense growth) 17. The period of recovery 18. Chronic stress
Increased elimination	19. Vomiting 20. Prolonged diarrhea 21. Laxative abuse 22. Kidney diseases 23. Renal salt loss and kidney acidosis 24. Chronic alcoholism 25. Diabetes 26. Therapy with diuretics 27. Anti-tumor or anti-autoimmunity drugs (cyclosporin, cisplatin) 28. Estrogen-based drugs or contraceptives
Endocrine dysfunction	29. Hyperthyroidism 30. Hyperparathyroidism 31. Hyperaldosteronism

3.2. PRIMARY AND SECONDARY MAGNESIUM DEFICIENCY

The primary (or constitutional) magnesium deficiency is the most common. In 2/3 of the cases, it involves individuals who demonstrate obvious clinical manifestations of the magnesium deficit but seem to have magnesium in blood plasma within the normal range or marginally low. However, normal momentary levels of magnesium in plasma and in erythrocytes do not exclude possibility of magnesium deficiency since the seemingly normal concentration of the magnesium in the plasma can be the result of depletion of the magnesium depot in bones and other tissues. In such a case, the magnesium load test is necessary to ascertain the diagnosis (Chapter 9).

The primary magnesium deficiency is linked to inborn hyperactivity of the transmembrane exchange of Mg^{2+} characteristic for patients with congenital tubulopathy or with hereditary abnormalities of the inverse resorption of magnesium, calcium and phosphorus in the kidney. People with a genetic deficiency that affects magnesium homeostasis are in lifetime need of nutritional and pharmacological support. The genetic causes can include the rare genetic defects in the genes involved in magnesium homeostasis (TRPM7/TRPM6 *etc*, Appendix IV). A significant reduction of the erythrocyte magnesium occurs, for instance, in some cases of autoimmunity, such is the case of carriers of the allele bw35 of the HLA gene as well as other alleles (Durlach, 1989).

A typical form of primary magnesium deficiency was described under various names: essential Mg-deficiency, spasmophilia, constitutional tetanus. It occurs as a result of a chronic magnesium deficiency. Calcium-based therapies can strengthen clinical manifestations of spasmophilia and convulsions. Treatment of spasmophilia consists in entering an extended course of magnesium preparations. At a considerable hypocalciemia, therapy might begin with magnesium-only preparations during 1-2 weeks, followed by gradual introduction of calcium preparations with the proportion of Ca:Mg equal 2:1 (eg 800mg of calcium salts and 400 mg of magnesium salts).

The secondary magnesium deficiency occurs when there the balance of adsorption-elimination is shifted towards elimination because of some disease or condition. Insufficient replenishment of the eliminated magnesium results in magnesium deficiency. The mechanism of such a condition is, thus, not only insufficient intake of magnesium but also abnormalities in magnesium adsorption and an increase of the magnesium loss with urine. A lack of magnesium in the body can occur as a result of disease that affects gastrointestinal tract (for example, patients with bowel truncations) which disturbs absorption of magnesium in intestines through generally poor absorption of food, chronic diarrhea, and dysbiosis. Other causes of the secondary magnesium deficiency may be medications, intoxications, usage of calcium or phosphates in large doses, chronic alcoholism, diabetes *etc*. Secondary magnesium deficit might also arise if there is a abnormality of regulation of ion metabolism and is characteristic for endocrine disorders (hypercalcemia, hypoparathyroidism, hypethyreosis, hyperaldosteronism, *etc*). The state of chronic stress also leads to depletion of Mg^{2+}, because stress results in active removal of magnesium ions from the cells.

In cases, when the main cause of magnesium deficiency is established, it might suffice to remove the cause (for example, if excess of diuretic drugs was the cause, then it is enough for the patient to cancel diuretics). In the case of healthy people, the only cause of magnesium

deficiency is unbalanced diet (except pregnant women who are at a greater biological need of magnesium). A magnesium deficiency that arose merely as a result of insufficient intake of magnesium with food products can be relatively quickly restored with the help of a healthy diet which meets the needs of the body.

3.3. Clinical Symptoms of Magnesium Deficiency

Clinical signs of magnesium deficiency are very diverse and include:

- cardiovascular abnormalities: tachycardia, hot flashes, hypertension or hypotension;
- respiratory disorders: faster breathing rhythm, a sense of suffocation (mostly under stress);
- digestive abnormalities: diarrhea, constipation (irritated colon), abdominal pain, the feeling of "lump in the throat";
- hemolytic anemia;
- hyperactivity: patient can not stay long in one place, constantly moves, even in sleep;
- chronic anxiety irritability, nervousness, a reduced ability to focus and impaired functioning of memory;
- abnormalities with sensitivity of the skin, paresthesia (sensation of tingling, pricking, or numbness of a person's skin) which comes from overexcitement of the sensitive nerve endings
- teetering during walk, dizziness;
- spasmodic seizures of muscles and, in particular, of the leg muscles;
- urination disorders: frequent urination (pollakiuria), pain in the bladder (cistalgia);
- pain in the back/the lumbar;
- lowered body temperature;
- nystagmus;
- hair loss, fragile nails.

The deficit of magnesium can also lead to increased intracranial pressure, diskinesia of the bile ducts, heart disease, vascular spasms, immunodeficiency, nephropathy, and anemia. It is possible to group the clinical signs of magnesium deficiency according to the most frequently observed phenotypes (as in the table 3-3). However, it should be remembered that a patient may manifest a combination of "phenes" from different phenotype groups which corresponds to an individual clinical portrait of the magnesium deficiency.

More detailed descriptions of diagnosis and of the clinical symptoms of magnesium deficiency are presented below. For the most parts, the symptoms are those reflecting an increased neuromuscular excitability coupled with vegetative dysfunction. The increase in neuromuscular excitability is reflected in shakes, twitching, knee jerk reflex, and Chvostek sign/test.

Table 3-3. Clinical manifestations of magnesium deficiency (Spasov, 2000; Gromova, Rebrov, 2005)

Phenotype	Symptoms
Cardiovascular	Pain in the heart region, arrhythmia, tachycardia, hypertension, hypotonia, myocardial ischemia
Cerebral	Headaches, dizziness, abnormal cerebral blood flow, abnormalities of memory operation, sense of fear, depression, irritability, sleep disorders
Muscular (tetanus)	Tingling, pricking, muscle cramps, a deficiency in the sense of touch, muscle weakness, tremor, pollakiuriya
Visceral	Poured pain, abdomen spasms, nausea, vomiting, constipation, pollakiuriya, successive diarrhea, bronchial spasms
Metabolic	Lowered body temperature, an increase of the interval in daily temperature range, feeling of cold, low tolerance to cold, the propensity to edema, hyperaldosteronism, decreased tolerance, alcohol, nicotine, drugs, thickening bile, gall bladder stones, kidney stones, abnormality of connective tissue formation, cystosteopenia, osteoarthrosis, osteochondrosis, calcification of the Mg-deficient tissues (atherosclerotic plaques, plots placenta, etc.), post-stress poliuriya, the accumulation of toxic metals (V, Pb, Cd, Al, Ni and others)

Generally, we can also subdivide the signs of magnesium deficiency into subjective and objective:

Subjective symptoms (complaints reflecting patient's perception of the quality of life)

- Living in a state of stress
- Increased mental fatigue
- Increased physical fatigue
- Persistent "bad mood"
- Chronic anxiety
- Headaches
- Spasmophilia (lump in the throat)
- Pains in the lumbar
- Pains in the abdomen

Objective symptoms (doctor's observations and tests)

- Muscle cramps
- Irritability

- Apathy
- Twitching of individual muscles (in particular, facial muscles)
- Movements of fingers reflecting tetanus
- Augmented knee reflex
- Trousseau symptom
- Chvostek test
- "Obstetrician's hand" test (tetanus contracture after blocking shoulder with tourniquet)

Shakes (tremors) represent a kind of hyperkinesia which is characterized by involuntary, excessive and violent movements of the muscles which impede implementation of the deliberate motions. In the case of neuroses and magnesium deficiency, hyperkinesias are most often observed in the fingers, has small amplitude and changing rhythm. Often, the tremors manifestations show considerable variability throughout the day. They become more apparent after physical exercise, physical training, even a slight hypothermia, emotional stress, or after intake of even low dose of alcohol or strong coffee.

Tic (twitching) is inadvertent, rapid contraction of muscles (most often, of the circular muscles around the eyes or of the muscle around the mouth that causes twitching of the corners of the mouth). Neurotic tics are different from organic in terms of their volatility and lack of a stereotype in manifestation.

Knee-jerk reflex test is one of the most wide-spread methods to assess the neural excitability of the patient. Too acute reaction during the test suggests abnormally high excitability of the neural arc and is one of the indirect indications of the magnesium deficiency. The test consists in an accurate observation of extension of the knee joint upon striking the quadriceps tendon directly with the reflex hammer. There are several ways to study the knee reflex. In the sitting position, the patient places the lower limbs in such a way that the shin would hang freely with legs bent at an obtuse angle. In lying position of the patient, the doctor brings left arm under the knee joints of the subject extends the legs also at an obtuse angle. Knee reflexes of some healthy people can be somewhat inhibited. To facilitate a genuine knee jerk, doctor might consider diverting patient's attention: by asking some questions, asking to count (silently), to take a deep breath *etc*.

Chvostek test can be considered as one of the signs of tetanus seen in hypocalcemia and hypomagnesemia. It can be observed by light tapping with finger or the reflex hammer of the stem of the facial nerve in front of the external auditory passage, 1.5-2 cm below the zygomatic arch. Alternatively, the test is performed by tapping with finger the bifurcation of the facial nerve (the middle of the line connecting corner of the mouth and tragus of the ear (as shown in the figure 3-1). During the test, all of the muscles innervated by the facial nerve can contract (Chvostek symptom I), muscles of the nose and corner of the mouth (Chvostek symptom II) or only muscles at the corner of the mouth (Chvostek symptom III). Despite apparent and considerable non-specificity of the Chvostek test, it is positive in 4 out of 5 patients with a deficit of magnesium.

In addition to the symptoms and tests dealing with neuromuscular excitability, it is important to assess the vegetative background of the patient. Following is a questionnaire we normally use to identify the signs of abnormal vegetative changes.

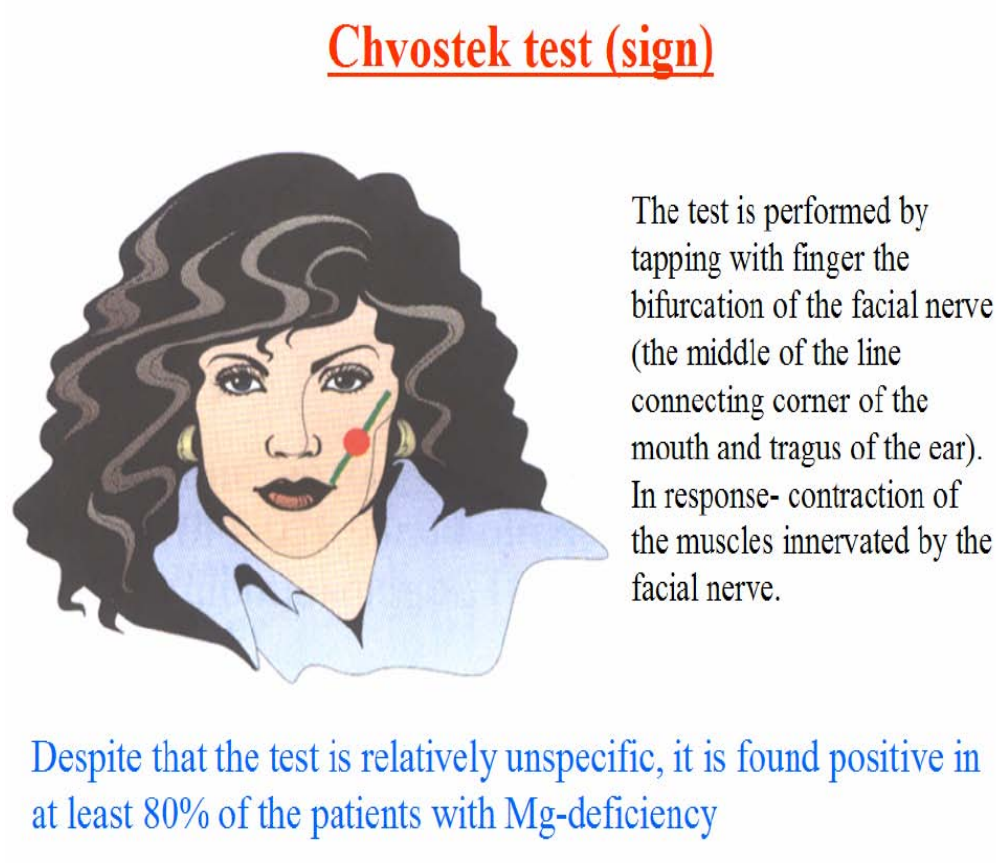


Figure 3-1. Chvostek sign.

Questionnaire to identify signs of vegetative dysfunction (after Wein, 2003)

Patient's name_____ Date _____

1. When agitated, do you have a propensity to:

A) redding of the face? Yes 3 No

B) pale face? Yes 3 No

2. Do you observe numbness or subjective feeling of cold

A) of the fingers/toes? Yes 3 No

B) of the entire palms/feet? Yes 4 No

3. Do observe changes in the color of your skin (pale, red, bluish)

A) of the fingers/toes? Yes 5 No

B) of the entire palms/feet? Yes 5 No

4. Do you sweat too much? Yes 4 No

If "Yes" - underline: constantly or when agitated

5. Do you often feel palpitations, "freezes", or "stops" of the heart? Yes 7 No

6. Do you often feel trouble breathing: sense of shortage of the air, too frequent breath? Yes No 7

7. Do you have regular problems with gastrointestinal tract (constipation, diarrhea, abdominal pains)?

Yes 7 No

8. Do you have a tendency to faint (i.e., sudden loss of consciousness or a feeling that you can lose it?) Yes 7 No

If "Yes", underline the relevant: stuffy room, excitement, prolonged stay in an upright position.

9. Do you have headaches? Yes 7 No

If "Yes", underline the relevant: diffuse, "whole head" aches; squeezing pain; only half of the head

10. Did you observe recently a decline in your efficiency or a rapid fatigue? Yes 5 No

11. Do you have sleep disturbances? Yes 5 No

If "yes" then specify: A) difficulty in getting asleep, B) shallow sleep in) a feeling of insufficient sleeping or fatigue when awakening

Doctor's key: Sum the points for each answer. In the case of 15 or more points, syndrome of vegetative dysfunction is a likely diagnosis.

3.4. CONSEQUENCES OF MAGNESIUM DEFICIENCY

There are immediate and long-term consequences of the magnesium deficiency. The *immediate consequences* are largely reflected in the higher excitability of the neuromuscular system because of overactive NMDA receptors and increased levels of catecholamine neurotransmitters (Chapter I). At magnesium deficiency, muscle cells suffer disruption of the normal depolarization pattern which leads to an excess of muscular contraction in comparison to relaxation. Clinically, this manifests as muscle twitching and convulsions, especially in the case of the gastrocnemius (calf muscle). In the case of cardiomyocytes, this manifests as low efficiency of diastole, in the case of the smooth muscle - as spastic processes (sphincter spasm of the gall bladder, bowel cramps, bronchial spasms *etc*). In the case of the nerve cells, abnormal depolarization interferes with the conductance of the nerve impulse so magnesium deficiency results in a redundant nerve signaling and a lack of the rest phase in the electric signal transmission. In this sense, magnesium can figuratively be compared with electric insulation material while calcium, sodium and potassium would comprise the wire itself. Clinically, this "lack of insulation" will manifest as convulsions, persistent state of overexcitement, sleep disorders, arrhythmia, apnea (suspension of external breathing).

Eclampsia is a serious complication of pregnancy characterised by convulsions and pregnancy-induced or pre-existing hypertension. Etiology of eclampsia includes subclinical or clinical forms of the magnesium deficiency. Eclampsia can be treated with magnesium sulfate although there are definite problems of using this and other inorganic salts due to their extremely low bioavailability. During eclampsia, the levels of magnesium can drop several times below the norm.

The *long-term consequences* of magnesium deficiency include formation of the metabolic and connective tissue abnormalities. Metabolic abnormalities include formation of pathological compartmentalization of the trace elements in various organs, tissues and biological fluids under the influence of the lack of magnesium in those biological substrates. For example, tissues that were hypomagnemic for years accumulate excess of calcium present as insoluble forms. The precise mechanisms for this calcification process are not known and can include, in particular, slower calcium exchange caused by the magnesium deficiency and abnormally high blood coagulation. The process of calcification involves calcification of the joints, bones and other forms of connective tissue, atherosclerotic plaques in aorta and other vascular locations (which is worsened when magnesium deficiency is combined with a deficit of pyridoxine), formation of the "stones" in bile, kidney and bladder (also potentiated by pyridoxine deficiency).

The long-term effects of magnesium deficiency also include accumulation of toxic trace elements (Ni, Pb, Cd, Be, Al), development of arterial hypertension, cardiovascular pathologies, increased risk of myocardial infarction, stroke, diabetes and of certain cancers. In children, magnesium and pyridoxine deficiency stimulates development of autism, dyslexia, deviant forms of behavior, attention deficit syndrome with hyperactivity. Adolescents with a long-term magnesium deficiency are at an increased risk of formation of the early cerebrovascular pathology (Andreev, 2001).

In the process of intensive growth of embryos, infants, children and adolescents lack of magnesium leads to the formation of insufficiency of the connective tissue. We systematically analyzed the molecular mechanisms of the formation of the connective tissue insufficiency

(undifferentiated dysplasia) elsewhere (Torshin, Gromova, 2008). The connective tissue insufficiency manifests as formation of the defects of the mitral valve, of the joints, hyperelasticity of the skin *etc*. During the period of rapid growth, adolescents with insufficiency of the connective tissue might lead to overstretched tendon in girls (thighs, chest and abdomen) and in boys (sides, lower third of the back). During pregnancy, magnesium deficit can provoke overstretching of the chest and abdomen because of the rapid growth of the breast and the increase in the uterine mass. During childbirth, women with a deficit of magnesium are characterized by a higher frequency of the tears of perineum (Kosheleva, 2001, 2005; Uvarova, 2006). Molecular mechanisms linking the magnesium deficiency with the structure of the connective tissue were thoroughly analyzed in our paper (Torshin, Gromova, 2008) and are briefly summarized in the following chapter.

Chapter 4

4. CONDITIONS AND DISEASES ACCOMPANIED BY MAGNESIUM DEFICIENCY

With a few exceptions (chronic hunger or congenital abnormality of the magnesium homeostasis), magnesium deficiency represents a satellite diagnosis that accompanies a considerable number of diseases. Chronic shortages and, therefore, an increased demand for magnesium are typical for diseases and conditions such as:

- Injuries,
- Infections
- Magnesium maladsorption in intestines (because of vomiting, diarrhoea, intestinal parasites or bowel tumor);
- Renal failure;
- Hyperpaathyreosis
- Hyperthyreosis
- Hypercalciemia
- Primary aldosteronism;
- Arrhythmia;
- Hypertension;
- Myocardial infarction;
- Stroke;
- Atherosclerosis;
- Diabetic acidosis;
- Dementias;
- Neuroses;
- Epilepsy and convulsive states;
- Brain dysfunction in children and adolescents (especially in combination with hyperactivity);
- Autism;
- Dependency diseases (alcoholism, drug addiction, nicotine dependence);
- Premature delivery;
- Prolonged lactation;
- Spontaneous abortion (especially in 2^{nd} and 3^{rd} trimesters);

- Premenstrual syndrome;
- Osteoporosis and bone fractures;
- Deficit of vitamin D (rickets in children, osteomalacia);
- Acute and chronic pancreatitis;
- Hereditary hypophosphatemia;
- Kidney stones;
- Prolonged course of diuretics;
- Bronchial asthma;
- Immune deficiency;
- Toxic accumulation of lead, aluminium, nickel, beryllium, cadmium;
- Treatment with cytostatics, immune suppressors, cyclosporin (which prevents normal resoprtion of magnesium in glomerules);

Once again, all of these conditions and diseases are characterized by clear component of the magnesium deficiency. It is not always clear whether the magnesium deficiency is the cause of the disease or simply one of the consequences. Often, however, magnesium deficiency does not simply accompany the disease but either was one of the factors that provoked the disease or, at least, one of the factors that affected the course and severity of the disease. In the latter case, magnesium deficiency can lead, for instance, to transition of a disease into its chronic stage.

The concept of “magnesium deficiency” includes both (1) lowering of the overall content of magnesium in the body and (2) the clinical manifestations in the form of the symptoms. The term "magnesium deficiency" has to be distinguished from the term “hypomagnemia” (“hypomagnesemia”) which implies a significant reduction in the serum concentrations of magnesium (normal levels are held to be 0.75 - 1.26 mmol/L). In pregnant, the lower limit is slightly higher (0.8 mmol/L) and sensitivity of the pregnant even to borderline hypomagnesemia dramatically increases.

The effects of magnesium deficiency are related, in most of the cases, to increased neuromuscular excitability, impaired energy metabolism and worsened mechanical and structural properties of the human tissues. However, as show the results of the systematic analyses of molecular function, presented further in this chapter, the impact of magnesium deficiency at the molecular level can be much more diverse and include also influence on the DNA repair, apoptosis and cell proliferation.

Anyway, increased neuromuscular excitability results from disturbed depolarization and leads to such patient-specific manifestations such as oversensitivity of the nerve cells partially reflected by hysteria, irritability, irritability, disturbing, oppression, sleep disturbances. Magnesium participates in the energy metabolism as cofactor of the metabolic enzymes (enolases, fatty-acid synthases) and also in the form of ATP-Mg complexes. The magnesium deficiency in this case is reflected primarily in the form of increased fatigue (mental and physical) under normal conditions and by inadequate heat transfer. The magnesium-related abnormalities are also related to the role of the cation in synaptic neurotransmission (NMDA and other receptors) and neuromediator metabolism (catecholamines, tyrosine, dopamine, norepinephrine, serotonin, gamma-aminobutyric acid) which manifest as depression, loss of the coordination of movements, impaired attention and memory, increased stress, epilepsy, autism, all kinds of phobias and manias. In particular, poor response to stress on the background of magnesium deficiency, aggravated by irrational diet and chronic stress, is

likely to be one of the major causes for the development of so-called “diseases of civilization” (IHD, hypertension, diabetes *etc*).

Neglecting this secondary diagnosis (ICD-10 “magnesium deficiency E61.3”) in the case of the common primary diseases can result in many side effects of these diseases which will seriously complicate the treatment and recovery. *Omission of treating magnesium deficiency can lead to "inexplicable" ineffectiveness of treatments and the drugs used in therapy of the main disease,* lengthening of the time to recovery or shortening of the time before remission. It might not be possible to help a patient if hypomagnesemia is neglected and only the primary disease is allegedly “treated”.

In the following sections of this chapter, we will consider the detrimental effects magnesium deficiency exerts upon reproductive system (pregnancy, premenstrual syndrome), neural system, cardiovascular system (atherosclerosis, hypertension, coagulation), urinary system, endocrine system (diabetes), the integumentary system/connective tissue (pathologies of the bone and cartilage), respiratory system (bronchial obstruction) and immune system. Finally, we consider relation of magnesium to oncology, professional pathologies and the role of magnesium in sports medicine.

4. 1. MAGNESIUM AND PREGNANCY

Pregnancy and lactation are very special physiological states which can become a serious test of the woman’s health when aggravated by an inadequate and substandard nutrition. The deficits of micronutrients (before all, iron, magnesium, calcium, vitamins C and B) during pregnancy are likely to cause a compensatory reaction which results in higher volumes of food consumed and, therefore, leads to an excess of weight that negatively affects pregnancy. A balanced homeostasis of the micro- and macro- elements in the body is a prerequisite for normal function of the female reproductive system including regular menstrual cycle, the ability to ovulate, to conceive and to maintain pregnancy. The elements which have the most important influence on female reproductive system are iron, calcium, magnesium, selenium, and copper (Yamada, 1998; TEMA-12, 2005). Given that before conception only very few women are interested at all in the vitamins and the minerals needed for the organism, mineral deficiencies in pregnant are quite frequent and the magnesium deficiency is no exception.

During pregnancy, magnesium deficiency contributes to the development of arrhythmia, gestosis (pre-eclampsia), miscarriages and premature births. A pregnant woman is naturally predisposed to magnesium deficiency as the fetus needs magnesium for the tissue growth and it is the mother’s body that should supply this and the other minerals. Moreover, the growing fetus is not the only cause of the increased demand of magnesium (see Box 2). The daily magnesium requirement for pregnant grows 1.5-2 times and makes 500-700 mg per day.

The effects of magnesium deficiency are diverse and relate to both to the mother and to the fetus. The deficit of magnesium during pregnancy is very dangerous and may be accompanied by a slowdown of the fetal growth which results in underweight births, premature births, eclampsia and, in some cases, miscarriage (figure 4-1). The most common clinical manifestation of magnesium deficiency in pregnancy is pathologically elevated muscular tone of the uterus (Box 3).

Box 2. Increased demand for magnesium in pregnancy

The increased demand of magnesium arises not only because of the fetal growth, but also because of the following changes in the female body:

-- Increase of the mass of the uterus from 100 to 1000 grams
-- Placental growth
-- Increase of the total mass of blood (number of erythrocytes) by 20-30%
-- Increase of the mammary gland
-- High levels of estrogen (which stimulate elimination of magnesium)
-- Raising aldosteron
-- Intake of antacid preparations which often inhibit magnesium adsoprtion

The increased demand for magnesium has to be compensated by adequate supply

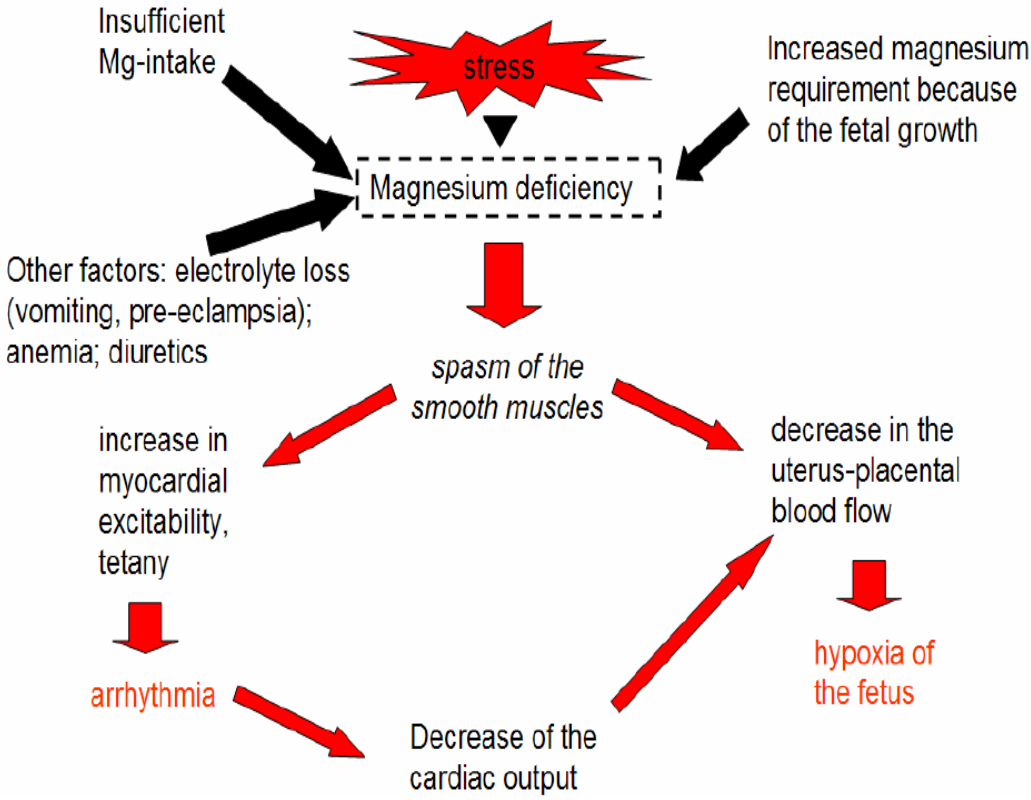


Figure 4-1. The magnesium deficit and arrhythmias in pregnant.

Box 3. Clinical consequences of hypomagnesemia in pregnant (after Kosheleva, 2006)

Elevated muscular tone of the uterus;
Hypovitaminosis D;
Pains in the back, the lumbar, and the pelvis;
Muscular spasms, most often of quadriceps;
Placental insufficiency;
Hypotrophy of the fetus;
Antiphospholipid syndrome (recurrent thrombosis);
The higher frequency of gestosis (pre-eclampsia);
Eclampsia;
Premature labor;
Reduced intensity of the uterus contractions in labor;
Insufficient cervix opening during childbirth;
The tendency towards miscarriages throughout pregnancy

Eclampsia is one of the most dreadful complications of pregnancy which corresponds to peak of magnesium deficiency. Fatality rate is 10% and each year 50 thousand women worldwide die from eclampsia. Diagnosis of eclampsia necessitates, in accordance with the WHO regulations, urgent treatment with magnesium preparations. Development of eclampsia is often preceded by gestosis (pre-eclampsia). Pre-eclampsia is characterized by levels of magnesium <0.72-0.75 mmol/L. The clinical features of the magnesium deficiency in gestosis (pre-eclampsia) include:

- Increase in blood pressure, proteinuria, convulsions;
- Pathologically elevated muscular tone of the uterus;
- Pains in the back, lumbar and pelvis;
- Tendency for swellings due to the imbalance Na/K, Na/Mg and Mg/Ca (because of the strengthening of aldosterone influences and also because of the increased retention of Na and water in the body)
- Premature births or miscarriages;
- Often combined with a deficit pyridoxine.

As indicated meta-analysis of 5 placebo-controlled studies of pregnant with distinct magnesium deficiency, usage of the magnesium preparations (magnesium citrate/lactate in 150 mg quantity, 2 times a day) starting with 4-5 weeks of pregnancy leads to a significant reduction in the rate of spontaneous miscarriages (Young, 2002). Our recent reviews (Gromova, Serov, 2008; Gromova, Gogoleva, 2008) presented a wealth of the data that illustrate the link between magnesium deficiency and the development of the states that complicate pregnancy. Using modern magnesium products for oral intake (such as Magne B6) is particularly important during pregnancy because of the high bioavailability of magnesium in these products, their high efficiency for prophylaxis and therapy as well as almost entirely absent side effects (Young, 2002). On the contrary, widely used magnesium

sulphate treatment is associated with high risk of complications and cannot be recommended except as a last resort (Gromova, Serov, Torshin, 2008).

Magnesium is a very important component in a balanced nutritive support to avoid pre-eclampsia as well as other forms of gestosis. In particular, magnesium is expremely important for the placentation process (Mozgovaya, 2007). In combination with vitamin B6, magnesium is better absorbed in gastro-intestinal tract, promotes natural and safe neutralization of the harmful effects of homocysteine and also has antioxidant effects. A study of 120 married women with a history of two or more spontaneous abortions (Tetruashvili, 2007) indicated that application of MagneB6 enabled the vast majority of patients to avoid tocolytic therapy with magnesium sulfate. Application of the drug resulted in a rapid normalization of tone the uterus in 83% of the cases and normalization of sleep, decreased anxiety in 79% of the cases. An integrated clinical and laboratory survey of 216 pregnant women with a history of spontaneous abortions (Strizhakov, 2008) indicated that complex therapy course that involved the use of MagneB6 corresponded to 1.8 times lower frequency of spontaneous miscarriages in pregnant women with recurrent spontaneous abortion.

4.1.1. Hypomagnesemia and condition of the fetus

Hypomagnesemia leads to hypotrophy of the fetus, underweight birth and a number of other clinical manifestations related to the fetus (Box 4). At least the three basic physiological mechanisms are involved in this process:

- lack of magnesium transmission from mother to fetus through the placenta;
- drastic reduction in the volume of plasma circulating between the fetus and placenta due to vasoconstriction which diminishes metabolic rate of the fetus,
- placental dysfunction accompanied by placental aging/calcification.

As we can see, all of these processes involve placenta- this unique organ through which the fetus protected and nurtured.

Box 4. Clinical features of hypomagnesemia of the fetus and the related molecular mechanisms

Chromosomal abnormalities (DNA repair is affected)
Defects in embryonic development (impaired connective tissue metabolism)
Fetal anemia (worsened blood circulation)
An increased risk of severe asphyxia (impaired energy metabolism)
Slower development of the fetus (vasoconstriction and reduced circulation)
Fetal edema (inflammation, sodium retention)
Premature birth (neuromuscular excitability)
An increased risk of intrauterine infection (immune system weakened)

From molecular point of view, the influence of magnesium on the placental function is many-sided. Our recent analysis of the human proteome indicated that at least ~100 out of 500 known Mg-binding proteins were found in significant levels in human placenta and are

important for the function of the placenta (Torshin, Gromova, 2008). Analysis indicated that magnesium-dependent proteins of placenta rarely comprise a singular molecular cascade (for example, apoptosis cascade, glycolysis *etc*) which makes it difficult to describe the effects of the Mg deficiency in terms of particular molecular cascades. Rather, Mg-dependent placental proteins belong to many different molecular cascades and the function of all of them is impaired when there is a deficiency of magnesium. We summarized the physiological effects of magnesium on various molecular cascades in the following diagram (figure 4-2). The three major ways in which activity of the Mg-dependent placental proteins affects physiology are 1. exchange of energy and substance in placenta; 2. condition of specific organ systems (muscular, immune, connective tissue) and, on the cellular level, 3. cell proliferation and apoptosis.

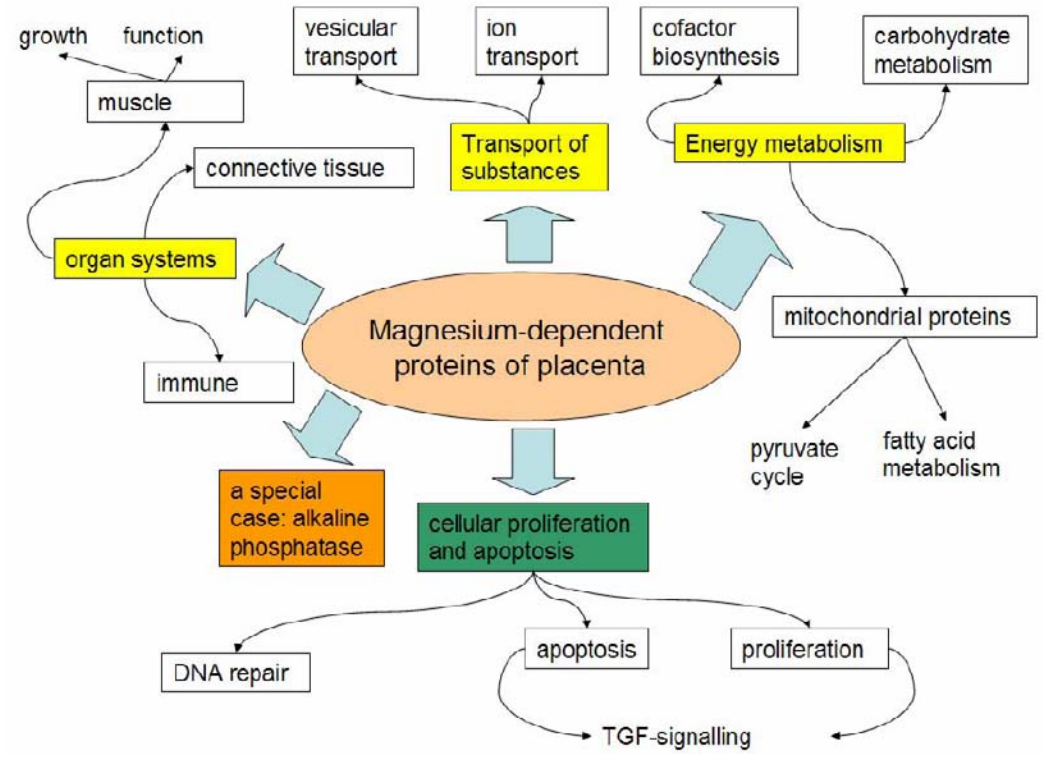


Figure 4-2. Molecular functions of the placental Mg-dependent proteins.

The less intense is *transfer of energy and substance* to the growing fetus through the placenta, the less intense will be the process of the fetal growth and the more likely will fetal malnutrition and underdevelopment. "Energy metabolism" is a broad notion that includes anabolic and catabolic processes of proteins, fats and carbohydrates, which ultimately lead to the accumulation of the cellular ATP, this universal molecule of the energy transfer in biological systems.

Mg-deficiency has a negative impact on the functioning of the placental proteins which support energy metabolism and require magnesium as a cofactor. These Mg-dependent proteins are involved in the synthesis of important coenzymes in metabolism of carbohydrates (in particular, glycolysis), and in the mitochondrial metabolism of pyruvate and of the fatty

acids. Reduced activity of the magnesium-dependent enzymes (most of all, glycolytic enzymes) is the most likely explanation for the formation of insulin resistance and of placental insufficiency in pregnant. For example, riboflavin kinase (gene RFK, figure 4-3) catalyzes phosphorylation of riboflavin (vitamin B2) with the formation of flavin mononucleotide (FMN), which is essential for the citric acid cycle and for the beta-oxidation pathway.

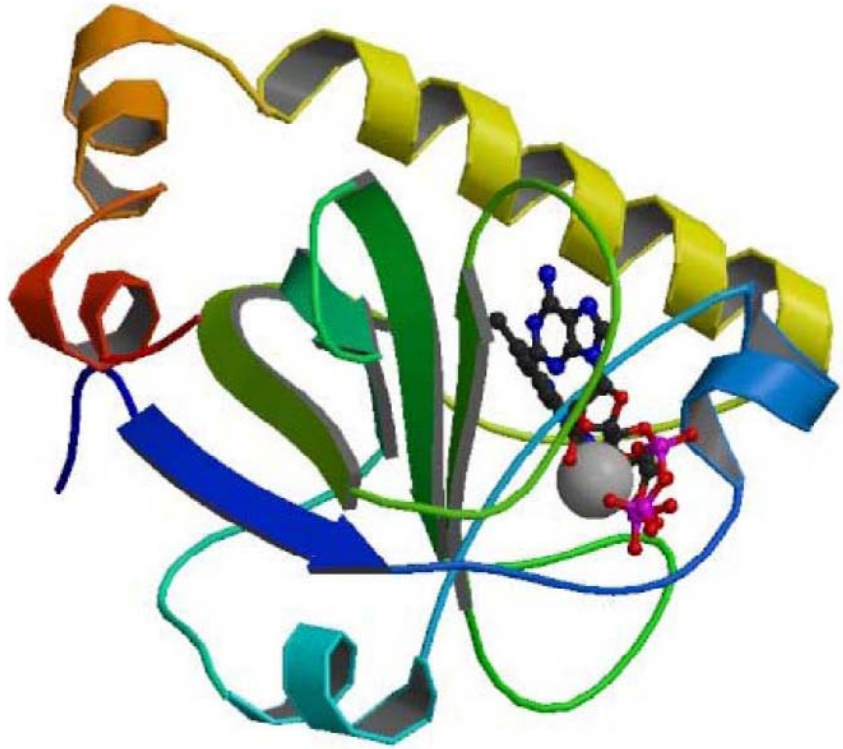


Figure 4-3. Riboflavin kinase (model based on PDB 1q9s). Magnesium (sphere) and flavin mononucleotide (wireframe) are shown.

Placenta is one of the tissues most saturated with mitochondria. Placenta does not only supply the energy to the developing fetus, but also to the pregnant. During pregnancy, the mother's body starts to depend on placenta. Indeed, the subjective maternal sensation of "cold", which continues for at least several hours or even days after the delivery, is well known in clinical practice. Magnesium deficiency often worsens the symptoms. Magnesium-dependent enzymes of mitochondrial fraction of placental tissue are involved in the fatty acid and piruvate metabolism. Deficiency of magnesium will reduce the activity of each of these enzymes, leading to a reduction in the amount of ATP produced in mitochondria.

Mg-dependent enzymes of the pyruvate cycle include, above all, the subunits of the NAD-dependent isocitrate dehydrogenase (genes IDH3A, IDH3B, IDH3G, IDH2) and two phosphatases of pyruvate dehydrogenase (PDP1, PDP2). These two phosphatases restore the activity of the pyruvate dehydrogenase molecular complex. Isocitrate dehydrogenases (figure 4-4) as well as the phosphatases interact with the pyruvate dehydrogenase, the central molecular machine of the energy metabolism.

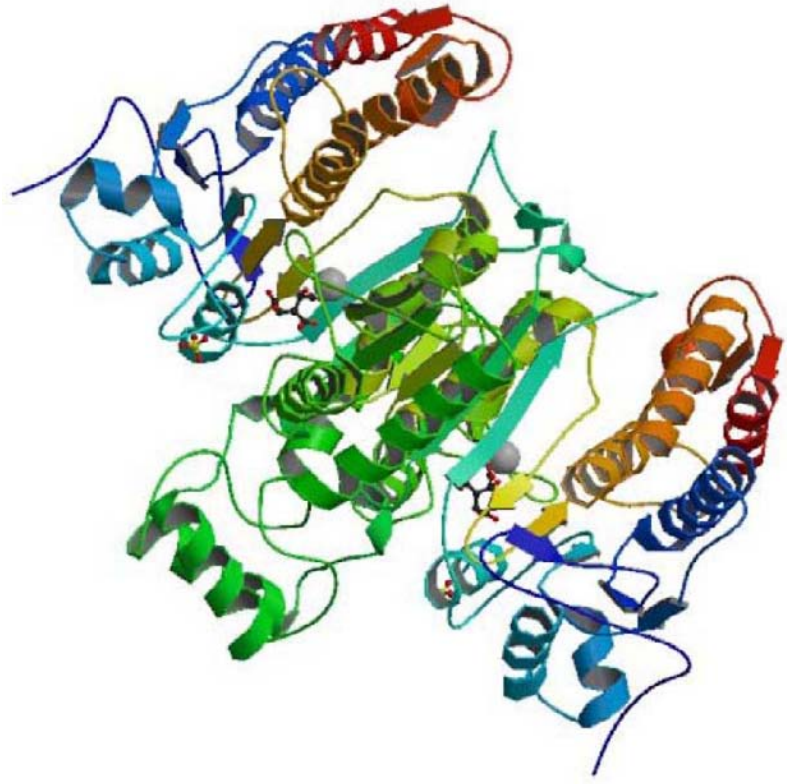


Figure 4-4. Dimer of isocitrate dehydrogenase (PDB 1cw7). Magnesium ions (sheres) and the molecule of isocitrate (wireframe) are shown.

During pregnancy, the importance of the *normal functioning of connective tissue* grows considerably. Connective tissue holds together various components of the placenta and forms the backbone of the chorion. A strong and flexible connective tissue prevents perineal tears during the childbirth, the development of hemorrhoides and flebopathies in pregnant. Later in this chapter, we consider the molecular mechanisms of magnesium's influence on the structure of connective tissue in greater detail (section 4.7). In brief, the mechanisms of influence of magnesium deficiency on synthesis and degradation of connective tissue include the activation of matrix metalloproteinases, lysyloxidases, glutaminases, slower synthesis of collagen, elastin and hyalouronan.

In the case of the connective tissue of placenta, magnesium deficiency results in so-called "calcification" and "aging" of placenta. These processes reflect not so much actual deposition of Ca in placenta but, rather, disruption of the mechanical structure of the connective tissue. This statement is confirmed by the fact that normal or even low level of calcium against the background of magnesium deficiency is characteristic for the formation of the "calcificated" regions in placenta. Moreover, formation of these "calcificated" regions can occur even at low levels of calcium in the maternal organism.

As we can see, magnesium is important for so many different aspects of the placental function that it becomes clear: magnesium deficiency will result in a considerable placental insufficiency since almost any aspect of the molecular physiology of placenta will be negatively impacted. Although all of the molecular mechanisms mentioned in the figure 4-2

are important for the normal placental function, the *placental alkaline phosphatase (PLAP)* represents an interesting particular case which can be important for both fetal and maternal tissues. PLAP is an enzyme that hydrolyzes organic ethers of the phosphoric acid. The enzymatic activity of PLAP requires two obligatory Zn ions and one Mg ion which participate in the active site of the enzyme. Magnesium ion plays is essential for activation of the enzyme and preserves functional geometry of the active site needed for catalysis (figure 4-5).

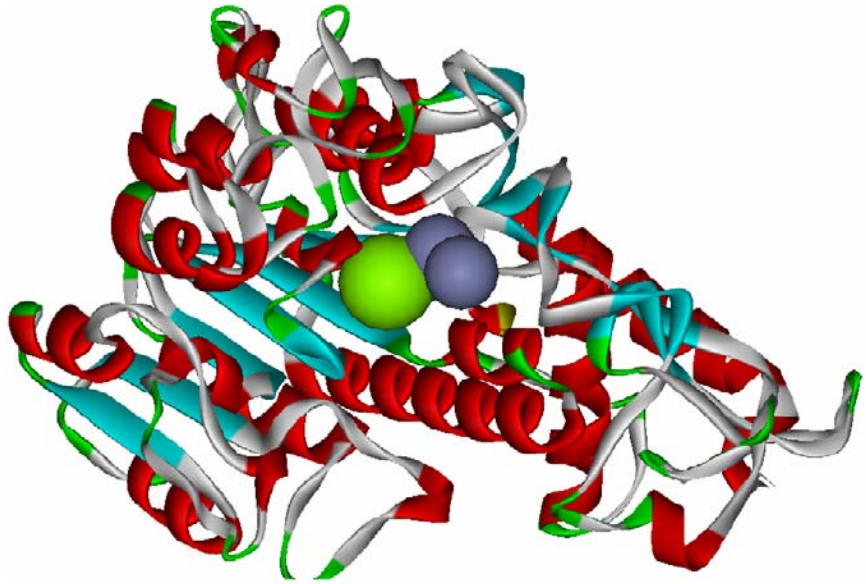


Figure 4-5. Placental alkaline phosphatase (PDB code 1zeb). The Mg ion (large sphere on the left) and the two Zn ions (smaller spheres on the right) are located in the active site of the enzyme.

The most interesting part about PLAP is that it appears to be a multifunctional protein. Higher ALP levels were observed in pregnant with female fetuses (Gol, 2006). Of particular interest is that serum alkaline phosphatase activity averages 2.1-fold higher in the late third trimester than in the first trimester (Choi, 2000). Although alkaline phosphatase test is widely used in clinical practice, physiological roles of the PLAP enzymes are not very well investigated. In addition to catalyzing hydrolysis of the esters of the phosphoric acid, placental alkaline phosphatase (PLAP) has the capacity to bind the Fc portion of human IgG which can be important for transfer of IgG molecules from the maternal circulation to the fetus. PLAP is necessary for the internalization of IgG (Makiya, 1992). The presence of large amounts of PLAP in clathrin-coated vesicles prepared from placenta strongly indicates that PLAP is involved in the vesicular transport endocytic machinery in this organ. In mouse embryos PLAP provides protection against serum starvation-induced cell death. PLAP can also regulate proliferation and remodeling of fetal tissues during the second and third trimester when it is expressed (She, 2000).

All of these data suggest that placental alkaline phosphatase is a multifunctional protein which can be very important for the normal pregnancy. A magnesium deficiency will cause lowering of the activity of alkaline phosphatase in placenta and a lower protein stability, which might adversely affect transfer of IgG, vesicular transport, will increase premature apoptosis of the placental tissues and will also decrease placental tissue proliferation.

While undertaking magnesium and calcium correction, it is important to take into account the rule of the proportional intake of the essential minerals: physiological need for calcium and magnesium are to be replenished by 800 mg/day of calcium and 400 mg/day of magnesium (that is, using the 2:1 ratio of Ca to Mg). Even if the levels of calcium are low, it is not recommended to start calcium therapy without the magnesium because this will result in further calcification of placenta, joints and atherosclerotic plaques. It's necessary first to restore both the levels of calcium *and* magnesium. In severe cases of hypocalcemia, a preliminary magnesium therapy course should be conducted for at least 2 weeks. Then, calcium is introduced (WHO, 1986) and the correction course is continued for at least 2 months in order to restore the magnesium and the calcium depot of the maternal organism. Prolonged Ca/Mg correction course restores physiologically normal distribution of calcium in the body: normalizes the levels of calcium in cells and in plasma, restores the calcium in the bone tissue and can also lead to disappearance of the calificates in the joints, of the stones in kidney and gall bladder *etc*.

As shows our experience, even a diet normalized in magnesium does not always provide enough magnesium because of the growing need of both fetus and mother. This fact necessitates the usage of magnesium preparations (*per os* and intravenous). Vomiting in the first trimester of pregnancy also exacerbates the deficit of both magnesium and pyridoxine. Critically low levels of pyridoxine are achieved during 9-14 weeks of pregnancy and have to be compensated along with the compensation of the magnesium deficiency. Magnesium, like all other cations, freely passes through the placental barrier and usage of high dosages of magnesium can cause fetal hypermagnesemia. Therefore, if a quick correction of magnesium deficiency in pregnant is required, it can be achieved by using slow intravenous introduction or automatic syringes.

4.1.2. Glucose Tolerance, Metabolic Syndrome, Gestational Diabetes

A number of enzymes involved in glucose metabolism require magnesium as a cofactor. Activity of these magnesium-dependent enzymes is reduced during magnesium deficiency and this contributes to the formation of the insulin resistance and glucose tolerance. In addition, magnesium is required for the insulin signal transduction (Takaya, 2004; Higashiura, 2005). There is a relationship between magnesium deficiency, excessive weight gain during pregnancy, metabolic syndrome in the postpartum period, and gestational diabetes (Feldeisen, 2007). Diabetes and pregnancy is accompanied by depletion of instracellular magnesium hypomagnesemia (Barbagallo, 2007).

An analysis of 608 cases of metabolic syndrome in women has shown that the expression of different manifestations of this syndrome (including level of insulin) is inversely proportional to the level of the dietary consumption of magnesium. In young women with sufficient consumption of magnesium the risk of metabolic syndrome decreased (He, 2006). In connection with the Westernization of the lifestyle, for example, Japanese sharply decreased consumption of magnesium (reduced quota of grain, barley, seaweed, vegetables, nuts). In parallel, the number of women with metabolic syndrome, hypomagnemia, obesity, hypertension, hyperglycaemia, hyperlipidaemia and cardiovascular disease has grown. Therefore, in Japan all the more urgent becomes the question of the prophylactic administration of magnesium drugs (Kumeda, 2005).

The level of magnesium in blood serum in women with diabetes negatively correlates with the lipid content of the blood and the manifestations of metabolic syndrome. Negative correlation was observed between the magnesium and the fat mass of patients (Randell, 2006).

Experimental magnesium deficiency in rat models induces clinically pronounced inflammatory response characterized by leukocytosis, activation of macrophages, secretion of inflammatory cytokines and proteins of the acute phase as well as by excessive production of the free oxygen radicals. Inflammation contributes to the proatherogenic changes in the endothelium of the vessels. Thus, magnesium deficiency sets the stage for the formation of the metabolic syndrome (Mazur, 2007). The study confirmed that hypomagnemia is characteristic for metabolic syndrome with obesity (Guerrero-Romero, 2006).

An examination of 290 patients with diabetes II indicated hypomagnemia in 143 (49.3%) patients. The levels of ionized magnesium were significantly lower in overweight patients, patients with high blood pressure, with proteinuria, with microalbuminuria, as well as those having high levels of plasma triglycerides (Corica, 2006). A study of 255 female patients with metabolic syndrome, hypophosphatemia was found in addition to hypomagnemia. The increased urine loss of magnesium correlated with hyperinsulinemia (Kalaitzidis, 2005).

4.1.3. Recurrent Pregnancy Loss

Spontaneous abortion during 1- 37 week, spontaneous abortion, premature contractions, and premature birth often occur against a background of profound magnesium deficiency. Premature birth is often associated with undesirable consequences for the child. However, magnesium sulfate therapy, often prescribed in these cases, severely interferes with the maternal and fetal homeostasis and, consequently, the balance of risk-benefits for this has to be carefully watched.

Excess of magnesium is toxic for the embryo and also slows down mineralization of the bone (Wedig, 2006). In premature infants, born from mothers treated with excess of intravenous magnesium sulfate, were observed the following conditions:

- hypermagniemia of up to 4.5 mg/dl,
- hypocalcaemia up to 6.0 mg / dL,
- increased activity of alkaline phosphatase up to 574 IU,
- diffuse osteopenia of long bones,
- fractures of the ribs (Kaplan, 2006).

These and other side effects lead physicians to weigh the risks of hypermagnesemia and its consequences more carefully. It is no coincidence that the practicing obstetricians call the tocolytic usage of intravenous magnesium sulfate as "North American anomaly" (Grimes, Nanda, 2006).

Careful review of Tan, Devendra (2006) provides clinical assessment of the following tocolytics used in premature birth: β-blockers, prostaglandin inhibitors synthetases, metindol, calcium channel blockers, nifedipin, oxytocin receptor blockers and magnesium sulfate. Side effects of beta-blockers in mothers include edema, arrhythmias, hypokaliemia. The results of the usage of magnesium sulphate were also quite ambiguous and, given the corrections for

prophylactic antibiotics, the tocolysis with MgSO4 does not appear to reduce the time spent by the infant and the mother in hospital (How, 2006).

Azria et al. (2004) analyzed data on the use of $MgSO_4$ solutions for the period 1966-2003. Magnesium sulfate was always used for the prevention of premature birth and eclampsia, and systematic reviews have demonstrated the effectiveness of magnesium sulfate in preventing eclampsia. By analyzing the data, however, it seems that magnesium sulfate is not so efficient in prevention of premature birth. Although the effectiveness of magnesium sulfate therapy for women with eclampsia and pre-eclampsia was confirmed many times, magnesium sulfate cannot be recommended as a therapy of choice for the prevention of premature labor.

4.1.4. Pre-Eclampsia, Eclampsia and Magnesium Sulphate Therapy

One of the most dreaded consequences of the deep magnesium deficiency in pregnant is eclampsia. It manifests itself with symptomatics similar to that of the hypertensive encephalopathy. During eclampsia, magnesium level may drop down several fold (3..10 times lower the normal level). In USA, for example, currently not less than 18% of deaths among pregnant are associated with hypertension and eclampsia. In the United States, magnesium sulfate is routinely prescribed to women with pre-eclampsia for more than three decades. The enormous amount of the accumulated clinical experience shows that magnesium sulfate is not always safe for the pregnant and for the fetus, raising the questions of the safe usage and of the alternatives to this drug (Belfort, 2006).

A study of 155 women (78 received the drug during 48 h, the control group of 77 persons did not receive magnesium sulfate) indicated that the group receiving magnesium sulfate, maternal morbidity was higher than in control though in infants all parameters were largely the same (Nassar, 2006). Excess of magnesium due to tocolysis with magnesium sulphate was also associated with atherolenic changes in the lipid profiles of newborns (Yavuz, 2006) and decrease antimicrobial ability of neutrophils (Mehta, 2006). These and other data underline once more that the magnesium sulphate should be used with caution.

4.1.5. Hypermagnesemia in Pregnancy

Except for hypothyroidism, adrenal insufficiency and severe dehydration, hypermagnesemia in pregnant is usually iatrogenic in origin. One of most common reasons for its etiology is intake of Mg-containing antacids which target reduction of the acidity of the stomach. The other is the usage of the magnesium sulfate as a tocolytic agent.

The common practice of treating eclampsia in pregnant includes intravenous or intramuscular injections of magnesium sulfate. This is 1^{st}-generation magnesium drug (that is, an inorganic salt) which freely passes through the placental barrier and tends to produce high concentrations of magnesium in maternal serum and in the fetus. Effects of magnesium excess on the fetus are negative and include hypotonia, hyporeflexia of the fetus and suppressed breathing function of the newborn. Magnesium preparations are strictly prohibited within 2 hours before giving birth, except in the cases of severe eclampsia. In the case when

newborn's breathing is suppressed because of hypermagnesemia, solution of calcium chloride is injected in the umbilical.

There is considerable amount of experimental and clinical material linking severe neurological disorders among newborns (such as cerebral palsy) with rapid parenteral injection of magnesium sulfate during pregnancy. Studies of (Grether, 2000; Mittendorf, 2006) showed that rapid infusion of magnesium sulphate leads to formation of excess Mg in newborns and provokes periventricular leukomalacia and intraventricular hemorrhages. A study of 4000 children with cerebral palsy indicated a strong correlation with the regular use of $MgSO_4$ during childbirth and even stronger correlation in case when there was uncontrolled use of magnesium sulfate (Ohta, 2002). Usage of $MgSO_4$ leads to episodes of sinus bradycardia (Hallak, 1998) and lowered levels of reduced parathormone of the fetus (Pantonen, 2001). Data from American Association of Obstetricians and Gynecologists (2004) suggest that long-term use of magnesium sulfate without control of the plasma magnesium during pregnancy leads to a 4-fold increase of the risk of the cerebral palsy in newborn. Studies of Matsuda et al (2000) indicated that combination of the fast infusion of $MgSO_4$ with urogenital infections that cause kidney disfunction among pregnant further increases the risk of birth of children with cerebral palsy.

These and other studies indicate that usage of $MgSO_4$ pregnancy should be considered as an extreme life-saving measure and not as a regular form of therapy. The prescription criteria are continually refined and supplemented by new conditions, such as the speed of infusion and monitoring the concentration of magnesium in blood in order to ensure safe levels of magnesium. Usage of $MgSO_4$ solutions is strictly contraindicated in patients with oligonuria, bradycardia, hereditary myopathy, thrombophilia or thrombocytopenia. The usage of the magnesium sulfate is also contraindicated in the case of ketoacidosis, diabetic nephropathy, proliferating nephropathy in diabetes mellitus, and renal failure.

The criteria for the therapies based on solution of $MgSO_4$ differ among the countries. The strictest approach is implemented in Japan. While in many countries the safe limit of Mg in the blood is set to be within 2.5-3.5 mmol/L, in Japan it is stipulated that concentration of magnesium in serum of the pregnant should not exceed 1.8-3.0 mmol/L as the latter figure is safer for the fetus. While levels of 3.5-5 mmol/L increase health risks for the fetus, levels higher than 5-6.5 mmol/L can result in breathing paralysis and death of the fetus in utero. However, even at the level of 1.8-3 mmol/L, the usage of the magnesium sulfate can already induce transient disorders of the brain functions in mother. At the levels higher than 3 mmol/L, fetus might develop irreversible brain lesions in the form of microhemorrages (mostly intraventricular) and mosaic leukomalacia. Additionally, during the infusions of magnesium sulfate it is quite informative to evaluate:

1. Urination rate (should be >30 ml/h);
2. Breathing frequency (should be >15-16 times per minute);
3. Pulse (should be at least 60 beats per min);
4. The knee reflex (suppression of the jerk comes much earlier than that of the breath).

Presently, the 2nd generation of the magnesium preparations are used for the correction of hypomagnesemia. The 2nd generation magnesium drugs are characterized by much higher bioavailability which is the result of using salts of magnesium with organic acids (magnesium

lactate, magnesium pidolate, magnesium citrate, magnesium orotate, magnesium asparaginate etc) and combinations of the organic salts with pyridoxine and other bioligands. Usage of the pelleted and drinking forms of these drugs in therapeutic doses almost entirely excludes the danger of even transitory hypermagnesemia in pregnant.

4.2. Magnesium and Pyridoxine in Premenstrual Syndrome; Influence of the Steroid-Based Drugs

PMS (premenstrual syndrome, premenstrual stress syndrome, premenstrual disease) is one of the most common neuroendocrine syndromes that occur at frequencies of 25-75% in different female populations (Mezhevitinova, 2005; Prilepskaya, 2005; Smetnik, 2005; Deuster, 1999). Premenstrual syndrome is clinically polymorphic condition which occurs among regularly menstruating women 3-14 days before menstruation and disappears in the course of the first days of menstruation.

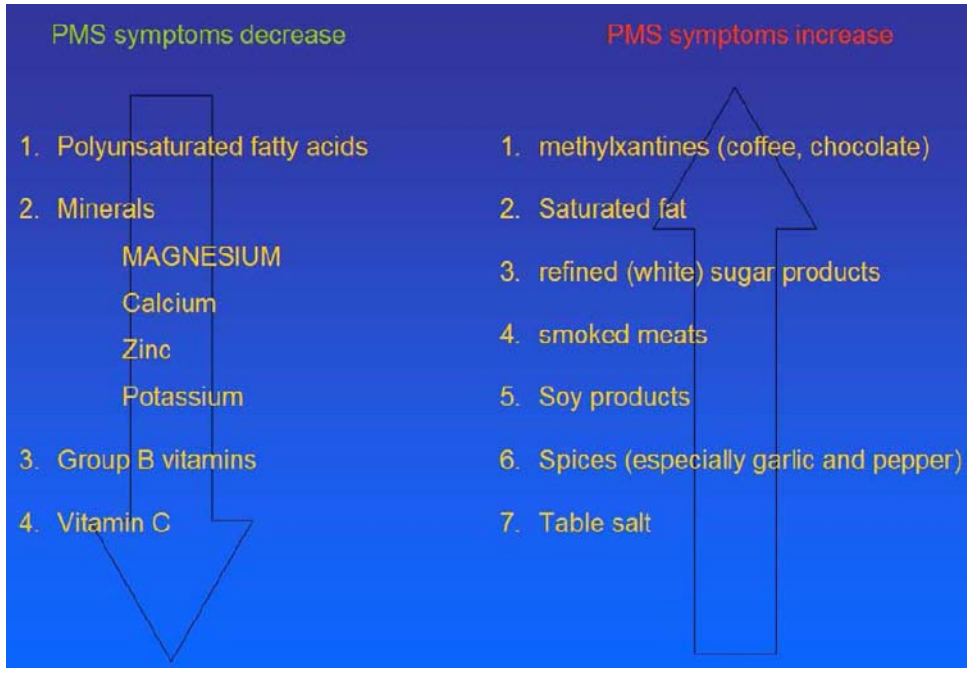


Figure 4-6. The nutritional factors and PMS (Horrobin, 1983).

PMS is influenced by a number of nutritional factors (figure 4-6) and manifests through neurophysical and vegetative symptoms, accompanied by an increase in the body weight (figure 4-7), hormonal irregularities (in particular, hyperestrogenia in the luteal phase of the menstrual cycle) as well as imbalances of neurotransmitters and of minerals. According to our own records, up to 65% of women with premenstrual syndrome have border-line hypomagnesemia (blood, hair) confirmed by clinical manifestations of magnesium deficiency.

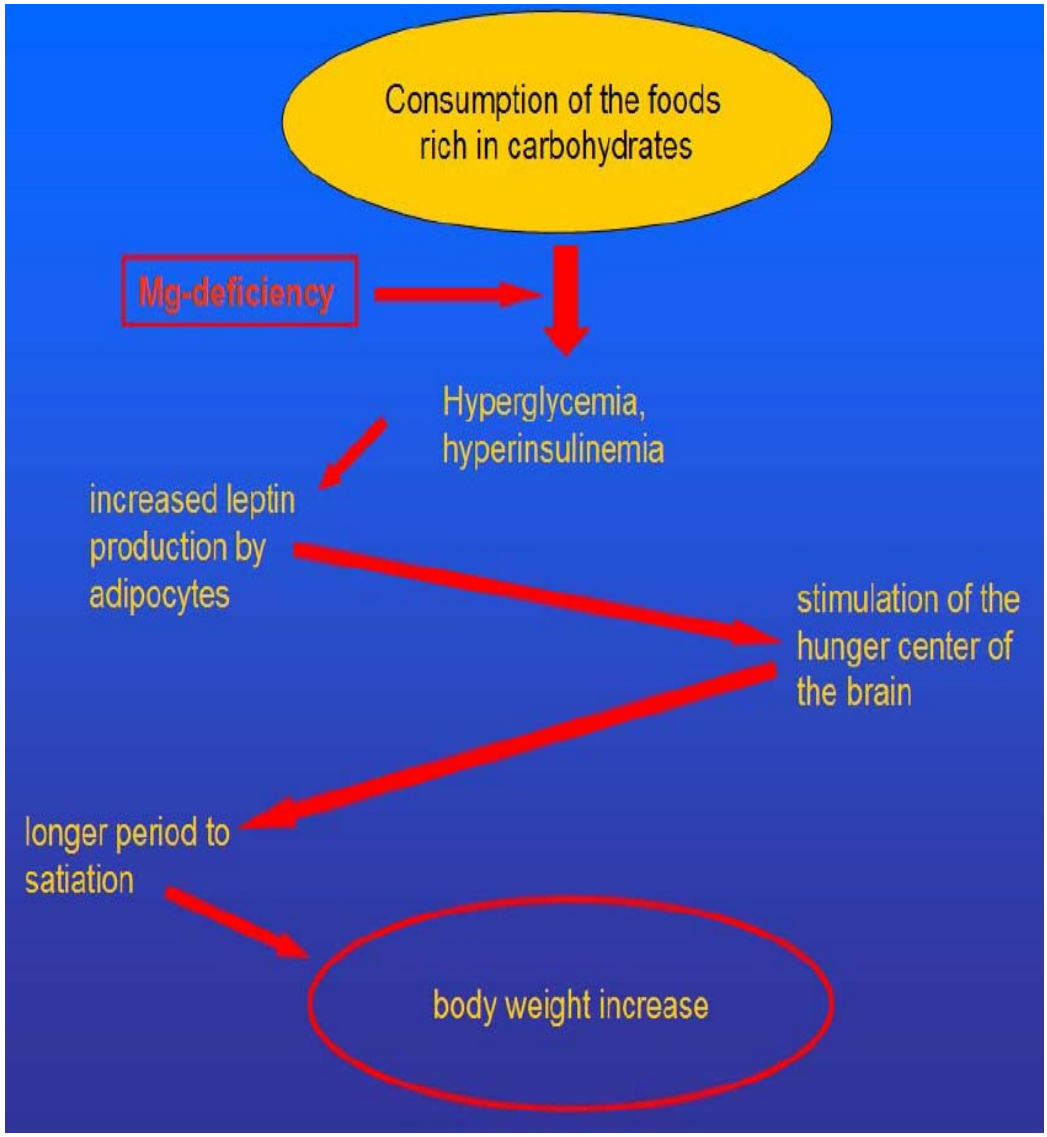


Figure 4-7. Magnesium, PMS and the body weight.

An increase of the estrogen levels in the blood serum causes the retention of sodium ions which results in accumulation of intercellular fluid and, therefore, tissue swelling (figure 4-8). Dropsical forms of PMS are associated with excessive levels of estrogens and lower than normal progesterone during luteal phase, imbalance of renin-angiotensin-aldosteron and a moderate excess of prolactin.

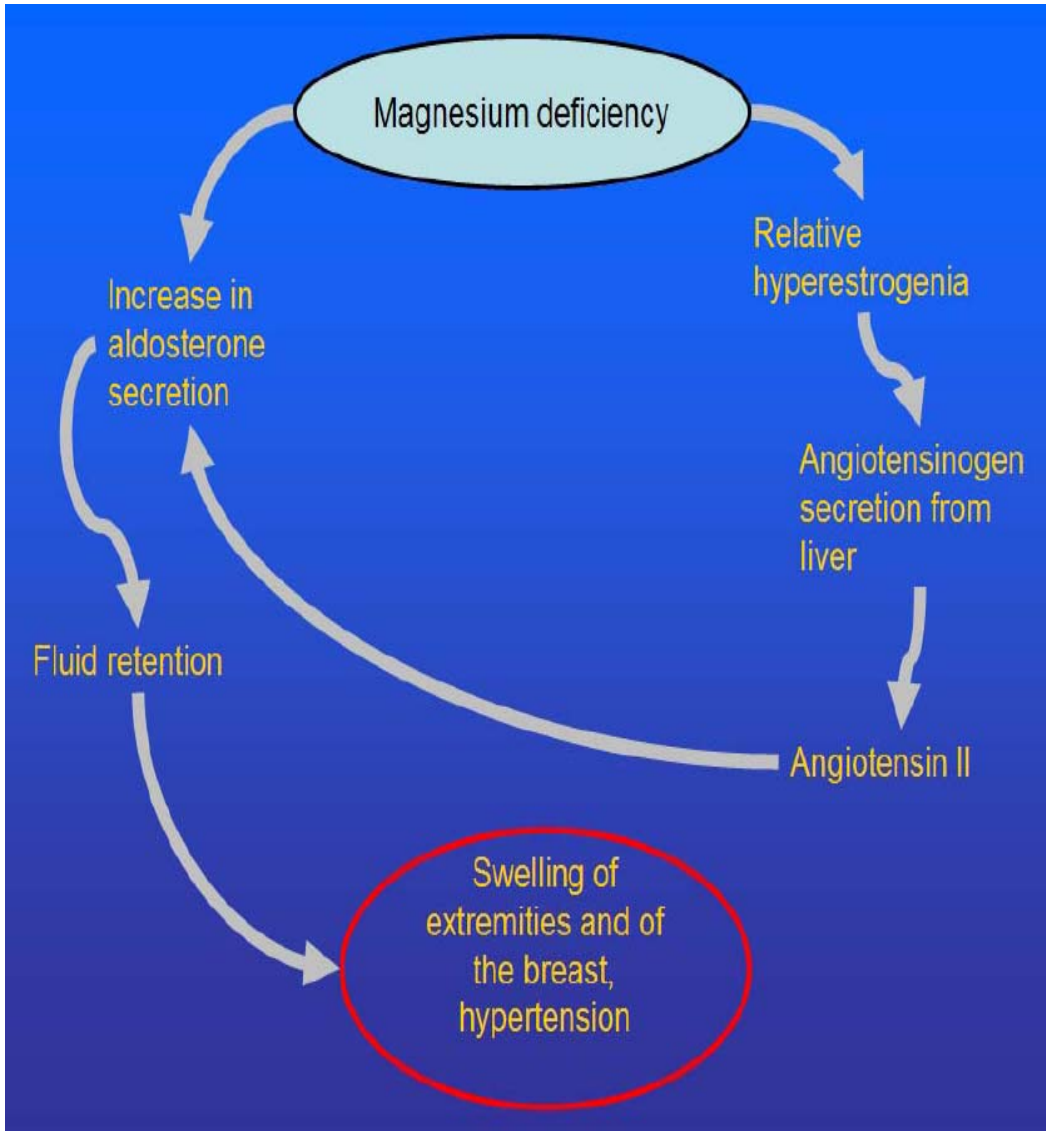


Figure 4-8. Excess retention of bodily fluids in PMS.

More than 200 symptoms were attributed to PMS in its various forms, the three most prominent are increased irritability, stress/anxiety and dysphoria (unhappiness) just prior to menses. Other common symptoms include abdominal cramps, constipation and possible hemorrhoids due to water retention, food cravings, joint or muscle pain, headache, fatigue and acne. These symptoms are likely to be related to reduced production of serotonin, prostaglandin PgE1, Leu- and Met-encephalin, opioid neurotransmitters accompanied by overproduction of prostaglandins PgE2 and PgF2-alpha. These imbalances in hormones and neurotransmitters are likely to determine the extent of neuropsychological deviations as well as lowered pain threshold (figure 4-9).

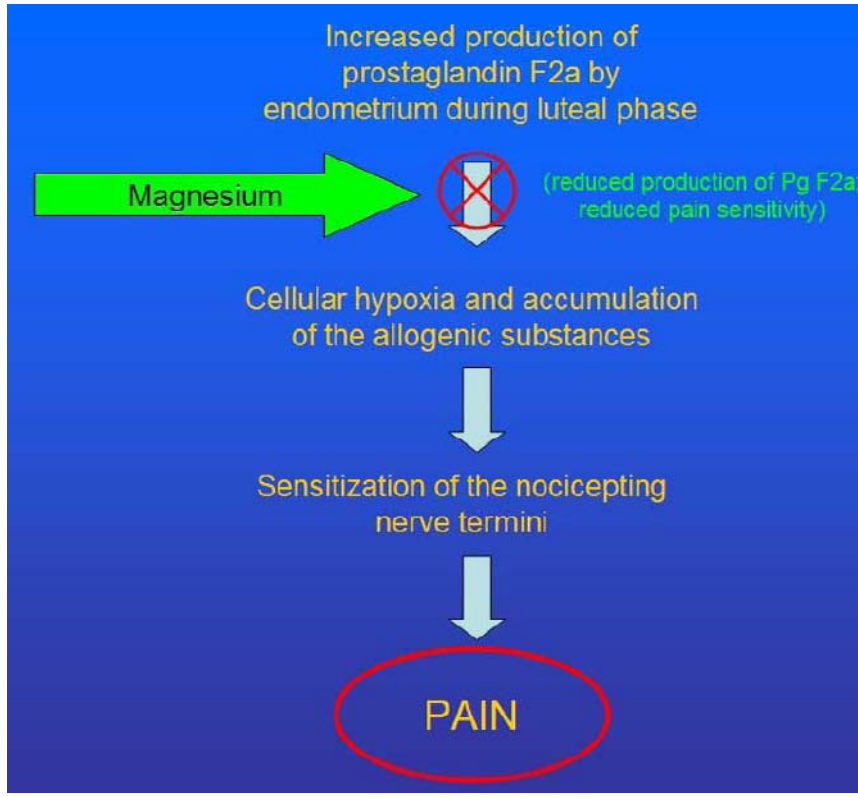


Figure 4-9. Pain sensitivity in women with PMS.

With the deficit of magnesium, the production of the protective prostaglandin PgE1 is lowered while replenishment of the magnesium depot in patients with PMS reduces production of the pro-inflammatory PgF2 (Seifert, 1989). Magnesium is involved in the processes of degradation of numerous neuromediators: catecholamines, acetylcholine, glycine and, along with other trace elements (Zn, Se, Cu), is also important for the levels and activity of the endorphins, encephalins and hypothalamic releasing factors. Mg-dependent enzymes are involved in numerous reactions of the energy metabolism, so magnesium deficiency results in weakness, fatigue, chilliness prior to and during menstruation. It is known that in the period during PMS and during menstruation women are more susceptible to viral and infectious diseases, especially when there is magnesium deficiency. Magnesium preparations stimulate energy metabolism and, by normalizing neurotransmitter metabolism and neural excitability, result in sedative and neuroprotective effects along with moderate immunostimulation (Buharina, 2006).

There exists a distinct correlation between the monthly biorhythm of the reproductive hormones and the levels of magnesium during the menstrual cycle. In healthy women, the erythrocyte magnesium has a clear monthly rhythm: lower in 1st (estrogen) phase of the menstrual cycle and higher during the 2nd (progesterone) phase. On the contrary, in women with premenstrual syndrome, the erythrocyte magnesium levels decrease during the 2nd phase of the cycle (Aghajanyan, 1996) so the lack of magnesium and of vitamin B6 in women with PMS might make a significant contribution to the pathogenesis of the syndrome.

Recent studies attempted cyclic therapy of PMS with magnesium-pyridoxine drugs. The cyclic therapy is synchronized with the period of the maximum need for magnesium in women with PMS (that is, during the 2nd phase of the menstrual cycle) which correspond to the peak of the PMS symptoms and to the peak of magnesium deficiency. In addition, it was found that correction of magnesium deficiency in women with PMS may potentiate other drugs which are prescribed with the aim of treating PMS (such as hypothiazid, remens, grandaxine, preparations containing extracts of *Agnus Castus etc*) by optimizing pharmacokinetic response (Prilepskaya, 2006). 25 placebo-controlled double blind studies have shown the effectiveness of using high doses of vitamin B6 (up to 100 mg/day) for the treatment of PMS (Higdon, 2005). A study of 48 women with premenstrual syndrome (Lebedev, 2008) indicated a significant decrease in clinical manifestations of PMS after a course of Magne-B6 therapy (1 tablet, 3 times daily, for 6 months). The main complaints of the patients were irritability (89.3%), bloating (68,4%), mastalgiya and mastodiniya (82.6%), depression (47.3%) and they decreased observably after the course of therapy (see figure 4-10)

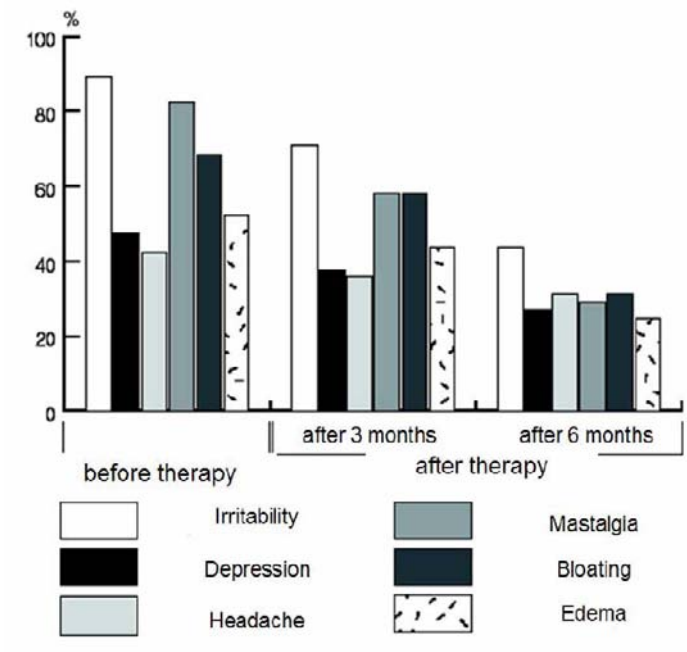


Figure 4-10. MagneB6 therapy in PMS (after Lebedev, 2008).

It should be borne in mind that *up to 40% of women taking oral contraceptives develop observable deficiencies of both magnesium and pyridoxine* during the first months of the drug intake (since estrogens and their derivatives show strong antagonism towards both magnesium and pyridoxine). At least 12 double placebo-controlled studies have demonstrated that oral contraceptives lead to a sharp drop in concentrations of vitamin B6 during the first 1-3 months. Pharmacologic restoration of magnesium and pyridoxine in women with magnesium deficiency significantly raises the quality of life and alleviates the magnesium deficiency symptoms (Prilepskaya, 2005; Smetnik, Butareva 2004).

4.3. Magnesium Deficiency and Pathologies of the Central Nervous System

The magnesium deficit is often found in patients with paresthesias, tremors, spasmodic states, ataxia, autism, nystagmus, migraine, hearing loss, emotional disturbances, depression, neurodegenerative diseases, and those under chronic stress (chronic fatigue syndrome). The deficit of magnesium is also an additional risk factor for stroke (WHO, 1996).

In brief, we considered the effects of magnesium on the neural system in the Chapter 1. We also mentioned that inhibition of the NMDA receptors and catecholamine degradation are the two most important mechanisms through which levels of magnesium affect the neural function. These two mechanisms are, apparently, not the only ones linking magnesium with the functional state of the neural system. The other mechanisms include action of magnesium on other kinds of the receptors (such as AMPA receptors), participation of magnesium in the enzymes of the energy metabolism, in the protein synthesis (stabilization of RNA, in particular), stabilization of the spatial configuration of neuropeptides, stabilization of neurofilaments (which is important for the vescular transport along the axon) and interactions with neurotransmitter amino acids. Indeed, amino acids can chelate the magnesium cations and, for instance, neuroprotective effects of glycine cannot be fully realized when there is a deficiency of magnesium (Gorbacheva, 2007). In addition, magnesium promotes sequestration of the neurotoxic trace elements (Be, Ni, Pb, Al, Cd) and prevents their accumulation in nervous tissue. Combined action of these and other molecular mechanisms is important for the neuromuscular, neurodegenerative and other manifestations of the magnesium deficiency.

4.3.1. Neuromuscular Manifestations

Neuro-muscular manifestations are the earliest signs of the magnesium deficiency and are, generally, associated with the increased excitability of the neurons. These manifestations include:

- tingling in the feet and palms (paresthesia, results from overexcitation of the sensory nerves);
- hyperactivity: patient can’t stay long in one place, constantly moves, even when sleeping (syndrome of the "troubled feet", linked to increased excitability of the skeletal muscles);
- convulsions (difficulties in cell repolarization), the feeling of “lump in the throat” (spasm of the pharynx);
- cardiovascular disturbances (augmented heartbeat, tachycardia);
- digestive disturbances (diarrhea, constipation, irritated colon, abdominal pains)
- respiratory disturbances: increased breathing rhythm, a sense of suffocation when under stress;
- urinary disturbances: frequent urges, pain in the bladder;
- pain in the back and lumbar;
- disturbed sleep;
- chronic feeling of fatigue, even after sleep.

Magnesium therapy is known to alleviate these symptoms and, in particular, to normalize night sleep and to restore the REM stage of dreaming. Group B vitamins (B1, B2, B6) also assist restoration of the normal dreaming, including colorful dreams (Held, 2003).

4.3.2. Stress, Chronic Fatigue Syndrome and Vegetative Dystonia

Stress of different nature (physical, mental) increases demand for magnesium and causes intracellular magnesium deficiency. Stress and magnesium deficiency are mutually linked processes which aggravate each other (figure 4-11).

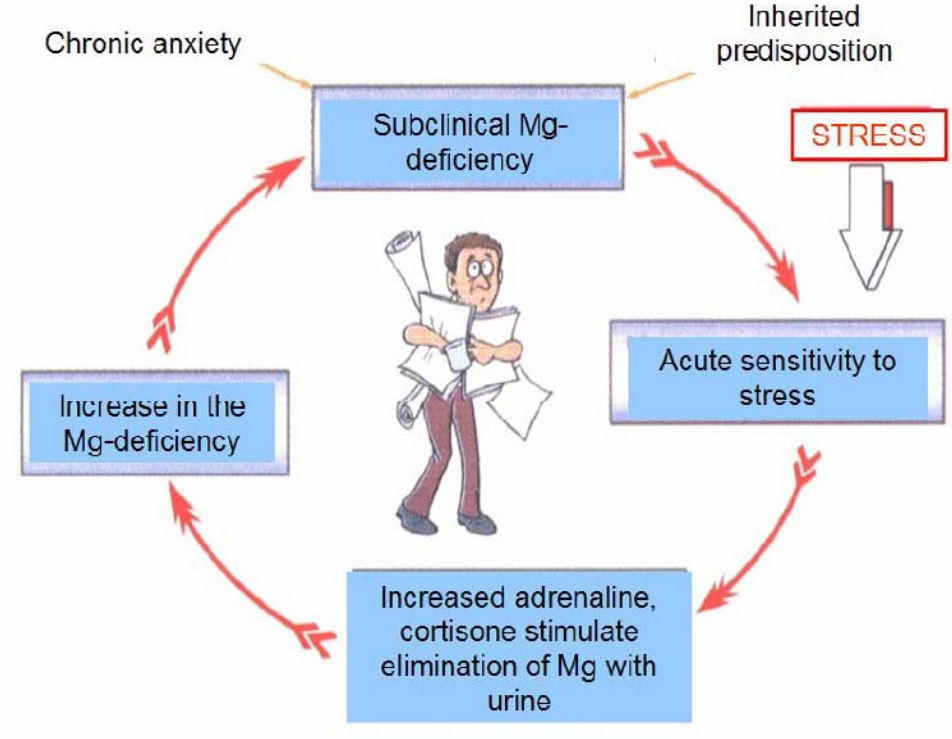


Figure 4-11. Stress and magnesium deficiency: the vicious circle.

In stressful situations, greater amounts of adrenaline and noradrenaline are secreted into bloodstream and this contributes to the depletion of intracellular pool of Mg^{2+} and to a higher elimination of magnesium with urine. Catecholamines, in particular, stimulate catabolism of fats and the free fatty acids tend to form insoluble salts with magnesium which then saturate primary urine. In kidney glomeruli, insoluble magnesium salts are not reabsorbed and thus are lost with urine.

Urine saturates with magnesium during and after strong emotions such fear or anxiety (exam, competition, making the career). The level of plasma magnesium lowers and this stimulates depletion of magnesium from the depots in muscle, myocardium, and bones. When the magnesium is supplied sufficiently with foods, supplements or magnesium drugs, the depots are not emptied and the normal intracellular levels of magnesium are maintained. In

this case, the negative influences of catecholamines on the magnesium levels are buffered. Moreover, magnesium participates in the degradation of catecholamines (through the COMT enzyme, Chapter 1) and sufficient magnesium keeps the COMT activity at the proper levels. As a result, the patient's ability to tolerate stress increases.

Magnesium-pyridoxine preparations can be successfully used to compensate magnesium deficiency and increase patient's ability to manage stress (Gromova, Kalacheva, 2009). We analyzed the molecular basics of the anti-stress effect of the magnesium-pyridoxine preparations elsewhere (Torshin, Gromova, 2008) and summarize the known molecular mechanisms in the figure 4-12.

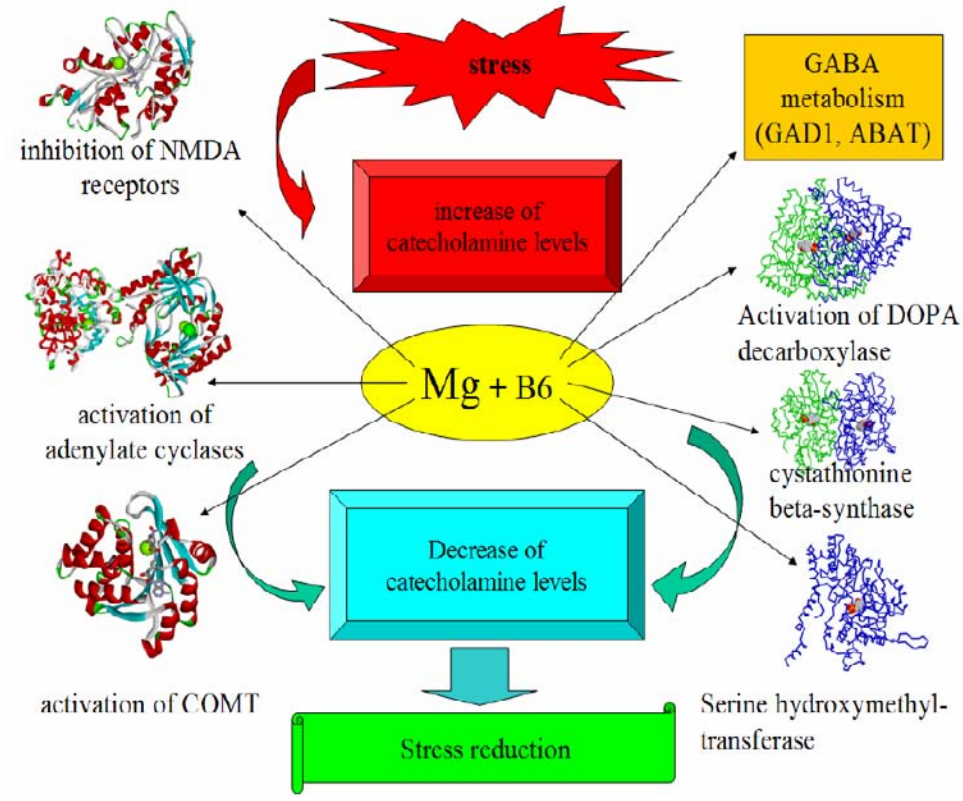


Figure 4-12. Molecular mechanisms of action of the magnesium-pyridoxine drugs.

The method of the functional linkage analysis (Torshin, 2009) allowed identification of proteins and genes through which the magnesium might affect the function of the nervous system. These include catechol O-methyltransferase involved in the regulation of catecholamine levels, N-methyl-D-aspartate receptors (NMDA-receptors or glutamate receptors, crucial for the hippocampal activity) and adenylate cyclases which activate G-protein signaling. Adenylate cyclase enzymes catalyze the transformation of adenosine monophosphate (AMP) into the cyclic AMP (cAMP), an important signaling molecule. All known adenylate cyclases have very similar spatial structure (figure 4-13) which contains two magnesium ions in the active site.

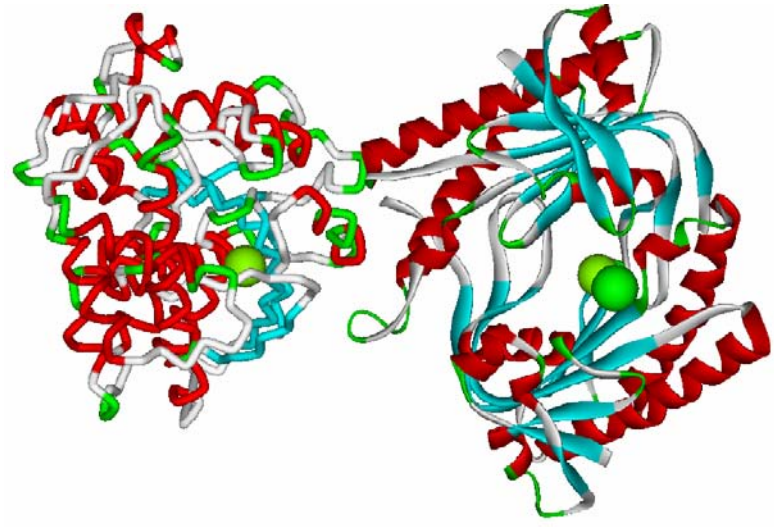


Figure 4-13. Spatial structure of the adenylate cyclase 5 in complex with the G-protein (PDB file 1CJK). Both adenylate cyclase (right), and G-protein globule (left) bind magnesium ions (spheres).

Magnesium deficiency leads to a systematic reduction in activity of all varieties of the adenylate cyclases and, consequently, to quite a range of neurological effects. Thus, reduced activity of ADCY1 and ADCY8 adversely affects the functioning of memory; reduced activity of ADCY2, ADCY3 and ADCY4 leads to a reduction in olfactory responses. The deterioration of the functioning of memory and suppression of the olfactory responses were in fact observed in magnesium deficiency (see above the clinical picture of the magnesium deficiency). Decreased activity of the cAMP signal system (in particular, ADCY7) might predispose to depression while reduced activity of ADCY6 leads to suboptimal functioning of the thyroid gland. In terms of impact on stress, the most interesting are the effects of magnesium on the activity of adenylate cyclases 5 and 9. The latter influence the signal transduction from β-adrenergic receptors while the former mediate the opioid receptor signalling. Functions of the known adenylate cyclases are summarized in table 4-1.

Table 4-1. Genes of adenylate cyclases and the functions of the corresponding proteins

Gene	Function of the protein
ADCY1	long-term memory
ADCY2	olfactory behavior
ADCY3	olfactory behavior
ADCY4	olfactory behavior
ADCY5	mediator of opioid receptors
Gene	Function of the protein
ADCY6	functions of the thyroid gland
ADCY7	signal transduction from neurotransmitters
ADCY8	memory operation
ADCY9	signaling from β-adrenoreceptors

Functional linkage analysis of the relation between pyridoxine and neural function pointed to several possible molecular mechanisms through which pyridoxine might exert its antistress and antidepressive effects (Chapter 8). Pyridoxine deficiency reduces the activity of cystathionine beta-synthase (CBS, gene CBS, figure 4-14) resulting in hyperhomocysteinemia.

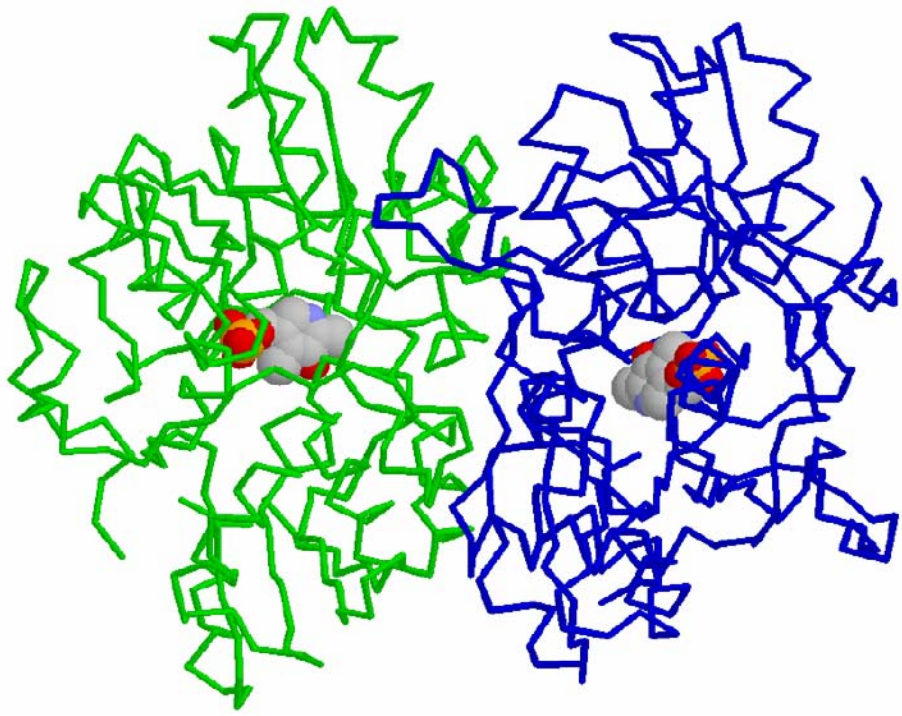


Figure 4-14. The dimer of cystathionine beta synthetase (PDB code 1jbq).

In a survey of 64 patients (age 20-49 years) with symptoms of chronic stress, the positive results of MagneB6 therapy become apparent since the second week of therapy and reached maximum by the end of the month (Akarachkova, 2008). There was a statistically significant improvement in both subjective sensations (affective complaints, complaints about cardiovascular and respiratory systems, and the quality of life according to MOS SF-36, Ware JE et al. SF-36 Health Survey: Manual and Interpretation Guide / MA: Boston, Nimrod Press, 1993), the clinical symptoms of stress (symptoms of autonomic dysfunction, Chvostek syndrome) as well as estimates of the mental state of the patients according to CGI scale.

Magnesium deficiency is found in up to 70% of children with attention deficit hyperactivity disorder (ADHD), prominently presented in patients with early forms of cerebrovascular diseases, as well as in patients with ischemic stroke (Gromova, Burtsev, 1998; Avdeenko, 1998; Andreev 1999; Fedotov, 2003; Limanova, 2004; Gromova, 2006; Gromova, Uvarova, 2006). Most children with ADHD and hypomagnesemia manifest symptoms of cerebral angiodistonia (headaches, dizziness, fainting) and transcranial ultrasound dopplerography shown a shift of autoregulation thresholds towards greater extent of vasoconstriction. Many cases of the sudden breath stop during sleep in children, along with sudden infant death syndrome, can be looked at as heavy chronopathologies of magnesium.

The latter also include development in children of extreme hypomagnesemia during night sleep (Durlach, 2002).

In a comprehensive clinical survey of adolescent patients (16-21 years), we have obtained interesting results indicating the role of magnesium in the *vasocontrictory reactions of the cerebrovascular system*. We have examined 232 adolescents with early forms of cerebrovascular diseases (Gromova, 2007), which included inadequate blood flow and discirculatory encephalopathy (criteria of Schmidt, 1975). Low levels of plasma magnesium (<0.75 mmol/L) were detected in 148 (63.7%) patients. These patients received a 30-day therapy with Magne-B6.

The patients undergone a comprehensive survey, which included ultrasound transcraneal dopplerography before treatment (day «0») and after 30 days of therapy (day «30») using ulstrasound devices «Labodop» (France) and "Premier" (Russia). The condition of the vascular regulation was determined using functional dopplerography under different test loads: apnea, hyperventilation, compression tests, orthostasis (Andreev, 1995). The following reactivity indices were calculated:

- Reactivity ratio for hypercapnic load: $Kp(co_2) = Vco_2/Vbckg$,
- Coefficient of reactivity at hypocapnic load: $Kp(o_2) = 1 – V(o_2)/Vbckg$,
- Index of cerebrovascular reactivity $ICR=(Vco_2 - Vo_2)/Vbckg$,
- Time transients after hyperventilation (TTo_2) and apnea ($APco_2$).

According to the values of these indices and additional neurological symptoms, the 148 magnesium-deficient patients had three types of pathological responses to hyperventilation test: hyperconstrictory, hyperdilatory and hyporeactive. Hyperdilatory reaction was observed only in 9 (6%) of 148 patients and hyporeactive response was found in 21 (14.18%) patients. *The most frequently observed response was hyperconstrictory, identified in 118 (86%) of the magnesium-deficient patients*. In hyperconstrictory patients, migraine usually arose after emotional stress, was localized to the frontal-temporal region, lasted 2-3 hours and dissipated after rest or sleep. Dopplerography shown distinct hyperconstrictory reactions (decrease of $Kp(co_2)$ to 1,3 ± 0,06 and increase of $Kp(o_2)$ to 0,63 ± 0,06, normal values of ICR) along with the formation of the hypercnstrictory vasoreactive stereotype (torpid nature of the transition process, TTo_2=38,3±0,7s). The treatment with the magnesium preparation (Magne-B6) considerably decreased hyperconstrictory reaction ($p < 0.02$) as indicated by the reduction of torpidity of the transition process (ΔTTo_2=5s) and increase of ICR (ΔICR=0.2) to the day 30 of the therapy. The neurological symptomatics and, before all, of migraine, was also decreased after the therapy (Gromova, 2007).

4.3.3. Magnesium and Neurodegenerative Diseases

A significantly reduced level of intracellular magnesium is characteristic for such mental and neurological conditions as schizophrenia, epilepsy, hypothalamic syndrome, hearing loss and disturbances of fine motorics including disgraphiya, as we attempted to shown in the following picturesque illustration (figure 4-15). Inclusion of magnesium in the treatment of these diseases leads to a partial regress of the neurologic symptoms (Johnson, 2001).

Figure 4-15. A representation of the neurological consequences of the magnesium deficiency.

Neurodegenerative diseases are also often accompanied by lower levels of magnesium. Meta-analysis of placebo-controlled studies that dealt with application of vitamin B6 in elderly (60-90 years) with dementia and Alzheimer's disease has shown that up to 90% of the patients had deficit of both pyridoxine and magnesium prior to the treatment. The use of vitamin B6 for not less than 12 weeks has shown effectiveness in patients with dementia (Malouf, 2003). The work (Pamphlett, 2003) demonstrated that in animal models of lateral amyotrophic sclerosis the long-term (4-5 months) intake of the magnesium pidolate solution considerably improved the clinical manifestations of the disease in animal studies. Generally, clinical studies and animal research indicate that magnesium pidolate tends to accumulate in the neural tissue and this explains high efficiency of magnesium pidolate for neurology (treatment of neurodegenerative diseases), psychiatry (treatment of generalized anxiety, autism) and narcology (delirious states, withdrawal syndrome).

4.3.4. Magnesium and Diseases of Dependence (Alcohol, Narcotic and Nicotine Dependence)

Low levels of magnesium in the body, along with lithium and zinc deficiencies contribute to drug, alcohol and nicotine addiction. Caffeine, teobromin, other xantine derivatives, as well as alcohol, cocaine, morphine, heroin, marijuana, synthetic drugs of the “ecstasy” stimulate loss of magnesium from the cells and increase the urine excretion of the cation. Magnesium preparations decrease the intensity of the dependency symptoms, primarily through inhibition of the NMDA receptors in the central glutaminergic synapses (Nechifor, 2008).

Hyperglycemic diet, which leads to an increase in the magnesium loss, is one of the factors that aggravate dependency diseases. In patients with genetic defects in tryptophane

dioxygenase enzyme, hyperglycemic foods lead to an increased loss of magnesium and are considered as aggravating factor in drug addiction (Marshak, 2005). Defects in this enzyme necessitate the consumption by the patient of the increased amounts of the tryptophane-containing foods (chocolate, cacao, banana, dates, cheese, sesame). When the tryptophane intake is insufficient, the patients can experience considerable anxiety and depression which result from the lowered levels of neuromediators. Magnesium and pyridoxine deficiency further aggravate these symptoms.

Mg^{2+} and Ca^{2+} ions have considerable affinity for opioid receptors and strongly inhibit binding of morphine to the receptors which results in slower development of the drug addiction. On the contrary, against the background of low Mg^{2+} and Ca^{2+}, dependence on morphine appears to develop faster (Zaitseva, 1993).

Chronic ethanol poisoning also affects metabolism of magnesium. Alcoholics tend to have a diet even more unbalanced than most of the patients. The alcohol itself, especially strong (such as vodka, scotch whisky *etc*) dramatically slows down the process of magnesium absorption in gastrointestinal tract. Any alcohol drink that stimulates diuresis will also increase the loss of magnesium. With the increase in magnesium deficiency, the alcoholics begin to demonstrate many of the nonspecific neuromuscular symptoms of magnesium deficiency which we mentioned earlier: anxiety, depression, sleep disturbances, cramps, and upon leaving the period of binge drinking - a pronounced weakness, lowered body temperature (34.5-36.0 C), ice limbs, and chills.

Long-term alcoholism exacerbates continuous stimulation of the adrenal glands which continuously secrete aldesterone. The excess aldosterone leads to an increase of elimination of magnesium from the blood stream. Reduction of the level of magnesium in blood is in proportion to the dose and duration of the binge period. While determining the balance of magnesium, it is not enough to rely solely on the level of plasma magnesium since it may remain normal even when 80% of the magnesium from the depot is lost. The most sensitive marker for determining magnesium status in aldosteronism and alcoholism is the lymphocyte magnesium (Delva, 2003).

Women develop alcohol addiction much faster than men. The clinical manifestations of alcoholism (oedema, aged skin, encephalopathy, the loss of cortical control, of memory and of abstract thinking, cirrhosis, Korsakov syndrome *etc*) also develop quicker in women. Women are also more susceptible to the toxic impurities in the low-quality alcohol which contains higher levels of mercury and cadmium. The latter toxic elements accumulate in the body and cause teratogenesis of the fetus.

The mental deviations in alcohol delirium are both magnesium- and pyridoxine-dependent. Pyridoxine is important not only for the delivery of magnesium inside cells but also has its own antidepressant properties due to interference with the GABA and catecholamine metabolism (figure earlier). Especially in the case of alcoholics in the state of *delirium tremens* (an acute alcoholic poisoning characterized by increased physical activity, hallucinations, drivel and raving), the pyridoxine levels strike the all time low, so the protocols for treatment of such patients often include not only magnesium drugs, but also high doses of pyridoxine (30-100 mg/day). When an alcoholic reaches the stage of delirium, a rapid correction of the pyridoxine level often provides an acute sobering effect. Pyridoxine also reduces the state of panic, fear and anxiety. Use of the magnesium salt bishofit (MgCl2x6H2O) in conjunction with pyridoxine increases the level of erythrocyte magnesium in the course of treatment of alcoholism (Iezhitsa, 2002).

The greater is the dose of alcohol, the less are the levels of the blood magnesium. One of the very sensitive markers of the magnesium content under the conditions of alcoholism or aldosteronism is the magnesium contents of the lymphocytes (Delva, Lechi, 2003). Magnesium deficiency also observably affects the cardiomyocytes which leads to the alcoholic cardiomyopathia (Patel, 1997; Avsaroglu, 2005).

In general, magnesium and pyridoxine reduce cravings for alcohol, drugs and nicotine. There is a connection between the micronutrient quality of the diet and the formation of the alcoholic dependency (Campbell, 2001). At present, adequate nutritional support is a mandatory component of the long-term rehabilitation of alcoholics and drug addicts. Nutrition and significantly reduces the formation of dependency increases the resistance to alcohol, restores levels of Mg, Zn, Li, Group B and other vitamins. Magnesium and pyridoxine preparations are indicated in chronic alcoholism (in particular, during delirium tremens), to stimulate sobering after an acute alcohol poisoning, to prevent the development of the alcoholic dependency (especially in women and in patients with hyperaldosteronism) and to reduce cravings for nicotine and drugs.

According to the WHO classification adopted as early as 1985, alcohol and nicotine dependence are particular cases of "drug abuse" in which the "drug" is either ethylalcohol or nicotine. There are significant differences in the erythrocyte levels of minerals and trace elements between smokers and non-smokers (Sakamoto, 1999). In particular, erythrocytes of smokers have lower total levels of phosphorus, sulphur and potassium. At the same time, the smokers' erythrocytes are enriched in toxic elements. Magnesium deficiency, however, is the most prominent in current smokers and the extent of the deficiency depends on the pack-years of smoking.

4.4. MAGNESIUM AND CARDIOLOGY

According to numerous large-scale biogeochemical studies (Kousa, 2006; Yang, 2006 and many others), cardiovascular disease (and, in particular, myocardial infarction) is endemically associated with geographic areas characterized by low content of magnesium in the drinking water. Clinical studies have shown that in patients with myocardial infarction the content of magnesium in blood serum falls to critically low values in 24-48 hours after angina (Weiss, 1995).

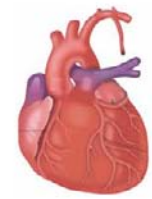
Patients with hypertension, especially women, often suffer from salt-dependent hypertension and produce more pronounced response both to diuretic therapy and to magnesium drugs. It was suggested (Branchevsky, 1985) that an effective replacement for table salt can be composed of magnesium and potassium salts in specific proportions. Our experimental and clinical research strongly indicates that without normalization of the balance of minerals and trace elements, treatment courses that rely solely on cardiovascular drugs of choice tend to be much less successful. The underlying cause for this lack of therapeutic effect of the common cardiovascular drugs is not only because disturbed mineral balance contributes to CVD pathogenesis, but also because the imbalances of minerals alter the pharmacokinetic and farmacodynamic responses to the drugs.

Magnesium deficiency increases with age. Magnesium deficiency results in cardiovascular dysfunction (such as arrhythmia) and leads to a higher risk of CVD. The

magnesium deficiency occurs frequently enough not only among elderly. A survey of the magnesium levels in 16,000 people from Germany indicated suboptimal levels of magnesium (<0.76 mmol/L) in at least 33% of the population sample. The level of magnesium in blood plasma below 0.76 mmol/L is considered as a significant risk factor for stroke and MI in European populations (Schimatschek, 2001).

Lower Mg^{2+} levels increase vasoconstriction of the coronary vessels and the reduced lumen size predisposes to ischemic damage even without thrombus formation, especially when there is considerable atherosclerosis. Therefore, introduction of sufficient quantities of magnesium, calcium and potassium, unsaturated fats, as well as enough antioxidants is important for reduction of cardiovascular mortality. The data on cardiovascular pathophysiology associated with magnesium deficit are summarized in the table 4-2.

Table 4-2. Major morphological manifestations of the cardiovascular damage in magnesium deficiency

Coronary vessels		Heart	
			
1. intima	Edema Hypertrophy Hyperplasia Calcification of the atherosclerotic plaques	**1. perivascular myocardium**	Infiltration Edema Necrosis
2. smooth muscle	Thinning Spasms Connective tissue displasia	**2. fibrosis**	
3. media	Edema Necrosis Hyperplasia	**3. valve defects** **4. endocardiac fibrosis**	

4.4.1. Molecular Effects of the Magnesium on the Biochemistry of the Cardiac Tissue

The detrimental effects of magnesium deficiency on the cardiovascular system are mediated by numerous types of proteins. The magnesium deficiency leads to the loss of function of these proteins and, as a result, to the physiological and clinical manifestations. According to the annotated portion of the human genome, there are at least 500 known magnesium-binding proteins. As show proteomic studies, more than 100 of these proteins are expressed at high levels in various cardiac tissues and, most important, in myocardium. Analysis of the functions of magnesium-dependent cardiac proteins (Torshin, Gromova,

2009) indicated that these proteins can be divided into 8 functional classes: support of the myocardium function, connective tissue of the heart muscle, energy metabolism, intracellular transport, cell cycle, DNA repair, apoptosis and cell proliferation (figure 4-16).

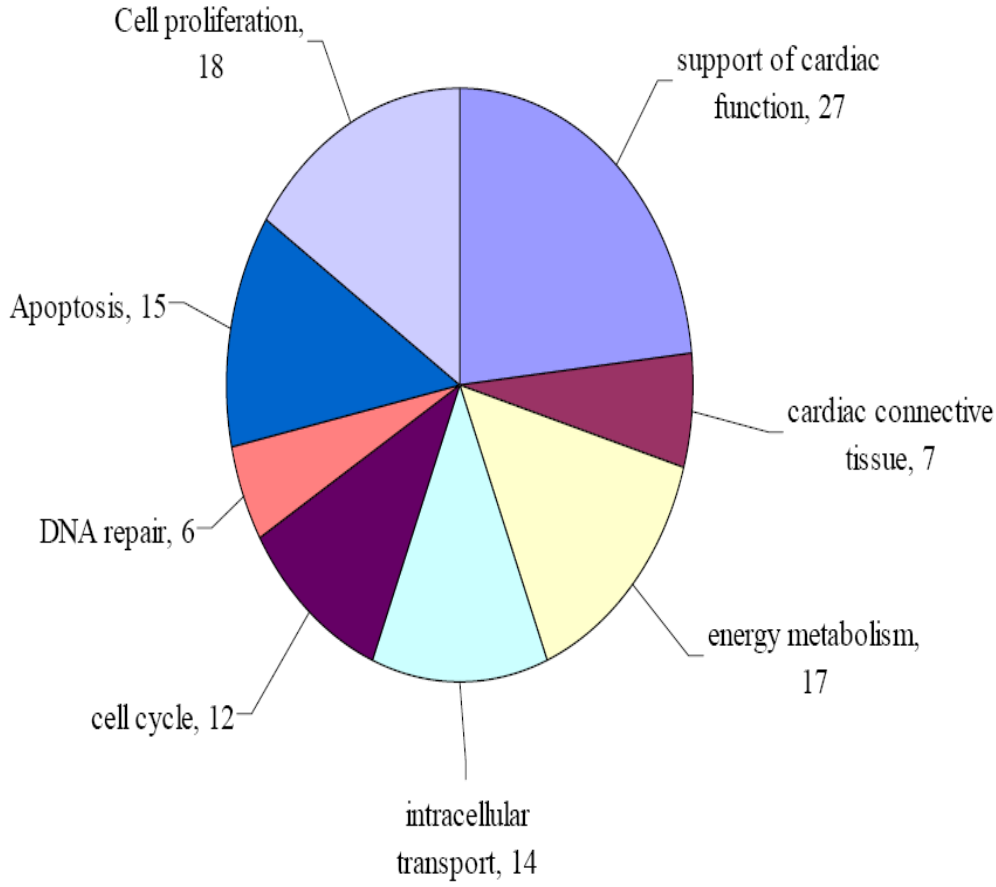


Figure 4-16. Functional classes of the Mg-dependent proteins of the heart tissue. The number of proteins is indicated for each class. For simplicity, each protein was related to only one class.

Many of the proteins belong to several of these functional classes. For instance, MAP kinases (MAPK, MAP2K etc.) are involved not only in the process of apoptosis, but also in cell proliferation and DNA repair. The three functional classes that include the largest number of magnesium-dependent proteins are (1) support of the myocardium function, 27 proteins; (2) energy metabolism, 17 proteins and (3) cell proliferation, 18 proteins. A more detailed classification of the functional properties of these proteins is presented in the figure 4-17. As shown in figures 4-16 and 4-17, the molecular mechanisms of the cardiac effects of magnesium are quite numerous. Systematic analysis and presentation is available in our recent paper (Torshin, Gromova, 2009), below we present some of the particular examples of the Mg-dependent proteins which are important for the condition and the function of the myocardium and of the other cardiac tissues.

Sufficient levels of magnesium are required to control *ion channels of cell membranes* and, most of all, the ATP-dependent potassium inward rectifier channels (KCNJ1, KCNJ2,

KCNJ3, totally 10 genes). These potassium channels are activated by G-proteins (which, in turn, are activated during cellular signal transduction from adrenergic and other types of receptors) and participate in adjusting excitability of the nerve and muscle tissue.

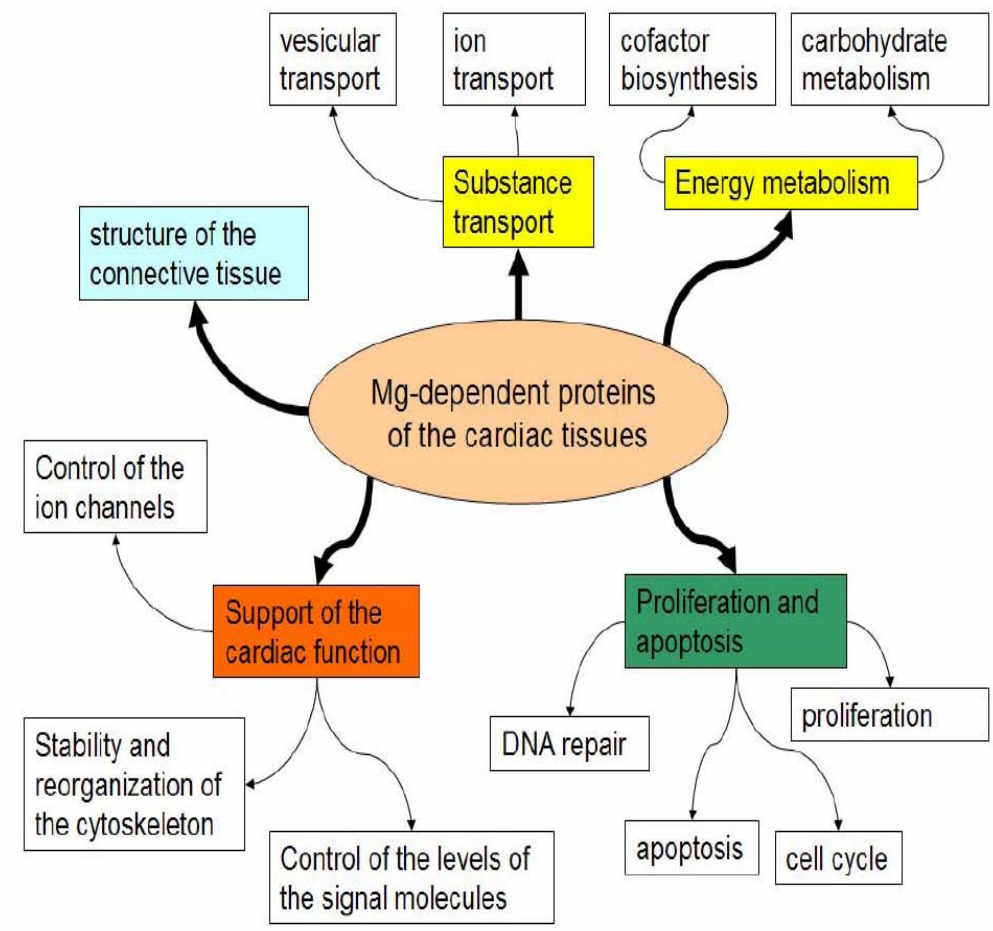


Figure 4-17. Detailed functional classes of the Mg-dependent proteins of the cardiac tissues.

Regulation occurs through «rectifier effect» (i.e., increase of the flow of potassium into the cells) which is based on magnesium's blocking of the potassium transport from inside the cells (figure 4-18). Genetic defects in KCNJ2 and other rectifier potassium channels are the cause of the «long-QT» syndrome, a periodic cardiorhythmic paralysis which can result in sudden cardiac death (Schimpf, 2008).

Magnesium impacts the *energy metabolism of the cardiac muscle* by interacting with specific enzymes as a cofactor essential for catalytic activity. These Mg-dependent proteins are involved in the synthesis of important coenzymes, in carbohydrate metabolism and, inside mitochondria, into pyruvate and fatty acid metabolism. Nicotinamid mononucleotide (NAD), flavin adenine dinucleotide (FAD) and coenzyme-A (CoA) serve as non-protein cofactors for many different enzymes, and, for enzymes involved in energy metabolism.

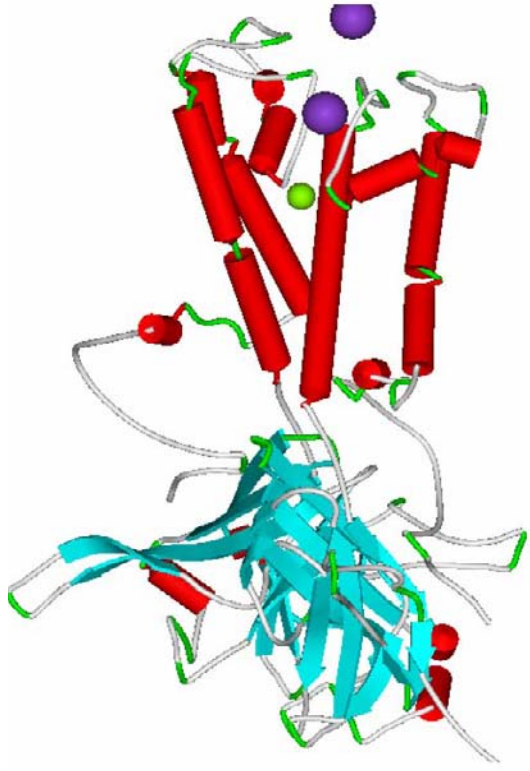


Figure 4-18. The spatial structure of the rectifying potassium channels. Magnesium ion (smaller sphere) and transported potassium ions (two larger spheres) are shown. The model was prepared on the basis of PDB file 1xl6.

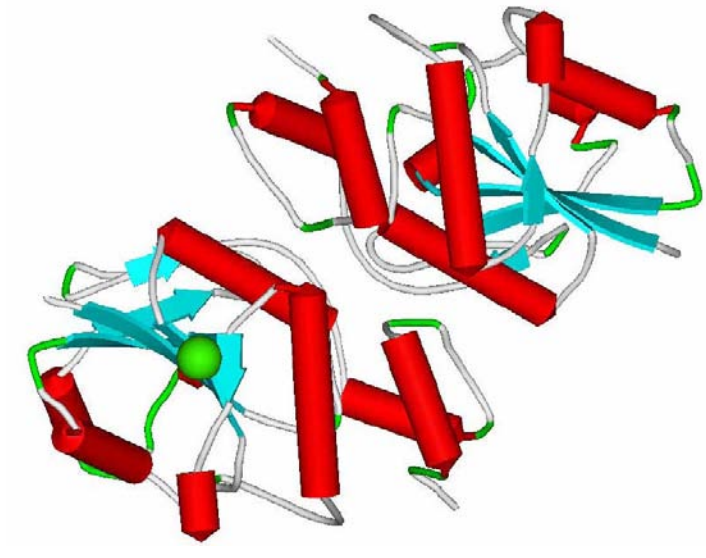


Figure 4-19. Nicotinamide mononucleotide adenyltransferase. Approximate location of the magnesium ion in the structure is shown with a sphere (PDB file 2h2a).

Nicotinamide mononucleotide adenyltransferases 1 and 2 (genes NMNAT1, NMNAT2) catalyze the formation of NAD and ATP (figure 4-19). Peroxisome coenzymes A diphosphatase (NUDT7) regulates the levels of CoA and acyl-CoA in response to metabolic demands. Coenzyme A plays a key role in the synthesis and oxidation of fatty acids as well as pyruvate oxidation in the citric acid cycle.

Glycolytic enzymes enolase (gene ENO2), phosphoglucomutase (PGM3) and phosphofructokinase (PFKP) require magnesium as a cofactor (figure 4-20). Enolase, in addition to playing a prominent role in the final stage of glycolysis, is also involved in a number of other processes such as monitoring the cell growth, response to hypoxia and immune response. Phosphoglucomutase-1 and phosphofructokinase are important enzymes in the glycolytic degradation of carbohydrates.

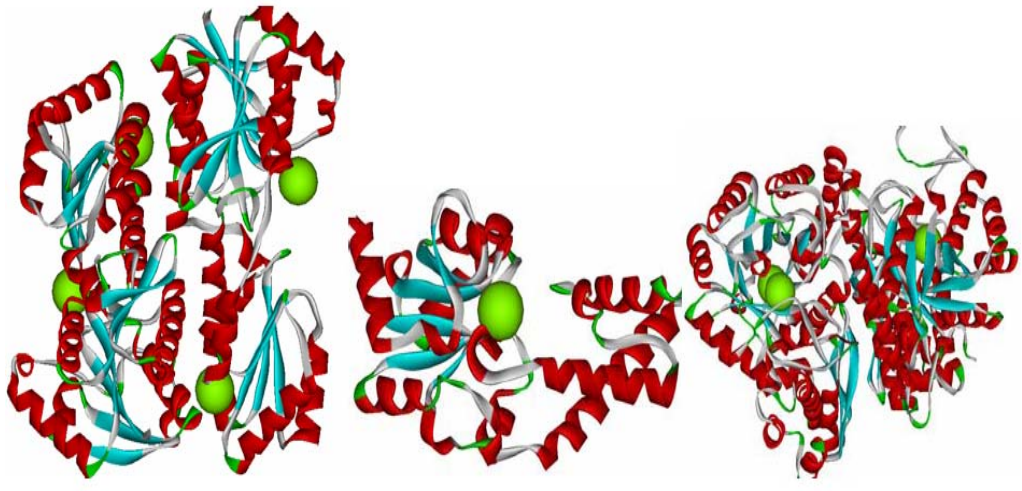


Figure 4-20. Mg-dependent glycolytic proteins abundant in cardiac tissue. Magnesium ions in the active sites of enzymes are shown as spheres. a) phosphofructokinase dimer (model based on PDB 1pfk), b) phosphoglucomutase (PDB 1zol), c) enolase dimer (PDB 2akm).

Levels of magnesium affect *immune response and apoptosis*. A balanced magnesium homeostasis is important for the function of the immune system, apoptosis and cell survival (Tam, 2003). These processes are controlled by signaling proteins called cytokines. In particular, the cytokine TNF («tumor necrosis factor») is a multifunctional pro-inflammatory cytokine. TNF can cause loss of cells through apoptosis, induce cell differentiation, proliferation and inflammation. Disturbances in the TNF levels and activity were associated with different diseases (Locksley, 2001). Signalling from TNF and other cytokine receptors is a multi-stage process. First, the receptor is activated by the related cytokines and initiates the JAK/STAT or MKK (MAPKK) signal transduction. Magnesium is particularly important for the final stage of tranducing the signal through the MAPKK cascades which involve mitogen-activated kinase kinase MAP3K4 and dozens of other Mg-dependent MAP kinases (figure 4-21). Magnesium deficit would prevent the signal transduction thereby reducing the influence of TNF and other cytokines on target cells violating, thus, the balance of apoptosis.

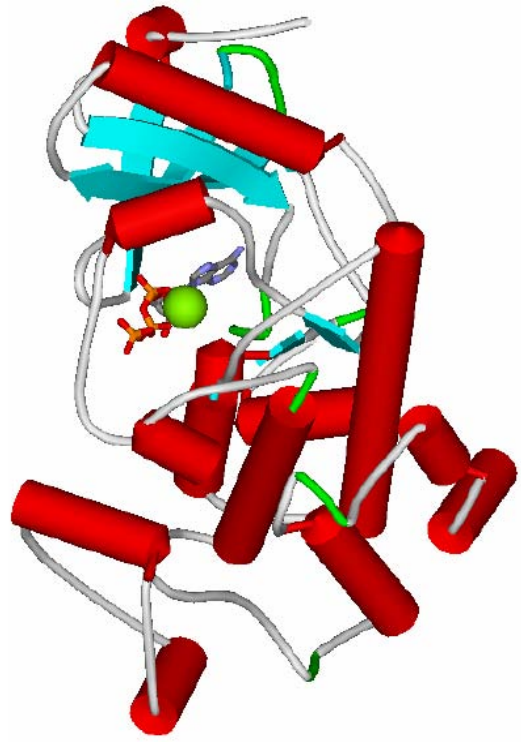


Figure 4-21. The spatial structure of mitogen-activated kinase-kinase enzymes. Magnesium ion (sphere) in the active site is shown along with the ATP molecule (wireframe).

The molecular mechanisms considered above indicate a wealth of influences of magnesium on the cardiac tissue. Taking into account that magnesium deficiency is population-wide phenomenon (16-45% in different geographic regions), it is clear that many cardiovascular patients have this condition. Accordingly, many of the above-mentioned molecular functions will be inhibited which will increase patients' susceptibility to the cardiovascular incidents and aggravate consequences of the incidents. The analysis of the molecular functions also suggests that the rehabilitation of the cardiovascular patients will be more difficult against the background of the magnesium deficiency.

4.4.2. Magnesium and Myocardial Infarction (MI)

In the past 30 years, cardioprotective properties of magnesium came very much under light. A pioneering large scale study was made in early 1960s (Voss, 1962; figure 4-22). More recently, the results of an international multicenter meta-analysis indicated a strong correlation between magnesium deficiency in patients and higher incidence of myocardial infarction (Ioannidis, 2001) as well as between the pharmacological correlation of magnesium deficiency and lower MI incidence. Similar results were reported by Cardiological Society of Finland in 2005 which indicated that the government program for prevention of magnesium deficiency among the population lead to almost 2-fold drop in the incidence of MI over the past 15 years.

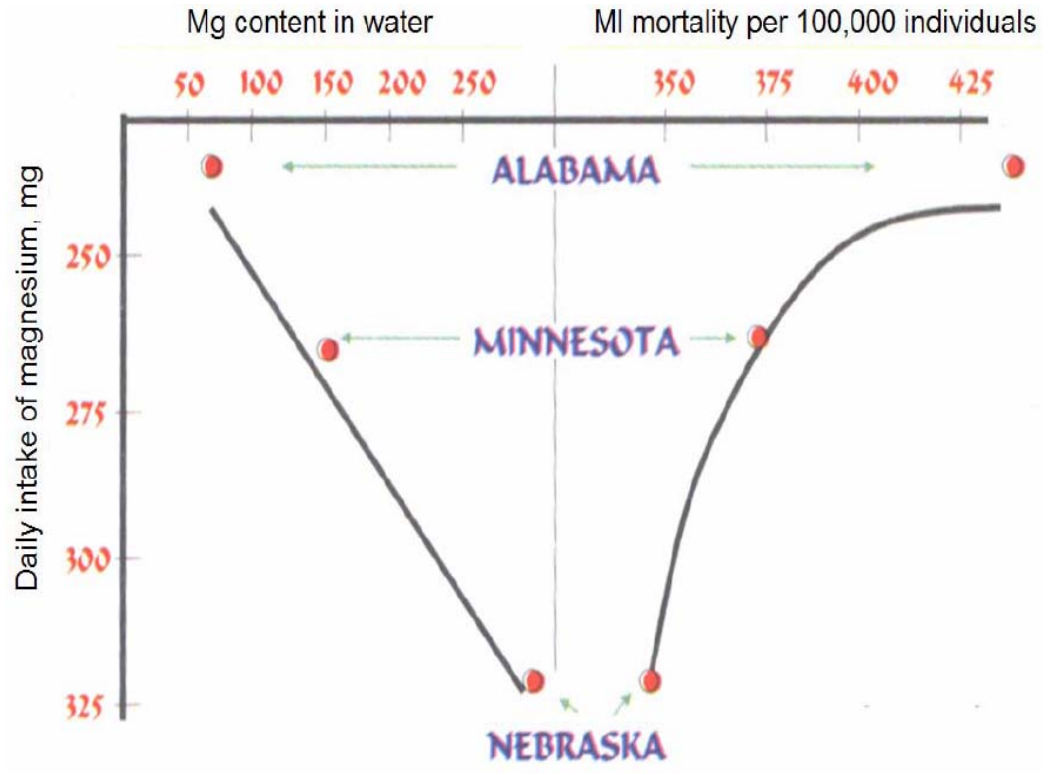


Figure 4-22. The deficit of magnesium and myocardial infarction (Voss, 1962).

One of the first studies that proven that magnesium therapy influences MI outcomes was Leicester Intravenous Magnesium Intervention Trial (LIMIT-2) performed in 1987-1992 (Woods, 1992). In this placebo-controlled, randomized, double-blind study 2316 patients with suspected MI received, in addition to the standard cardiovascular treatment course, received 8 mmol magnesium sulfate intravenously during 5 minutes and then 65 mmol of magnesium sulfate during the day. In control group, saline was used as a placebo instead of the magnesium. During the period the patients were in the cardiac emergency department, the incidence of the left atrial failure was 25% lower in patients that received magnesium treatment ($P = 0.009$) as compared to the control group. Among the controls, the fatality rate during the first 28 days post-MI was 10,3% while in the group after magnesium treatment-7.8% ($P = 0.004$, figure 4-23). The total IHD mortality was 21% lower ($P = 0.001$).

By the standards of those years, these results were so impressive that magnesium infusions were introduced by American Association of Cardiologists (1994) as one of the recommendations for the treatment of MI. In subsequent studies of the era of powerful drug combinations (nitrates+ ACE inhibitors + beta-blockers + thrombolytics) supplementation of the drugs with magnesium did not have a significant effect on the reduction of the mortality rate. The results of these studies indicate, however, that in patients with magnesium deficiency the magnesium correction improved survival, removed diverse symptomatics of magnesium deficiency (cramps, arrhythmia, depression and anxiety), reduced the intensity of the chest pain, improved sleep and emotional balance.

The results of the LIMIT-2 study are still important: they distinctly outline the favorable impact magnesium treatment has on the course and outcome of MI. *In numerous cases when*

implementation of the modern standards of pharmacological treatment does not seem possible for various reasons (for example, contraindications of drugs, old age, persistent arrhythmias, socioeconomic status etc), a magnesium therapy may be the means of saving the life of the MI patients.

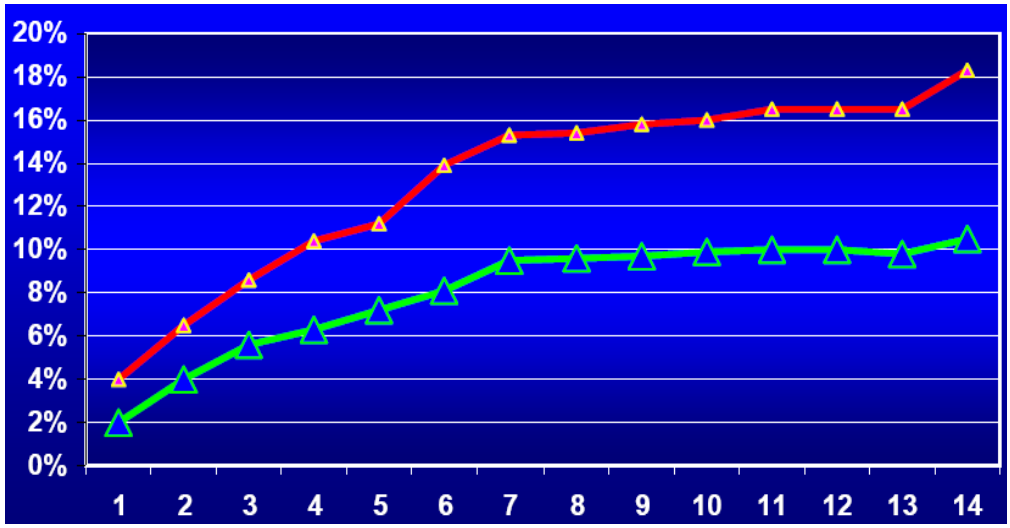


Figure 4-23. Study LIMIT-2. The upper curve: mortality rate of the patients receiving placebo; the lower curve: mortality rate of patients who received magnesium treatment.

On the contrary, the 2002 study called "MagNET" (Spatling, 2004) showed no difference in MI mortality between the group that received magnesium intravenously and the controls. However, the international community has since recognized that the MagNET study does not meet the standards of the evidence-based medicine in a number of ways (Spatling, 2004), in the same way as the MAGIC study we have mentioned earlier. Firstly, the prescription of the magnesium dosages for particular patients and the subsequent data analyses were carried out without consideration of the blood magnesium levels. Secondly, the adjustments for pharmacotherapy with drugs that can seriously affect magnesium levels (diuretics, beta-blockers, ACE inhibitors, antibiotics, antacids *etc*) were not made. The same consideration concerns the drugs that substantially affect the MI mortality. Thirdly, the regimen of the drug injection does not appear to be adequate. Due to these and several other flaws of the MagNET study, a number of subsequent studies were performed which presented many positive influences that magnesium therapy has upon cardiovascular health (Shiga, 2004; Shechter, 2005; He, Liu, 2006 etc).

More thorough biochemical research in animal models and human cells shows, time and again, that low levels of plasma and erythrocyte magnesium accompany myocardial infarction. While sodium and calcium content in the damaged cells of myocardium increase, potassium and magnesium levels decrease. Using a large dose of magnesium in the first hours of acute MI allows to limit the size of the infarct (Svyatov, 1999). The maximum effects were observed with organic magnesium salts (magnesium adipinate, magnesium asparaginate, magnesium levulinate, magnesium nicotinate, magnesium orotate, magnesium pidolate, magnesium citrate). Work of Svyatov (1999) demonstrated that painless myocardial ischemia is a characteristic feature of the clinical course of MI against the background of magnesium

deficiency. Albeit the word "painless" suggests some positive effect, the phenomenon of the painless ischemia poses, actually, considerable difficulties in accurate diagnostics and timely assistance. In other words, proprioception and nociception are dulled because of the magnesium deficiency and, as a result, the patient is not able to feel the early signs of the ischemia (such as chest pain, for instance).

As biochemical studies indicate, the development of clinical MI is, as a rule, accompanied by steady decline of the magnesium levels. In the acute phase of ischemic myocardial infarction, magnesium levels in plasma can drop to as low as 0.45 mmol/L (while the norm is ~0.8 mmol/L). Already during the first hours of acute MI, the elongation of the QT interval (i.e., predictor of fatal arrhythmia) can be often observed. The longer QT correlates with a massive loss of intracellular magnesium during the first hours after the infarction. The low concentration of intracellular magnesium augments the tendency for vascular spasms thus increasing excitability of smooth muscle in terms of their sensitivity to vasopressors. Moreover, the patients with magnesium deficiency also have somewhat suppressed diastole (since myocardial relaxation is more difficult because of the lack of Mg^{2+} and Mg-ATP). And it is this difficulty in the relaxation of myocardium that is related to myocardial ischemia. The deficit Mg^{2+} and the growth of the tissue content of Ca^{2+} accompanies post-ischemic reperfusion syndrome and the critical imbalance in Mg^{2+} and Ca^{2+} results in cardiomyocyte apoptosis. At the same time, usage of the modern magnesium drugs (MagneB6) or even of the old-style magnesium sulfate can prevent development of severe arrhythmias, reduce size of infarct, and thus can prevent the recurrent MI.

The QT elongation is one of the signs of magnesium deficiency that is visible on ECG. Other signs include T-inversion, elongation of the S-T interval (or of the P-R and S-T), flattening and widening of the T-wave (Kurbanov, 2004) and an appropriate technique to calculate these parameters is described in the same work. In order to determine these parameters precisely enough, it is necessary to have ECG recording with time resolution of at least 50 mm/s and with amplitude resolution of at least 1 mV=10mm. It should be noted that manual measurements of the Q-T intervals can sometimes produce more accurate results than computer-based estimates. The QT interval is measured in seconds and is defined as the distance between the beginning of the Q-wave (initial indent of the QRS complex, figure 4-24) and the end of the T-wave (ventricular repolarization *per se*).

The "corrected QT interval" (QTc) is obtained by using Bazett formula: $QTc=QT/\sqrt{R}$, where R is the interval from the onset of one QRS complex to the onset of the next QRS complex. As abnormally elongated are considered QTc values longer than 420-440 ms (figure 4-25). Bazett formula is not very accurate at too low or too high heart rate. Another criterion defines "long QT interval" as the one exceeding by more than 50 ms the QTc values tabulated for given heart rate. Dispersion of QT interval (dQT) is defined as the difference between the maximum and the minimum values of the QT measured in the 12 standard leads.

End of the T-wave is defined as the point at which the T-wave returns to the isoelectric line T-P. In case when at the end of the T-wave the U-wave (repolarization of the Purkinje fibers) is recorded, the Q-T interval is measured until the minimum between T and U. The leads in which it is impossible to accurately determine the end points of the T-wave, ECGs showing signs of ciliary arrhythmia or QRS complexes broader than 0.11s are not analyzed.

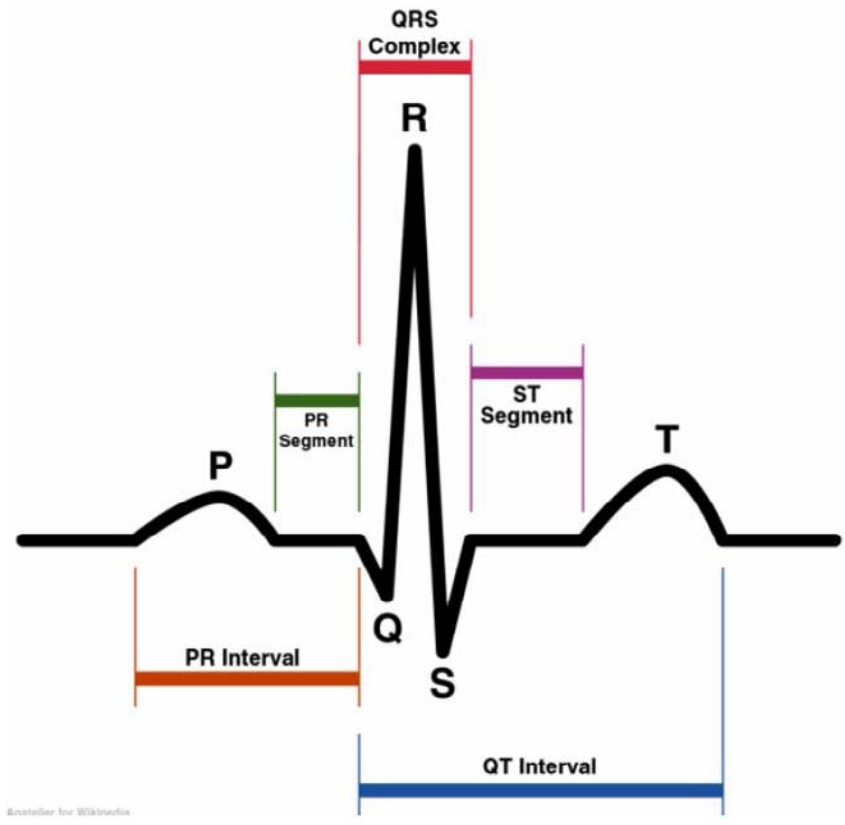


Figure 4-24. Schematics of the normal sinus rhythm of ECG.

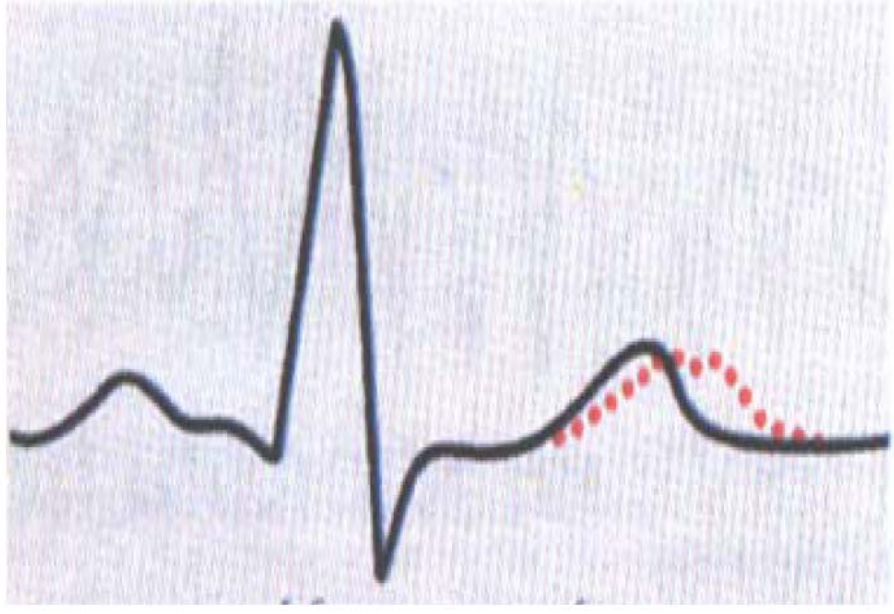


Figure 4-25. Widening of the T-wave of ECG.

Normally, dQT should not exceed 80 ms. During interpretation of the QT interval in respect to the magnesium deficiency, it is advisable to take into account the impact of the drugs listed in the table 4-3.

Table 4-3. Drugs that cause elongation of QT interval (Pickering, 2006)

Antiarrhythmics	Class I: hinidin, aymalin, prokainamid
	Class III: amiodaron, bretilium, sotalol, nibentan
	Class IV: verapamil, diltiazem
Psychotropic drugs	Fenotiazins: frenolon, triftazin
Antidepressants	imipramine, amitriptyline, pimozid
Beta-adrenomimetics	salbutamol, terbutanol, fenoterol etc (also increase Mg elimination)
Heart glycosides	strofantin, corglicon, digoksin (increase Mg elimination)
Arterial vasodilators	Dihydrolpyridins,fentolamin
Antibiotics	erythromycin, halofantil, chloroquine etc (rapid and irreversible withdrawal of magnesium from cells)
Antihistamines	astemisol, terfenadin, dimedrol, pipolfen, diazolin (*1)
Diuretics	Furosemide (*1) uregit (increases Mg elimination), hypothiazid, osmotic diuretics
Prokinetics	Metoklopramid, cizaprid
Purgatives	Slow down magnesium adsorption in the small intestine
Antitumor drugs	Cisplatinum (sharply increases Mg elimination and decreases readsorption in glomeruli)
Psychostimulants	Caffeine, ephedrine, nicotine (withdrawal of magnesium from cells)
Hormonal drugs	Insulin
Estrogen derivatives	Reduce intestinal adsorption of magnesium and increase demand for pyridoxine
Peroral Ca-based drugs	Calcium D3 etc (slow down Mg absorption through competition for the same transport systems)
Phosphorus-based drugs	slow down Mg absorption

(*1) Antihistamines of the 1st generation and furosemide are most dangerous since they lead to maximal observed elongation of the QT interval.

In urgent situations, reanimathologists can be recommended to determine the levels of magnesium every 2-3 hours. When planning surgery with artificial hypothermia, it can be helpful to use intravenous magnesium provided that the magnesium levels in blood are regularly monitored (since in the case of acute MI, complicated by rhythm irregularities, the use of magnesium can lead to lower morality).

4.4.3. Magnesium and Arrhythmia

Arrhythmia is one of the serious complications of MI that increases post-infarction mortality and which also greatly depends upon the magnesium levels in body. Magnesium regulates the potassium-dependent and other ion channels of myocardium. Competing with Ca^{2+} for the same binding sites, magnesium alters the speed of the release of calcium from interaction with molecular motors of the myocardium. At the same time, the levels of potassium and magnesium in blood plasma are interdependent: normal magnesium levels

prevent the loss of potassium while magnesium deficiency contributes even more to the development of arrhythmia when aggravated by hypokaliemia (often caused by diuretics). The normal balance of magnesium is a prerequisite for normorhythmia of the heart and also plays a synergic role in the cardioprotective action of the beta-blockers such as verapamil, nifedepin, and cinnarizine.

Due to the distinct positive correlation between physiological levels of magnesium and muscular relaxation, prolonged hypomagnesemia correlates with a higher incidence of ventricular extrasystolics, tachycardia and fibrillations. Antiarrhythmic effect of magnesium drugs was confirmed in a number of studies. Holter monitoring of healthy volunteers who undergone electric cardioversion (achieved through intravenous 500.0 ml solution of 0.9% NaCl) indicated that intravenous injections of magnesium solution (5g of $MgSO_4$) reduced supraventricular activity, ventricular arrhythmia, the incidence of atrial fibrillation as well as the emergence of ventricular extrasystols. The combinations of magnesium lactate, magnesium orotate and pyridoxine are known to increase the effectiveness and safety of the therapy with 3rd class antiarrhythmics (Leatham, 1993). These and other magnesium preparations can be used for a number of arrhythmia-related conditions (Box 5).

Box 5. Magnesium preparations and arrhythmia conditions

Magnesium preparations can be recommended when patient has:

- tachycardia and flickering
- ventricular arrhythmia with magnesium deficiency
- ventricular arrhythmia due to digitalis medication
- atrial tachycardia
- hypokaliemic arrhythmia
- ventricular arrhythmia with myocardial infarction
- tachyarrhythmia during treatment with antiarrhythmics
- atrial ciliary arrhythmia.

Proper application of the magnesium sulfate is quite efficient for the profylaxis of the post-operational arrhythmias (Mitchell, 2007; Shepherd, 2008). The high frequency of complications in the form of cardiac arrhythmia and ventricular tachycardia in patients who underwent artificial hypothermia (Polderman, 2001) can be attributed to the development of extreme hypomagnesemia: during the first 6 hours of the impact of low temperature, the level of magnesium in blood plasma lowers from 0.98±0.13 to 0.58±0.13 mmol/L, possibly through acute depletion of the Mg-containing energy substrates ATP, ADP and others.

Magnesium imbalance contributes to the development of cardiac tachyarrhythmia (abnormally fast heart rate). Intracellular magnesium deficiency stimulates an increase in the activity of sinus node, which shortens the time of atrioventricular transmission (Voelger, 1991), reduces absolute refractory period (during which a second action potential absolutely cannot be initiated) and elongates relative refractory period (during which initiation of a second action potential is inhibited but not impossible). As the result, various forms of arrhythmia develop.

Low level of magnesium in the blood of patients diagnosed with tachycardia is one of the causes of CVD mortality. Even using magnesium source as simple as intravenous magnesium sulfate restores sinus rhythm and transforms atrial tachycardia into sinus tachycardia (Stühlinger, 2000).

Magnesium preparations are most effective with ventricular tachycardia of the type *torsade-de-points* (figure 4-26). The *torsade-de-points* pattern is a sure sign of the ventricular fibrillation and occurs more frequently in arrhythmia patients who also have long QT interval. The physiological condition underlying this pattern is one of the main reasons for sudden death of the patients with elongated QT. This "dance" can accompany development of the proarrhythmic effects of the class III antiarrhythmics. Prescription of the intravenous magnesium appears to be the only effective method of treatment, with magnesium injections being prescribed A.S.A.P. regardless of the levels of magnesium in blood.

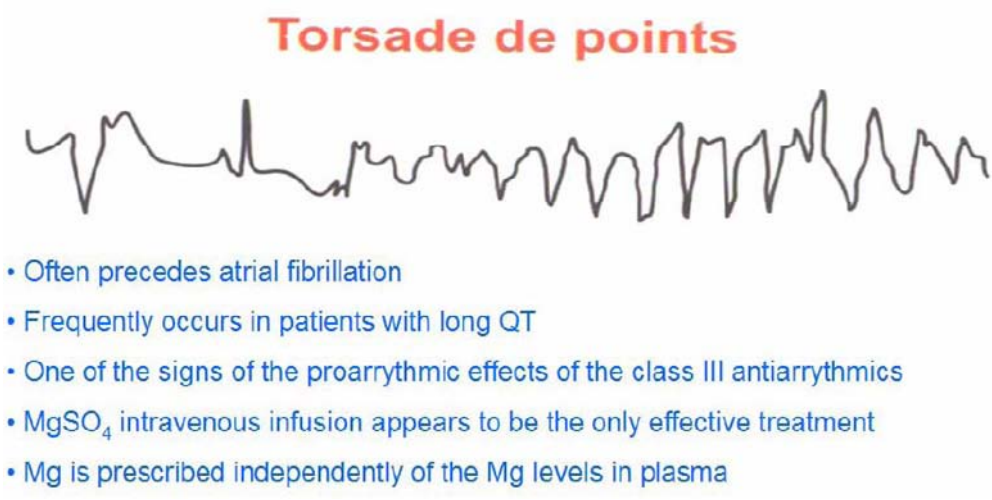


Figure 4-26. Arrhythmia *torsade-de-points* ("dance of the points").

As the result of adequately planned and successfully performed German multicenter randomized study MAGICA (Zehender, 1997), prescription of preventive magnesium and potassium medications became a standard arrhythmia treatment in a number of European countries. According to this standard, magnesium and potassium are introduced before or during the therapy with antiarrhythmics, diuretics, or cardiac glycosides. This study of the 322 patients with cardiovascular disease and suffering from ventricular arrhythmia has shown antiarrhythmic effect of 3-week magnesium/potassium therapy with dosage corresponding 150% of daily requirements (Mg asparate at 6 mmol/day and K asparatate 12 mmol/day). After 3 weeks, the number of ventricular extrasystols decreased by 12,2% ($p < 0.001$) in patients who took magnesium and potassium salts, while in the case of the patients who took placebo, the figure rose by 2.2%. The number of extrasystols in the group on magnesium reduced by 60-70% in comparison to the control group.

In many modern antiarrhythmic drugs, paradoxical proarrhythmic effects were identified. These effects increase several-fold mortality of the MI patients. Usage of the magnesium medications within a scheme of integrated treatment of acute MI in combination with

ventricular arrhythmia reduces mortality from the disease (Parikka, 2002). Magnesium therapy with the help of organic forms of magnesium like magnesium pidolate, magnesium lactate, magnesium citrate, and magnesium orotate can weaken these proarrhythmic effects (figure 4-27).

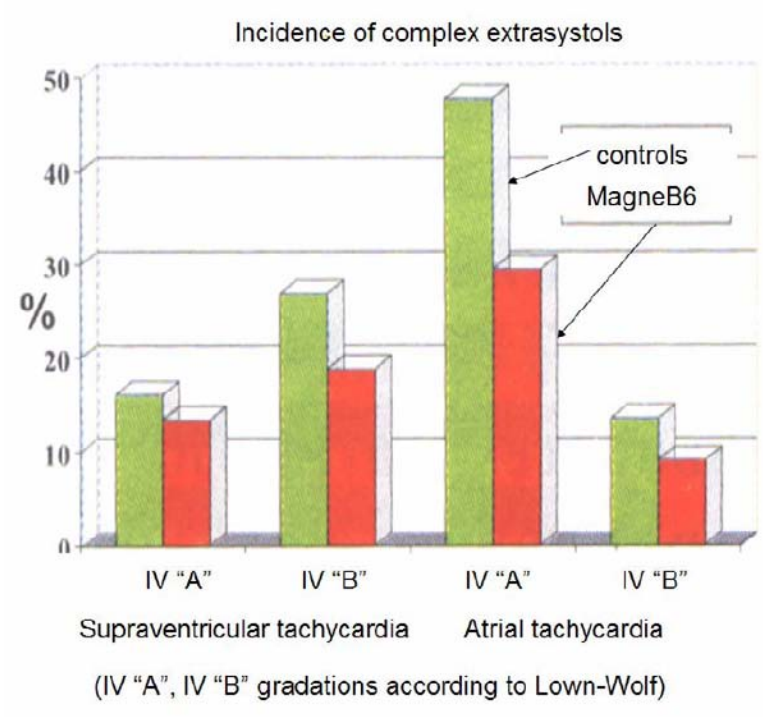


Figure 4-27. Impact of Magne B6 on the frequency and nature of disturbances of the heart rate (Kiyakbaev, 2001).

4.4.4. Magnesium and Arterial Hypertension

One of the major causes of arterial hypertension is the high level of stress associated with life in modern society. The detrimental influence of stress gets significantly aggravated by an improper schedule of the food consumption and the low nutritional quality of the common foods. A study compared 138 nuns and 126 lay women 35-60 years old which had similar ethnic roots (Italians), anthropometric indicators, and family history of hypertension (Timio, 1985). Differences between groups were (1) social environment (the nuns adhere to a quiet and secluded way of life without alcohol and tobacco, while the women of the control group lead typical western way of life); (2) diet (nuns observe fasts), (3) quality of food (the nuns do not consume junk foods or excessive salt, their diet consists of cereals, vegetables, fruit, nuts). In the group of nuns in 20 years of observation, there hasn't been any increase in the blood pressure (figure 4-28), while in the control group the typical age dependence of high blood pressure was observed (Timio M, 1985). Observations over the next 10 years showed a significantly higher mortality from cardiovascular causes in the group of "secular" women (Timio, 1999).

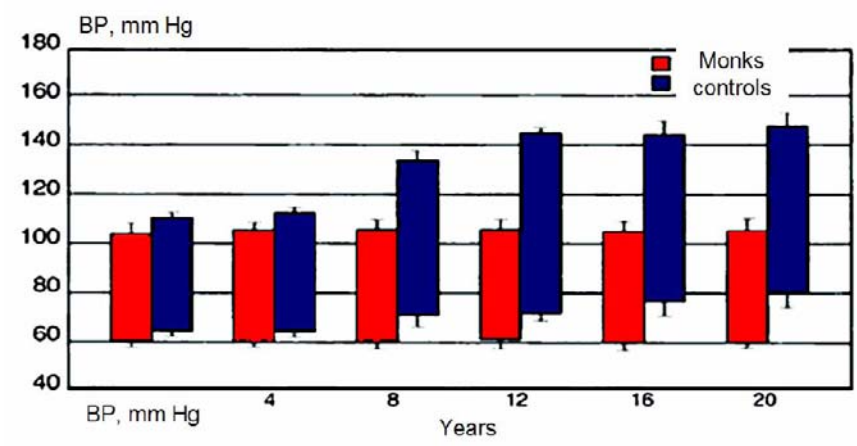


Figure 4-28. A study of nuns and lay women points to stress as a major cause of hypertension (Timio M, 1985).

Magnesium is a natural regulator of vascular tone. Shortage of magnesium and calcium in food and water is known to increase the frequency of hypertension (Singh, 1989). This result is also corroborated by biochemical and physiological studies which proven participation of magnesium in the regulation of arterial pressure. Firstly, Mg^{2+} ions, along with other essential trace minerals are involved in the regulation of osmotic balance. Secondly, magnesium affects the tonus of the vascular smooth muscle and is a well-known vasodilator. As simultaneous application of magnesium, nifedipine, indometacin blocks the relaxing effect of magnesium on the blood vessels, it is possible that vasodilatory effect of magnesium occurs through interactions of prostacyclins with calcium channels. Thirdly, magnesium reduces the effects of endogenous vasoconstrictors: adrenaline (through COMT enzyme, Chapter 1), aldosterone, vasopressin, and angiotensin-2.

Chronic magnesium deficiency against the background of essential hypertension is a significant risk factor for acute stroke as the blood vessels supplying the brain tissue are extremely sensitive to magnesium balance. At very low magnesium levels, cerebral vascular spasms can lead to ischemic damage even without obstructions of the blood flow in the form of thrombi. The best strategy to avoid Mg-dependent stroke is dietary correction (diet rich in fresh fruits, vegetables and seafood) along with rationally adopted physical exercise.

Hypertension can be reduced by lowering the blood aldosterone. This most efficient way to achieve this goal is to use diuretics. However, this procedure does not only increase excretion of aldosterone, but also of the mangesium which has detrimental effects on the cardiovascular system. Low concentrations of extracellular magnesium lead to vascular spasms and parenteral infusion of magnesium sulfate in hypomagnaemic patients significantly reduces blood pressure (by 20 mmHg). Generally, prescription of parenteral magnesium sulfate in patients with severe arterial hypertension is not sufficient in the form of monotherapy.

The negative side effects of diuretics can be reduced by using specific magnesium-preserving diuretics. For example, hypothiazid stimulates much less excretion of calcium, magnesium and potassium than furosemide. Potassium-preserving diuretics are, often, also magnesium-preserving (Shah, 2007; Skvortcova, 2008).

In the treatment of arterial hypertension, it is necessary to take into account the differences in the course of the treatment of hypertensive disease among the men and women (figure 4-29). In women, aldosterone fluctuations and the dependence of blood pressure on magnesium are more pronounced. Women also respond with more intense diuresis to magnesium infusions. There are a number of characteristics in pathogenesis and clinical course which occur more frequently in hypertensive women than in hypertensive men:

- Hypertension in more often Na-volume dependent;
- Increased rigidity of vessels (Ca:Mg imbalance);
- Disturbances of the circadian rhythm of blood pressure;
- Left ventricular hypertrophy, diastolic dysfunction;
- Orthostatic hypotonia (varicose veins, lack of sympathetic stimulation, vegetative neuropathy);
- Hypertension combined with metabolic syndrome;
- Hypertension in women in more sensitive to smoking and alcohol intake.

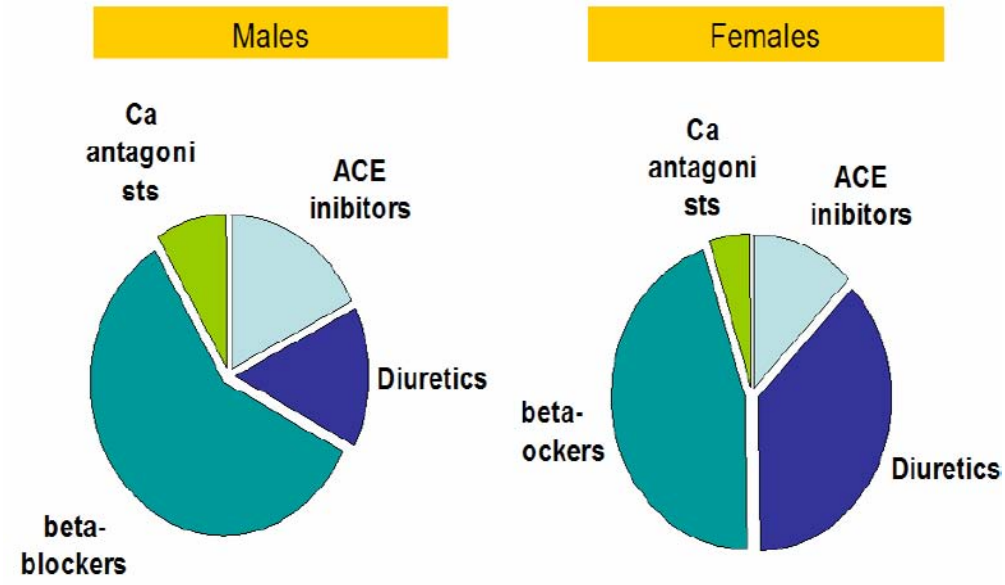


Figure 4-29. Differences in pharmacotherapy of men and women with arterial hypertension (Klungel, 1998), 56026 patients aged 20-59 years.

The choice of diuretics for the hypertensive patients is very important. For instance, hypothiazid has calcium-preserving effect and it is safer to use than furosemide because hypothiazid leads to considerably smaller losses of calcium, potassium and magnesium. Generally, potassium-preserving diuretics (veroshpiron *etc*) are, simultaneously, also magnesium-preserving ones. Unlike hypertensives and those with hypomagnesemia, infusion of magnesium sulfate solution in healthy subjects has little impact on the blood pressure. The reliable effect of lowering blood pressure by 20 mmHg or more is achieved only with introduction of therapeutic dosages of magnesium (200-500 mg of magnesium salts) in hypomagnemic patients. Therefore, adequate magnesium level is a necessary but not a sufficient condition for the normal blood pressure.

4.4.5. Magnesium and Atherosclerosis

A long-term magnesium deficiency correlates with decreased HDL, increased TG, LDL, VLDL particles in blood plasma and, accordingly, with a higher extent of atherosclerosis. From molecular point of view, magnesium is required, in particular, in the active sites of mitochondrial enzymes of fatty acid metabolism. The mitochondrial proteins of the *fatty acid metabolism* are, before all, the long-chain-fatty-acid--CoA ligases ACSL1, ACSL3, ACSL4, ACSL5, ACSL6 and the acyl-coenzyme A synthetases ACSM1, ACSM2A, ACSM2B, ACSM3, and ACSM5 (figure 4-30). Long-chain-fatty-acid--CoA ligases activate long-chain fatty acids for both synthesis of cellular lipids and degradation via beta-oxidation pathway. ACSLs differ in their substrate specificity: for example, ACSL1 preferentially uses palmitoleate, oleate and linoleate; ACSL3 preferentially uses myristate, laurate, arachidonate and eicosapentaenoate while ACSL5 has a wide range of saturated fatty acids substrates. Acyl-coenzyme A synthetases (ACSMs) link medium-chain fatty acids to coenzyme-A and also differ in their substrate specificity. Magnesium deficiency causes considerable drop in catalytic activity of these enzymes and results in the above-mentioned unfavorable changes in the lipid profile.

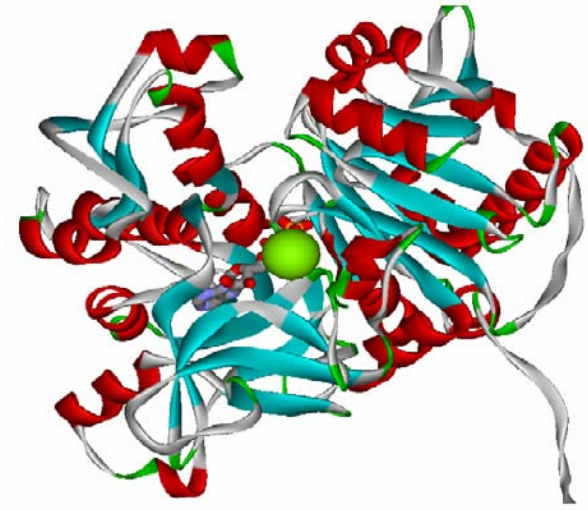


Figure 4-30. Model of the full atomic structure of an acyl-coA synthetase. Substrate analogue is shown in “wireframe” mode. All Acyl-CoA synthases have similar full atomic structure and require magnesium (sphere) in the active site.

As the result of impaired activities of long-chain-fatty-acid--CoA ligases and acyl-coenzyme A synthetases, deficit of magnesium reduces the content of phospholipids in biological membranes and also lowers plasticity of biological membranes. Lack of magnesium also lowers antioxidant response of the body. As the result, higher levels of the oxidized lipids will induce proiflammatory response of the immune system which will lead to the formation of the new atherosclerotic plaques. Prolonged hypomagnesemia does not only activate atherosclerosis, but also leads to fatty infiltration of the liver (Calderon, 2000).

Magnesium is a natural hypolipidemic agent. A dietary magnesium correction succeeds in improving triglyceride profile (see also section 4.6 further). The levels of triglycerides are

reduced more efficiently by using magnesium rich diet (or magnesium salts) and an appropriate physical exercise. Physical exercise appears to potentiate Mg-dependent enzymatic digestion of the excess of triglycerides (Nielsen, 2006).

4.4.6. Magnesium and Thrombus Formation

It is well known that magnesium deficiency increases the propensity for the thrombus formation (Vormann, 1998). And contrariwise – magnesium preparates can slow down formation of the arterial thrombi (Sheu, 2002). The inhibitory effect of magnesium on the thrombus formation is dose-dependent. The deficit of magnesium increases the levels of thromboxane A2, accompanied by vascular wall damage and, accordingly, blood coagulation through the intrinsic pathway. Magnesium may also delay the formation of arterial blood clot through inhibition of platelet activity.The Ca^{2+}: Mg^{2+} ratio shifted towards excess calcium is also a likely reason for the increased coagulation under conditions of magnesium deficiency.

A comparative study of the impact of magnesium sulfate and acetylsalicylic acid on the platelet aggregation in healthy subjects has shown that both magnesium and aspirin have equally powerful anticoagulant effect and inhibition of coagulation occurred after adding already at low concentrations (0.5-1.0 mM) of the magnesium sulfate solution (Kurup, 2003). Subnormal and low levels of magnesium are recognized risk factor of the "final thrombus formation" in patients with myocardial infarction and thromboembolia (Kumari, 1995).

In our recent study, we performed a systematic analysis of the major physiological and molecular mechanisms of thrombophilia under conditions of magnesium and pyridoxine deficiency (Torshin, Gromova, 2009). These mechanisms include synthesis of thromboxane, hypercoagulable states, inflammation of the vascular endothelium, atherogenic lipid profile, structure of connective tissue, and hyperhomocysteinemia. The results support the notion that magnesium exhibits both disaggregant and anticoagulant properties.

Hemostasis is a complex physiological process by which blood is converted from the liquid, flowing state into a condensed, non-flowing state. Hemostasis can be divided into four major stages: 1. local vasoconstriction to restrict blood flow at the site of damage, 2. activation of platelets and clot formation at the initial location of damage, 3. cross-stitching of the initial clot by fibrinous filaments (coagulation), and 4. partial, then complete dissolution of te fibrin clot to restore the normal flow of blood (fibrinolysis).

Although coagulation is one of the main processes of hemostasis, it is far from being the only factor influencing the formation of clot. For example, the presence of atherosclerotic plaque narrows the vessel lumen and makes the vessel blockage by a clot more probable; proinflammatory processes associated with atherosclerosis, may themselves initiate platelet aggregation *etc*.

In general, the physiological mechanism of thrombotic events can be described by three basic physiological mechanisms (the Virchow's triad):

1. changes in blood flow (vasoconstriction),
2. changes in blood coagulability (coagulation, fibrinolysis), and
3. changes in the vascular wall (atherosclerosis, inflammation, a violation of the structure of connective tissue).

These pathophysiological processes are summarized in the figure 4-31. These processes are interrelated and virtually all of the processes depend on the levels of magnesium in the blood (with the exception of antiphospholipid syndrome, hyperhomocysteinemia and, perhaps, endocrine dysfunciton). Hyperhomocysteinemia greatly depends on the levels of the group B vitamins: pyridoxine (vitamin B6), cyancobalamine (B12) and folic acid (B9).

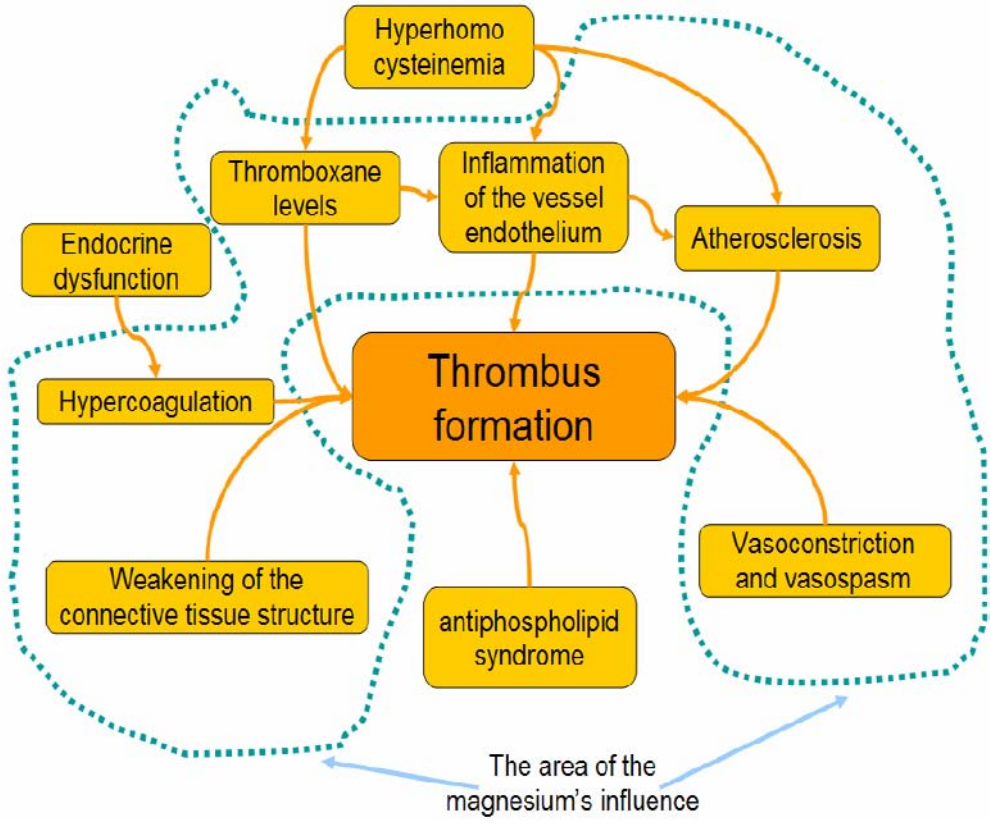
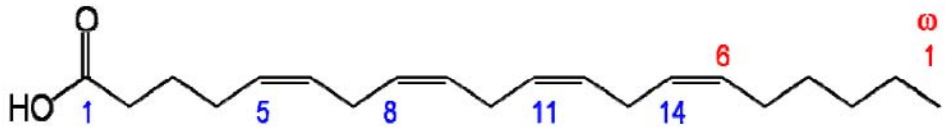


Figure 4-31. The basic pathophysiological processes contributing to thrombotic events. Magnesium has an impact on most of the processes.

Magnesium and Thromboxane Synthesis

Magnesium inhibits the effects of many agonists leading to platelet aggregation, and, above all, thromboxane A2 (Shechter, 2000). Decrease in the levels of magnesium deficiency leads to increased levels of thromboxane A2 in blood plasma, and urine. Thromboxane is a prostanoid synthesized in the arachidonic acid cascade (figure 4-32). Arachidonic acid - a kind of omega-6 polyunsaturated fatty acid (ω-6 PUFA) present in large amounts in the phospholipids of cell membranes. In the first stage, the arachidonic acid is synthesized from phospholipids by phospholipase which catalyzes hydrolysis of phospholipids. Then, arachidonic acid is converted to different classes of prostanoids (prostaglandins, prostacyclins and thromboxanes).

From the molecular point of view, the most likely mechanism of the magnesium's influence on thromboxanes is the direct inhibition of phospholipase by magnesium. Phospholipase enzymes contain two calcium ions in the active center (figure 4-33). Recalling the calcium-magnesium antagonism (based on physico-chemical properties of the two ions), we can assume that magnesium, substituting calcium at the active site, will inhibit phospholipase activity. Magnesium may also affect the activity of enzymes not directly, but through the antagonistic influence on the calcium homeostasis at the cellular level.



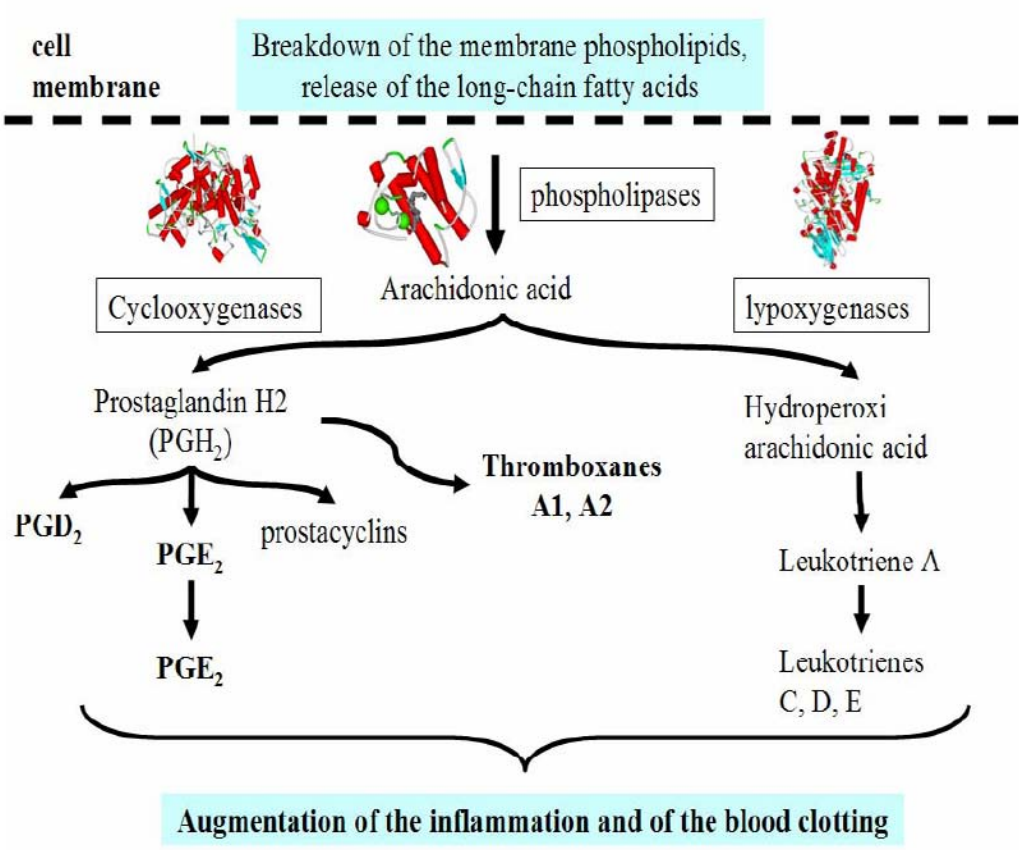


Figure 4-32. Arachidonic acid and the arachidonic acid cascade. A) The structure of arachidonic acid, at the bottom - the numbering of carbon atoms from the carboxylic end of the molecule, at the top ─ numeration from the omega-end. B) Cascade of biotransformations of the arachidonic acid.

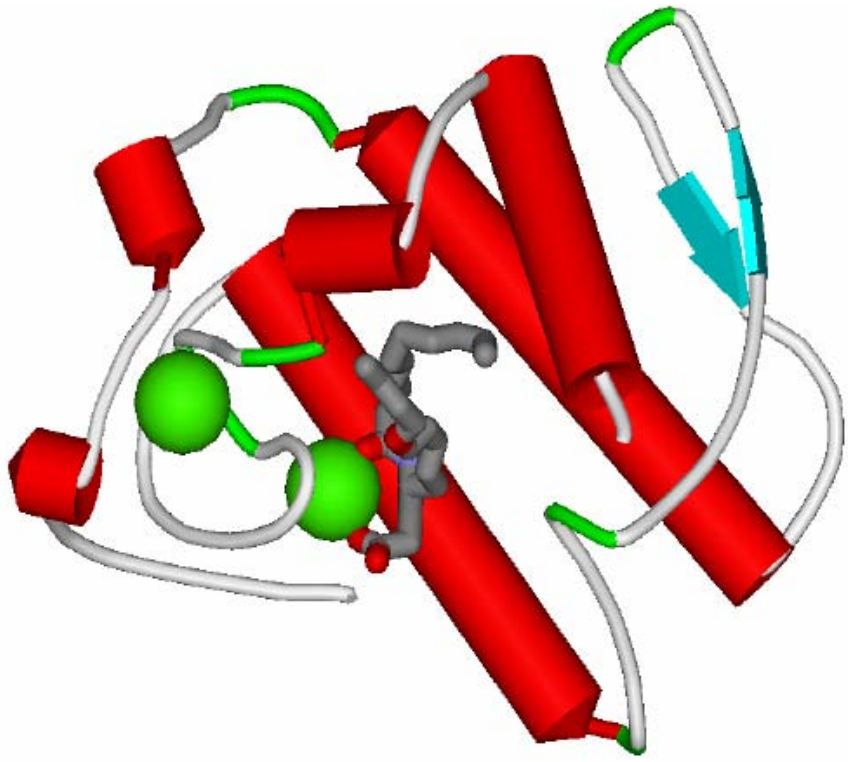


Figure 4-33. The spatial structure of phospholipase (PDB 1j1a). Two ions of calcium and the fatty acids in the active ite are shown. Magnesium can substitute calcium thus leading to a lowered activity of the enzyme.

Magnesium and Coagulation

Molecular mechanisms of blood coagulation are well known. These include the coagulation cascade proper (activation of which leads to the formation of fibrinous filaments and stabilization of the structure of the clot) and the fibrinolytic cascade (clot degradation through hydrolysis of the fibrinous mesh). The processes of coagulation and fibrinolysis have a special place in hemostasis because they lead to the formation of stable blood clots which, under appropriate conditions, are the direct cause of cardiovascular and cerebrovascular incidents. The essential molecular genes involved in the coagulation cascade are presented in figure 4-34.

It is believed that the process of coagulation is divided in two main ways: «extrinsic» mechanism of coagulation initiation and the «intrinsic» mechanism. The «extrinsic» coagulation occurs only with a significant injury of the vessel which leads to infiltration of the tissue factor (F3) into the bloodstream. Following the formation of the F3-F7a, clotting factors F9 and F10 then activate. In turn, F10a and cofactor F5a form prothrombinase complex which then activates thrombin, the central component of the molecular cascade of blood coagulation. As a result, activated thrombin (F2) catalyzes transformation of fibrinogen (genes FGB, FGA) into the fibrin.

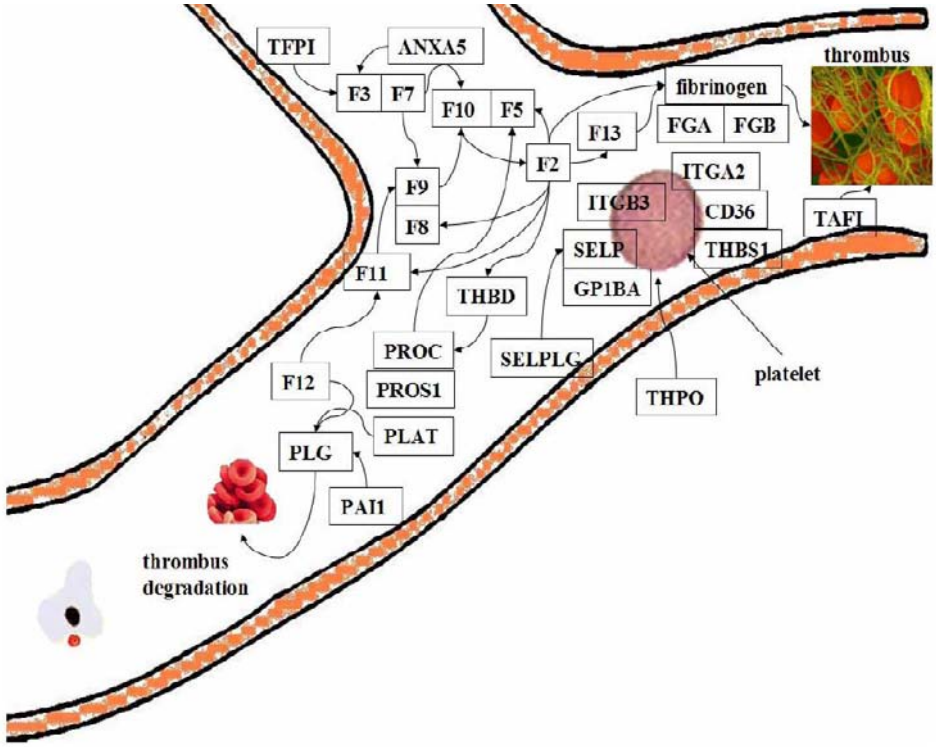


Figure 4-34. Genes and proteins involved in coagulation/fibrinolysis.

The «intrinsic» mechanism of coagulation is triggered in the absence of a significant tissue damage and represents a reaction to the presence of any alien material in the bloodstream or on the vessel wall (e.g., microinjuries of the vessel wall which expose collagen fibers to the vascular endothelium. The mechanism of coagulation involves the conversion of prekallikrein into kallikrein and activation of the clotting factor F12 («Hageman factor»). F12 activates factor F11, which in turn activates the factors F9, F8 and F10.

The most likely mechanism for direct effects of magnesium on coagulation is the *replacement of calcium in the structures of procoagulant proteins*. The antagonism between calcium and magnesium is well known. It is equally well known that many procoagulant proteins (prothrombin F2, blood clotting factors are F13, F10, F11, F7, F8, F9, protein C) are calcium-dependent protein with similar spatial structures. Calcium ions are bound by the N-terminal Gla-domains of these proteins. The levels of activity of these proteins may fall due to genetic defects, calcium deficiency or excess magnesium. Accordingly, the substitution of calcium for magnesium leads to a reduction procoagulant activity of these proteins and, consequently, to a reduced propensity for thrombotic events.

The Gla-domain is an essential piece of the spatial structure of coagulation factors F2, F7, F9, F10, PROC, etc. This fragment of the three-dimensional protein structure has the same spatial structure in all the proteins discussed (figure 4-35). The Gla-domains bind to hyalouronan of the extracellular matrix and are required for a reliable fixation of the clot at the damaged part of the vessel. The glutamic acid residues of these proteins are converted, with the help of vitamin-K-dependent proteins into gamma-carboxyglutamates (Gla), which

bind the calcium. Binding of calcium is necessary for the maintenance of the spatial structure of the Gla-domain (conformation). Substitution of the calcium with magnesium will prevent stable interaction of the Gla-domains with the hyalouronan thus impeding clotting.

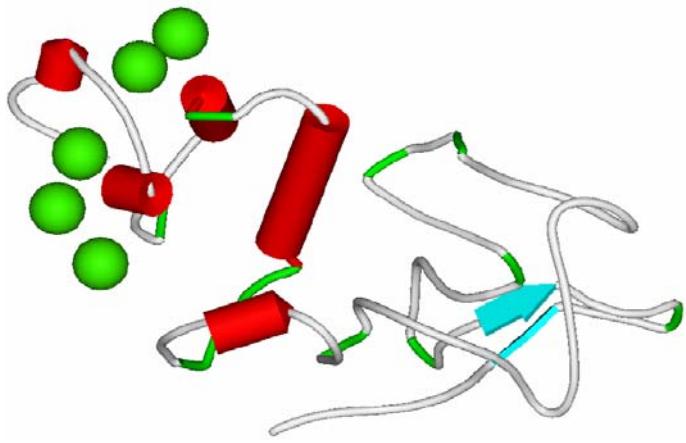


Figure 4-35. The spatial structure of calcium-binding Gla-domain procoagulant proteins. Showing calcium ions associated Gla-domain.

In addition to the Gla-domains, *magnesium might affect coagulation through proteolysis of the von Willebranda factor* (vWF). Magnesium sulfate reduces the aggregation of platelets through the acceleration of proteolysis and secretion of vWF. Proteolysis of vWF is carried out by ADAMTS-13 proteinase, also known as vWF proteinase. Increased activity of ADAMTS-13 predisposes to hypocoagulation due to enhanced proteolytic processing of vWF (Levy, 2005). Magnesium activates ADAMTS-13 thus increasing proteolysis of vWF (figure 4-36).

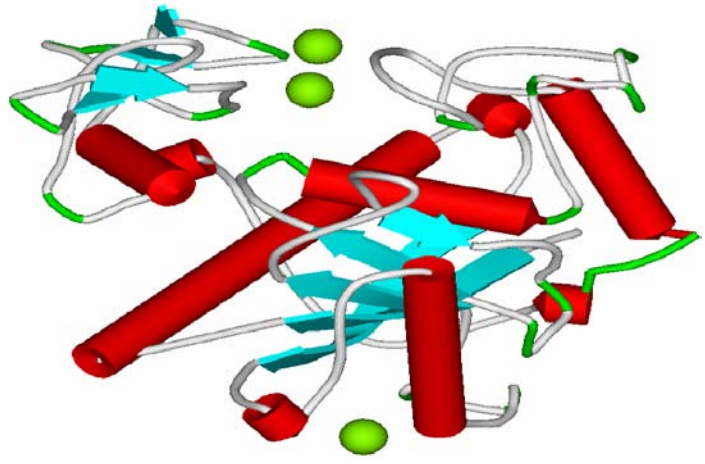


Figure 4-36. The spatial structure of the catalytic domain ADAMTS13 proteinase (model based on PDB file 2JIH) performs proteolytic transformation of the von Willebrand factor (vFW). The ions of magnesium (spheres) accelerate proteolytic processing of vWF.

Magnesium and Inflammation of Vascular Endothelium

Magnesium modulates the cellular events associated with inflammation. Experimental work on rats has shown activation of leukocytes and macrophages, secretion of inflammatory cytokines, acute phase proteins, and increased production of free radicals under the condition of magnesium deficiency. Low levels of magnesium in the tissues of the body stimulate the synthesis of interleukins IL-1a, IL-6 and VCAM (mediator of monocyte-endothelial interaction). In terms of magnesium deficiency, the most pronounced are increases of IL-6 and of acute phase protein MCP-1 (Bernardini, 2005). A number of the proinflammatory effects of the magnesium deficiency are the result of the excess activity of the arachidonic acid cascade mentioned earlier.

Thus, magnesium is natural disaggregant and anticoagulant. In addition, magnesium potentiates anticoagulant effects of acetylsalicylic acid and this allows to minimize the aspirin dosage. There are also synergic effects of magnesium with other anticoagulants (trental, heparin and medicinal plant extracts such as ginkgo biloba extract).

4.5. MAGNESIUM AND UROLOGY

Mineral exchange is controlled largely through kidneys and magnesium ions are involved in regulation of the osmotic balance. Infusion of magnesium salts is well-known to have diuretic effect. In women and patients with hyperaldosteronism, the diuretic response is more pronounced after the introduction of magnesium. Contrariwise, if the amount of magnesium in the blood decreases, the kidneys restore the balance by suspending excretion of magnesium and somewhat increasing elimination of calcium and potassium. In this way, magnesium deficiency gradually leads to the formation of calcium and potassium deficiencies.

An imbalance of calcium and magnesium in urine (more calcium, less magnesium) stimulates precipitation of the kidney stones. The process is aggravated through lack of physical exercise and insufficient consumption of liquids. Normally, an adult should consume not less that 1.5-2.5L of liquid per day. Calcium, magnesium, uric acid and cysteine feature in composition of many types of the kidney stones; the major materials comprising the stones are calcium oxalate and calcium phosphate.

To date, no pharmacological panacea against kidney stones was formulated. It is known that magnesium levels in plasma are lower in patients who have oxalate kidney stones. Low consumption of magnesium (<240 mg/day, $P<0.003$) is a risk for the formation of the kidney stones (Hall, 2001). The serum levels of magnesium were higher in healthy controls than in the patients with oxalate stones (Atakan, 2007). The latter fact suggests that it is possible to prevent formation of the kidney stone through the regular usage of magnesium preparations.

Indeed, routine magnesium treatment of patients with magnesium deficiency (250-500 mg of magnesium salts *per* the day) lowers the incidence of the formation of new kidney stones by 90% (Kerr, 1982). Usage of the organic salts of magnesium (citrate *etc*) is especially effective in lowering the risk of the kidney stone formation (Zerwekh, 2007).

In our records, approximately one third of patients with kidney stones manifest hypomagnesuria on the background of hypercalciuria. In patients with oxalaturia the levels of magnesium and pyridoxine in urine are lower than in patients with uraturia. Restorative therapy with magnesium drugs also influences favorably the ratio of magnesium and calcium.

An increased daily quota of magnesium (and, in particular, in combination with pyridoxine) might prevent deposition of calcium compounds and thus can serve as a prophylaxis which, at least, would slow down the emergence of stones.

To the processes of the kidney stone formation contributes not only the Ca:Mg imbalance but also abnormal P:Mg and Al:Mg ratios which provide conditions for the growth of phosphate- and aluminum-containing kidney precipitates. Compared to deficiencies of other elements (Mg, Ca, Fe, Se, Zn, Mn *etc*) phosphorus deficiency is quite rare. On the contrary, during last decades rather an excess of phosphorus can be observed in patients, partially due to artificial food additives such as moisturizers in meat products, orthophosphoric acid in various soda drinks, taste enhancers, artificial colors and other exciting components that are abundant in "civilized" diet. Food stuffs from natural sources do not contain excess phosphorus and contain just sufficient amount of phosphorus in chemical forms characterized by high bioavailability. Patients with tendency to form kidney stones should be aware of the presence of the phosphorous salts and other compounds in the food they consume. Aluminum also displaces magnesium from the tissues and contributes to the formation of kidney stones. The modern sources of the excess aluminum include antacids drugs (such as Almagel or Alumag), food additives, aluminum packaging (canned beer, foil), along with deodorants and cosmetics.

Preparations of magnesium (magnesium citrate, magnesium gluconate, MagneB6, *etc*) inhibit the formation of the calcium, oxalate, aluminum and phosphate kidney stones. Preventive effect of magnesium and pyridoxine preparations against kidney stones is potentiated by physically active lifestyle, proper diet and adequate drinking load. Especially important it is to maintain these preventive measures in children with dismetabolic nephropathy who feature polymorphic considerable mineral imbalances and, in particular, magnesium deficiency (Kuznetsova, 2006).

4.6. MAGNESIUM AND DIABETES

Magnesium ions directly participate in mediation of the effects of insulin. Without normal balance of intracellular Mg and Ca ions, control of glucose level in blood is impossible. Magnesium modulates transmembrane flow of glucose in muscle, hepatocytes, neurons, the cells of placenta and in other energy-rich tissues of the organism. Magnesium is also required as cofactor for the most important enzymes of carbohydrate metabolism: the glycolytic enzymes. The balance of intracellular Mg:Ca determines the sensitivity of cells to insulin and increased intracellular calcium accompanied by decreased magnesium is one of the markers of insulin resistance.

As we said earlier, glycolytic enzymes alpha-enolase (ENO1), phosphoglucomutase-1 (PGM1) and 6-phosphofructokinase type C (PFKP) require Mg as a cofactor (see figure 4-17). Enolase, apart from its well-known function in the final steps of glycolysis, also plays a part in various processes such as cell growth control, hypoxia tolerance and allergic responses. Phosphoglucomutase-1 is biosynthetic protein that participates in both the glycolysis and gluconeogenesis. The 6-phosphofructokinase enzyme catalyzes conversion of the D-fructose 6-phosphate to fructose 1,6-bisphosphate and is important for the glycolytic carbohydrate degradation.

Since glycolysis is the major biochemical pathway for the sugar consumption, one of the plausible physiological mechanisms underlying the interrelation between diabetes and magnesium is apparent: lower magnesium levels lead to lower rate of the carbohydrate metabolism. Lower rates of the carbohydrate metabolism are detected by the feedback loops through sugar sensors (such as glucokinase) and more insulin is secreted to stimulate catabolism of the carbohydrates. *As the carbohydrate metabolism is already impaired due to chronic lack of magnesium ions, the higher levels of insulin do not have much effect on the rate of the catabolism of the sugars.* Thus, hyperinsulinemia and, further, resistance to insulin are formed.

Almost any diabetic patient has, at the very least, a borderline hypomagnesemia while 30% of diabetics are characterized by acute hypomagnesemia (Chekman, 1992; de Valk, 1999; Spasov, 2000). It is partly because insulin increases the loss of magnesium with urine exacerbating the vicious circle. It was noticed that the body's need of magnesium increases with the growth of carbohydrate load. Restoration of the level of intracellular magnesium in diabetics is accompanied by normalization of sensitivity of peripheral tissues to insulin and reduction of glycemia. Large epidemiological studies indicated a significant correlation between higher mortality from diabetes in the regions of North American continent that feature too low magnesium content in drinking water (Foster, 1988). The role of magnesium deficiency in etiology of diabetes as well as efficiency of magnesium corrective therapies was also confirmed in animal studies on the models of alloxane diabetes (Hans, 2003) and in more recent clinical studies, such as that of McKeown *et al* (2008).

The magnesium deficiency during pregnancy appears to be an essential condition predisposing towards gestational diabetes. Moreover, infants born to mothers with diabetes are characterized by low levels of magnesium on the background of hypocalcaemia (Banerjee, 2003).

Normalization balance of magnesium in patients with diabetes mellitus is as important as *normalization of the carbohydrate consumption.* A four-week course of magnesium salts (400-550 mg/day) was accompanied by increased activity of magnesium-dependent lecithin cholesterol-acyltransferase (LCAT), an increase in HDL, apolipoprotein AI, as well as by reduction of the systolic and diastolic pressure and aldosterone secretion. Timely prescription and regular use of the magnesium drugs by diabetics prevent complications such as diabetic retinopathy. Restoration of magnesium levels is especially important in patients with high dietetic load of fats and carbohydrates which is often combined with high levels of triglycerides (Liese, 2003).

In patients with high triglycerides, simple advice to reduce consumption of animal fats, sugars, refined white bread and pasta is not enough. Replacing the common white sugar with glucose/fructose is also rarely effective (Busserolles, 2003). Patients shouldn't just exclude excess solid fats and carbohydrate consumption (potatoes, white bread, fructose, honey, glucose, candies) but also introduce magnesium-rich products or pharmaceutical preparations.

Dietary carbohydrate load can be reduced by including unrefined sugar (cane sugar, beet sugar), honey as well as sugar substitutes which do not negatively affect magnesium homeostasis and are safe long-term (table 4-4).

Table 4-4. Characteristics of sugar substitutes and sugars

Product	Safety note	Remark
Saccharin (Sugar twin, Sweet N Low)	Safety was not proven	300 times sweeter than sugar
Aspartame (Nutra Sweet, Nutra Taste)	The most thoroughly tested additive, safe with long-term use, except for patients with phenylketonuria	180 times sweeter than sugar
Acesulfam (Sunnette, Sweet One)	There are no apparent detrimental health effects	200 times sweeter than sugar
Cyclomat	In 1970s was banned in US as a potential carcinogen, now is allowed to use, but results of studies are controversial	30 times sweeter than sugar
Sucralose	Being tested	600 times sweeter than sugar
Alitame	Being tested	2000 times sweeter than sugar
Stevia	There are no apparent detrimental health effects	30 times sweeter than sugar, contains minerals
Natural honey	good for health in moderate quantities	contains vitamins, minerals, trace elements, plant hormones
Dark syrup	good for health in moderate quantities	contains vitamins, minerals, trace elements
Unrefined sugar	Intake has to be restricted	Contains traces of vitamins and minerals, stimulates excretion of Ca, Mg, Cr, Zn, group B vitamins
Refined (white) sugar	Intake has to be considerably restricted	Actively stimulates excretion of Ca, Mg, Cr, Zn, group B vitamins

Another important factor in preventing and overcoming insulin resistance is *regular physical exercise.* During the exercise the magnesium-controlled carbohydrate metabolism occurs with a maximum biological efficiency. Lack of exercise, on the contrary, stimulates insulin resistance of the muscle tissue which is also has lower metabolic rate and impaired glucose transport. The blood glucose is thus not used, its level increases and insulin resistance of other tissues develops.

Regular schedule of the food intake is also an important factor which, when properly implemented, can considerably lessen disability and improve the quality of patient's life. Against the background of hypomagnesemia, hyperglycemia on the empty stomach is characterized by considerably more difficult course. Irregular meals can lead to additional stress and increase blood pressure, apart from the point that 50% or more cases of diabetes are often combined with arterial hypertension.

For the treatment to be efficient, it is important to control the course of the disease. In the case of diabetes, this control is achieved using a series of the lab tests. Although blood glucose is the most widespread test related to diabetes, it isn't very informative by itself (Nordin, 2004). Additional tests include triglycerides (Appendix II), glycosylated hemoglobin (Appendix III), glycosylated albumin; the control of the levels of the plasma thiamine, ketoacidosis and creatinine is also highly desirable. In type 2 diabetes, reduction of

magnesium in blood correlates with the increase in the glucose levels in urine. High glucose makes urine hyperosmolar and this increases further loss magnesium and other electrolytes because of the lessened readsorption in the glomeruli. In severe diabetic cases, it is impossible to detect hypomagnesemia in endovascular fluids due to hyperosmolarity. In this situation, the definition of magnesium levels in erythrocytes can be helpful. The extent of the diabetes-associated ketoacidosis correlates with the extent of hypomagnesemia (Bauza, 1998). Ketoacidosis is also often combined with a deficit of zinc. The Mg/creatinine ratio in urine increases in proportion to the gravity of clinical course of diabetes.

The results of the lab tests can be helpful in the clinical management of diabetes. For example, glycosylated hemoglobin test (HbA1c) was recognized by WHO as being essential for the monitoring of diabetes mellitus more than a decade ago. HbA1c reflects hyperglycemia during the entire period of the life of erythrocytes (up to 120 days). Normally, HbA1c values are 4.5-6.5%. It is recommended to maintain the level of HbA1c <7% and to revise the current diabetes therapy if HbA1c goes >8%.

It is very important that the use of magnesium drugs be safe and suitable for long-term treatment courses (Spasov, 2000). Second-generation medications (see Chapter 5) such as MagneB6, magnesium pidolate, magnesium lactate, magnesium orotate, and magnesium asparaginate exclude the likelihood of side effects typical of the 1st generation magnesium drugs such as hypermagnesemia and gastrointestinal disorder. Since insulin-dependent diabetes remains, virtually, life-long, modern magnesium medications can be prescribed for life to prevent the emergence of diabetic complications. Correction of magnesium, zinc, chromium, vitamins B1, B6 can be an efficient profilaxis of the development of diabetic foot, diabetic cataracts, retinopathy, nephropathy and other complications of diabetes.

4.7. MAGNESIUM AND PATHOLOGIES OF THE CONNECTIVE TISSUE

Constituting about 50% of the body mass, the connective tissue is one of the four types of tissue in traditional classifications (the others being epithelial, muscle, and nervous tissue). Its major function is forming the structure and the support for the other tissues. Cartilage and bone are the major varieties of the connective tissue, other kinds include areolar connective tissue which holds organs and epithelia in place, dense connective tissue that forms ligaments and tendons *etc*. The role of magnesium in the connective tissue and, most importantly, in the skeletal system was underestimated for a long time. Here, we briefly consider the displasias of connective tissue and the molecular mechanisms through which magnesium deficiency affects the structure of connective tissue. A more detailed review of the problem is available in our recent work (Torshin, Gromova 2008).

4.7.1. Undifferentiated Connective Tissue Dysplasias

Non-differentiated connective tissue dysplasia (ndCTD) is a heterogenous group of disorders that are likely to present a basis for various chronic diseases. ndCTD is revealed frequently and corresponds to abnormal structural and functional changes in connective tissue. These changes often result in abnormal morphology and function of the organs

involved (Filipenko, 2006). Clinical and morphological manifestations of ndCTD range widely and, generally, include skeletal changes along with abnormal cartilage development such as disproportionably long extremities, arachnodactilia, deformities of the rib cage, scoliosis of the spinal column, flat foot (fallen arch), dental problems and pathologies of joints. However, the bone and cartilage abnormalities are only the most apparent features of ndCTD. Other clinical manifestations include hyperelastic and easily bruised skin, reduced muscle mass, lung and renovascular pathologies, genital prolapse, gastrointestinal disorders as well as abnormalities in the cardiovascular system: mitral valve prolapse, venous insufficiency, and varicose disease.

The diagnostics of the ndCTD is based on these symptoms and additional data such as anthropometric data (higher heights and decreased body mass index, lower parameters of physical fitness), impaired external respiration, decreased size of the heart, low arterial pressure, occlusive plethysmography, specific features of ECG and ultrasonic phleboscanning. According to the analysis of these phenotypic markers of ndCTD, the prevalence of ndCTD can be relatively high in the general population: for example, 8.5% in the population sample of 400 individuals (Golovskoĭ, 2002).

Connective tissue displasias in children and adolescents involve not only calcium deficiency, but also magnesium. The children of long-term existing magnesium deficiency remarkably often demonstrate funnel chest, scoliosis, flatfoot and hypermobility of the joints (Stepura, 1999). Recent reviews (Senni, 2003; Foucault-Bertaud, 2004) brought together numerous data indicating that Marfan syndrome, mitral valve prolapse, and congenital dysplasias of connective tissue (which manifest as low mechanical strength, sclerosis, fibrosis and susceptibility to chronic inflammation) depend on the duration of the state of long-term magnesium deficiency and require life-long magnesium/calcium correction. Application of magnesium orotate (50 mg/day during first week and 25 mg/day thereafter) appears to be a very effective therapy of children with syndrome of cardiac connective tissue dysplasia, mainly with mitral valve prolapse and anomalous chordae tendineae (Domnitskaia, 2005). The practice of using magnesium drugs during postoperative period with the aim of forming more aesthetic cicatricial elastic tissue is also related to the positive influence magnesium has on the condition of the connective tissue and its mechanical structure.

4.7.2. Magnesium and Molecular Cell Biology of the Connective Tissue

The term “connective tissue” belongs largely to the areas of physiology and biomedicine. In order to approach the Mg-dependent molecular mechanisms involved in CTD, we have to start operating the terms of the molecular cell biology such as “extracellular matrix”, “cell-cell adhesion”, “cell-matrix adhesion”, “hyalouronan”, “proteoglycans” and a number of others. An extraordinary molecular complexity that characterizes any tissue of the human body tends to overwhelm and the connective tissue is no exception in this regard. Albeit it can be said that the major components of the connective tissue are collagen fibres, elastic fibres, the cells and remodelling enzymes, this description does not reflect well the actual complexity of the cellular and molecular biology involved and the number of the genes implicated. Previously, we made a systematic analysis of the molecular mechanisms of the influence of magnesium on the structure of connective tissue (Torshin, Gromova 2008). A summary of the relevant molecular mechanisms is presented below.

Unlike, for instance, epithelium tissue in which the cells are tightly bound together through cell-cell junctions, the connective tissue shows an abundance of the extracellular matrix and very few cells. In molecular biology, the extracellular matrix is defined as the complex network composed of macromolecules such as collagens, proteoglycans and elastin that strongly interact with each other and with cells to maintain the structural integrity of the tissues (Alberts, 2002). The matrix helps to hold cells and tissues together and provides an organized environment within which migratory cells can move and interact with one another. The matrix consists of the three essential components: the gel-like medium, fibers and cells.

The most basic component of extracellular matrix is the *gel-like medium* formed by proteoglycans: the extended protein chains with numerous glycosaminoglycan polysaccharide chains covalently attached (figure 4-37). This polysaccharide "gel" resists compressive forces on the matrix while permitting the rapid diffusion of nutrients, metabolites, and hormones between the blood and the tissue cells. The structure of the gel is further strengthened by the fibers of which there are three major types: collagenous fibers (type I collagens, for the most part) that form the skeleton as it were of the connective tissue, the elastic fibers (elastin, fibrillins) that give the connective tissue it's elasticity and the reticular fibers (type III collagen) which cross-link all the other fibers and hold together the tissue components. The collagen fibers both strengthen and structure the matrix while elastin fibers give it elasticity. All the fibrous macromolecules that constitute the extracellular matrix are mainly produced locally by cells in the matrix. In most connective tissues, the matrix macromolecules are secreted largely by cells called fibroblasts, in specialized types of connective tissues such as cartilage and bone by chondroblasts and osteoblasts, respectively.

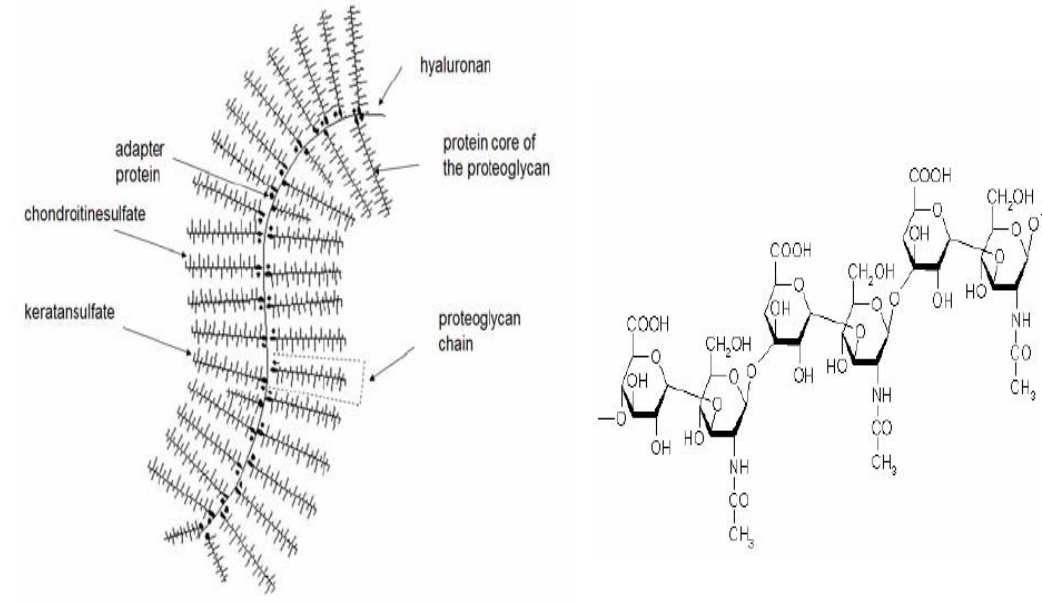


Figure 4-37. The major structural components of the glycoprotein complexes forming the gel-like medium of the connective tissue. A) General structure; B) A fragment of a hyaluronan chain.

The *gel-like ground substance* of extracellular matrix is formed by proteoglycans and multidomain glycoproteins. Proteoglycans are attached to the long treads of the hyaluronan (figure 4-37), each thread containing over 25,000 monomers of the hyaluronic acids and can be doznes of microns in length. Hyalouronan, the basis of the gel-like substance, is synthesized by means of hyalouronan synthases (genes HAS1, HAS2 и HAS3) and is degraded by means of hyaluronidases (genes HYAL2, HYAL3, HYAL4 и HYALP). It should be noted that hyaluronan synthase proteins contain a magnesium ion in the active site (figure 4-38) where as the action of the hyaluronidase inhibitors strongly depends on the magnesium concentration. Therefore, a *magnesium deficit will lower the rate of synthesis of the hyaluronan and, at the same time, will also increase the rate of its biodegradation.* Both these influences will result in dissipation and a sort of "drying" of the gel-like substance which will worsen the condition of the connective tissue.

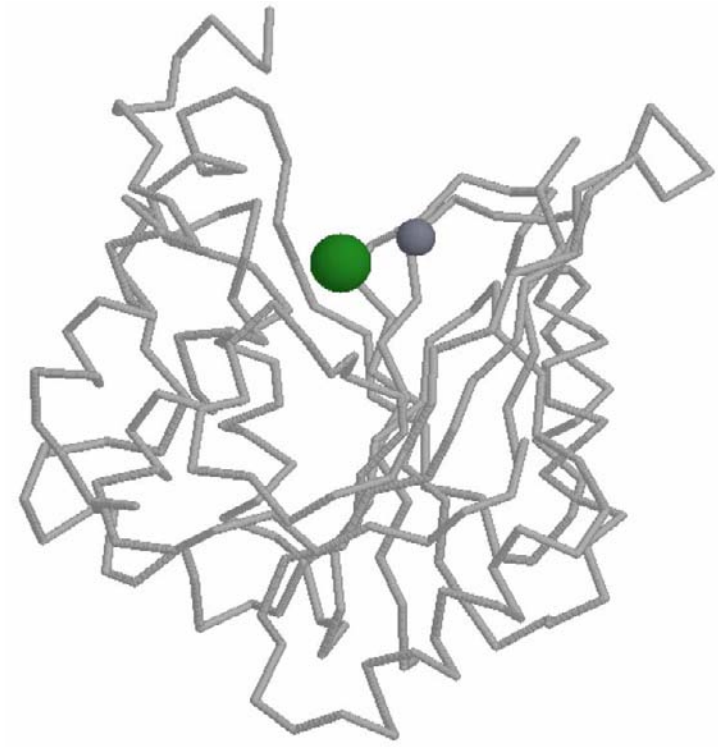


Figure 4-38. A model of the hyaluronan synthase 1 (based on PDB file 1qgq). The magnesium ion(larger sphere) and the manganese (II) ion (smaller sphere) indicate the location of the active site of the enzyme.

Proteoglycans contain long linear carbohydrate polymers that are negatively charged under physiological conditions because of the covalently attached sulphates and uronates. For each type of proteoglycan, there are numerous proteins that bind specifically to this proteoglycan as well as not less numerous syntetases that are involved in the synthesis of the glycoseaminoglycan chains and their attachment to the protein core. Chondroitin sulfate proteoglycans (genes CSPG1,2,3,4,5,6) and heparan sulfate proteoglycan (perlecan, HSPG2) are involved in formation of the gel structure and mutations in these genes result in skeletal dysplasia. The enzymes involved in the biochemical modifications and attachment of the

glucoseaminoglycan chains can significantly affect the structure of extracellular matrix. For example, deficiency of the Mg-dependent xylosylprotein 4-beta-galactosyltransferase 7(galactosyltransferase I, gene B4GALT7) was associated with connective tissue dysplasia (Okajima , 1999).

The *collagen fibers* give the connective tissue its strength and durability. Each collagen fiber is several micrometers in diameter and consists of thousands of the individual collagen chains densely packed together (figure 4-39). Collagens are the most abundant proteins in the extracellular matrix and in the connective tissue. There are about 50 collagen genes in the human genome which correspond to more than 20 kinds of the collagen fibers found in various tissues. Inherited mutations in collagens are known to lead to Ehlers-Dahlos syndrome and epidermolysis bullosa.

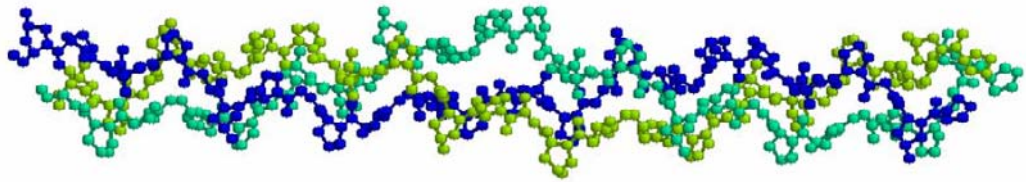


Figure 4-39. The triple collagen helix is the major component of the collagen fibers (PDB file 1cag).

Genetic collagen diseases, including the connective tissue dysplasias, arise not so much because of the defects in the collagen genes but, more commonly, from the defects in the genes implicated in biosynthesis, postranslational modification, secretion, assembly and remodeling of the collagens.

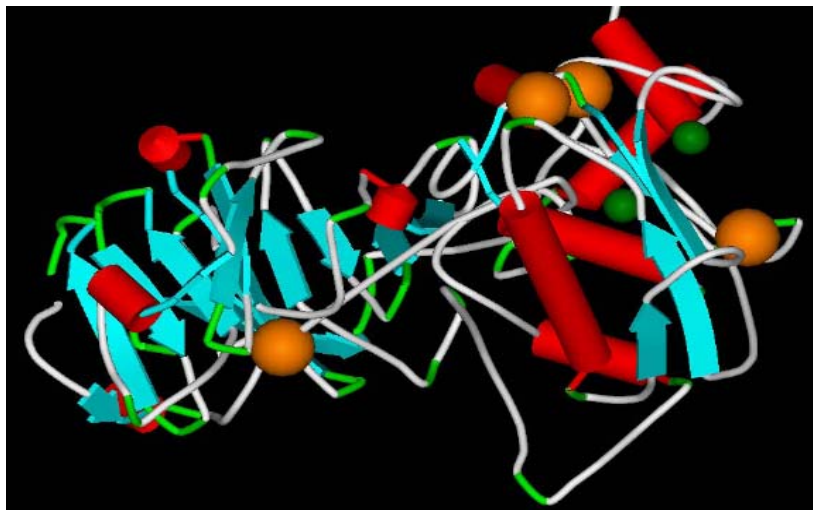


Figure 4-40. Three-dimensional structure of the human MMP1 proenzyme. Subunit consists of two domains, the N-terminal catalytic (right) and the C-terminal (left) which is involved in substrate specificity and in binding TIMP (tissue inhibitor of metalloproteinases). The four Ca^{2+} ions are show as the large orange spheres, the two Zn^{2+} ions as the small dark green spheres (PDB code 1su3). Figure is prepared with the help of Weblab Viewer (http://www.msi.com).

In particular, *remodelling of the fibers of extracellular matrix* is supported by the matrix metalloproteinase (MMP) enzymes. MMPs degrade collagen fibers thus removing structural supports of the connective tissue. It is important to notice that MMPs are characterized by uniform structure of the individual subunits and their subunits include four obligatory Ca^{2+} and two Zn^{2+} ions (figure 4-40) and can, potentially, be directly inhibited by magnesium by susbstituting one of the calcium ions at the active site. At the very least, increased serum Mg^{2+} reduces the production of IL-6 and MMP-1 (Ueshima, 2003). Extracellular folic acid and magnesium decrease the homocysteine-induced MMP-2 secretion (Guo, 2006). In rat vascular smooth muscle cells, magnesium reduced the MMP2 production dose-dependently through a signalling cascade that involves a tyrosine kinase (Yue, 2003).

The above description of the studies of extracellular matrix proteins suggests multiple molecular mechanisms through which the connective tissue displasias might emerge. Any disbalance in this finely attuned system, be it abnormality of the tissue proliferation, unbalanced collagenolysis, defects in the structural genes (proteoglycans, collagens) or abnormalities in the post-translational modifications can result in CTD. The readers, interested in the details of the analysis can refer to our paper (Torshin, Gromova, 2008). In brief, magnesium is required for the activity of the enzymes involved in biosynthesis and degradation of the the major protein elements of extracellular matrix: the gel-like medium, the collagen fibres, the elastic fibers. The magnesium deficiency results in (1) degradation of the gel-like medium of the connective tissue, (2) degradation of the collagen, the structural supports of the connective tissue and (3) an increase in the elastic fibres. All of these influence result in connective tissue which is amorphic and has poor mechanical qualities. The summary of these and other molecular mechanisms is presented in the figure 4-41.

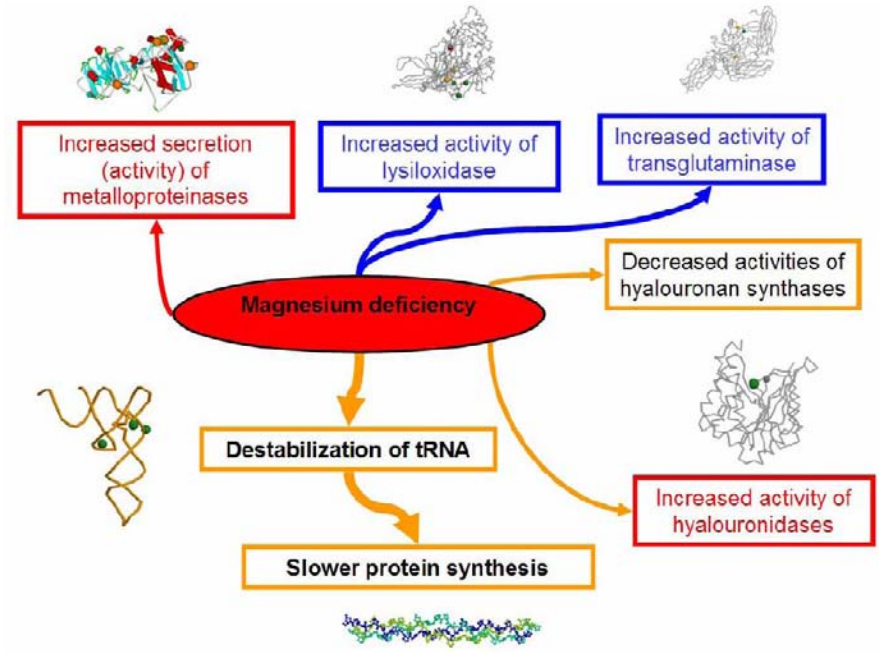


Figure 4-41. Potential molecular mechanisms of the Mg-dependent connective tissue displasias. The dots and spheres in the images of proteins correspond to the binding sites of magnesium and other minerals (Ca, Cu) in the three-dimensional protein structures.

4.7.3. Magnesium and Disorders of Skeletal System

Cartilage and bone represent specific types of the connective tissue, so the molecular mechanisms affecting the structure of connective tissue are also applicable to bone. There are several important distinctions of the bone from other types of connective tissues in respect to magnesium. More than 50% of the total amount magnesium in the human body is deposited in the bone. The Mg:Ca ratio in the normal bone is close to 1:55 so the amount of magnesium in the bone tissue is comparable with the levels of trace elements (figure 4-42). Apart from its beneficial influence upon the structure of the connective tissue, magnesium also preserves the normal level of calcium in bones thus preventing loss of the bone tissue and contributing to the bone renewal, it. As the result, the bone tissue is characterized by slower aging. On the contrary, magnesium deficiency is characterized by a wide range of the bone pathologies including:

- osteoporosis,
- osteopenia,
- degenerative diseases of bones and joints (osteoarthrosis, spondilesis etc.)
- rheumatoid arthritis,
- gout,
- rickets,
- scoliosis
- cicatricial dysplasia.

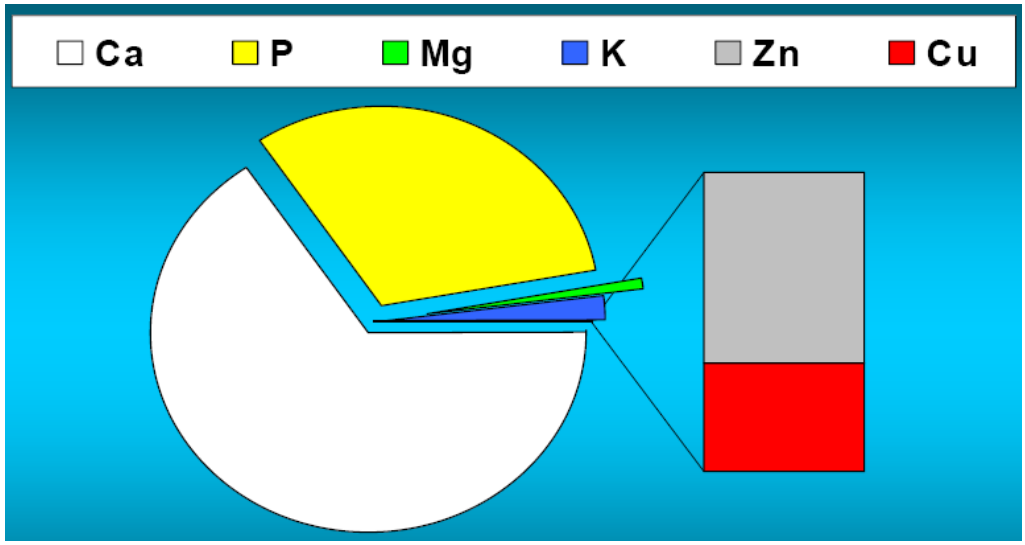


Figure 4-42. The normal ratio of minerals in the bone tissue (Swaminathan, 1999).

Magnesium in bone tissue is concentrated mainly on the surface of the apatite crystals. In patients with osteoporosis, normal levels of magnesium observed in the blood are accompanied by a deficit of magnesium in the bones (Driessens, 1990). A chronic magnesium deficiency disrupts the Mg:Ca balance of in the bone tissue and, especially in combination with hypodynamia and calcium deficiency, is one of the conditions for the formation of

osteochondrosis and scoliosis. The mechanism of preventive and therapeutic actions of magnesium against osteochondrosis is stoppage of the deposition of calcium phosphate in the soft tissues and on the surface of the joints.

In post-menopause period, an increased loss of magnesium in interverterbral disks and symphysis depends on the plasma estrogen and is proportional to the age (Takano, 1999). Magnesium therapy can be successfully applied as a part of the climacteric and senile osteoporosis (Seeling, 1990).

The levels of magnesium influence the effects of vitamin D on bone tissue. Vitamin D is known to enhance the absorption of calcium in bones. Magnesium is needed for intracellular signaling downstream the vitamin D receptor (Torshin, 2009). Rickets - one of the main nosological forms related to the pathological exchange of calcium and vitamin D_3 in bone. It is known for over 30 years that rickets is not only a violation of the calcium and D_3 metabolism but also is a consequence of magnesium deficiency (Reddy, Sivakumar, 1974). In some cases, long but unsuccessful treatment of rickets with calcium and vitamin D_3 might be the result of neglecting the magnesium status of the patient. And, contrariwise, including magnesium preparates can contribute to the patient's rapid recovery.

Long-term magnesium deficiency, especially in combination with sedentary lifestyle and calcium deficiency - a condition for the formation of scoliosis and spinal osteochondrosis. The positive impact of the introduction of additional magnesium (food, preparates, drinking water with the Mg-content of 0.05%-0.15%) was shown in experiments measuring metabolism in bone tissue and the mechanical proportions of the bones of ovaryectomized rats (model of hypoestrogen states during menopause). Additional dotation of magnesium against the background of low estrogens supports bone formation, prevents bone resorption and increases bones' dynamic force. Magnesium-augmented diet increases absorption of calcium and the levels of osteocalcin, a blood marker of osteogenesis (Tu, 2005).

A crucial impact on the development of osteoporosis has the Ca:Mg proportion of the regularly consumed food and water. Population-wide, the incidence of osteoporosis is lower when the ratio of magnesium to calcium in the diet is higher (table 4-5). Minimum incidence of osteoporosis is associated with ratio of Ca:Mg varying from 2:3 to 3:2. The ratios of 3:1 or higher a associated with progressively greater risk of osteoporosis. It also should be noted that the absolute amount of calcium consumed in countries with low incidence of osteoporosis is lower than in countries with very high incidence. Therefore, the nutritional standards should take into account the ratio and not just being limited to recommended daily allowances.

Table 4-5. The incidence of osteoporosis among women 45-55 years in different countries, Ca and Mg consumption (mg/day) and the Ca:Mg ratio (Swaminathan, 1999)

Country/region	Ca	Mg	Ca:Mg	Incidence (cases per 1000)
New Guinea	448	500	1:1	3,1
North Africa	196	300	2:3	7
Singapore	389	400	1:1	23
Finland	1332	200	6,5:1	111
Britain	977	250	4:1	119
Sweden	1104	200	5,5:1	188

4.8. MAGNESIUM AND BRONCHIAL OBSTRUCTION

Chronic magnesium deficiency is a predisposing factor for the formation of bronchial asthma and recurrent bronchitis. Due to prominent role magnesium has in the muscle relaxation, magnesium ions reduce spasm of smooth muscle cells surrounding bronchioli and of the bronchial smooth muscle. Magnesium ions also reduce the release of histamine by the mast cells thus reducing inflammation. In patients with bronchial asthma, severity of the disease often correlates with the level of magnesium in plasma and erythrocytes.

A dietary deficit of magnesium leads to accumulation in the blood of histamine. At the same time, the condition and treatment of the lung also influences the magnesium levels. For instance, inhalation of increasing doses of salbutamol (100-4000 microgram) by healthy subjects increases the concentration of Mg^{2+}, K +, cAMP and glucose in the blood. Inhalation of the sprayed magnesium sulfate can increase the vital capacity of the lung as a greater dose of histamine is required to reduce the vital capacity (Rolla, 1987). Magnesium preparations are effective in prevention of bronchospasm caused by histamine in patients with moderate to average reactivity of the bronchi. Intravenous infusion of the magnesium sulfate allows to block mild attacks of the bronchial asthma. A study of the magnesium levels in the moisture from exhaled air of asthma sufferers indicated low content of magnesium in the condensate when compared with healthy controls (Voskoboynik, 1989). While magnesium allays bronchial spasms, introduction of magnesium antagonists (a solution of the table salt) increases bronchial spasms, edema and inflammatory processes in bronchitis. Bronchospasm profilaxis greatly benefits not only from magnesium preparations *per os* aiming at the restoration of the magnesium levels throughout the entire body but also from inhalations of water with high levels of magnesium.

Hyperventilation syndrome (another term - «neurocirculatory dystonia») is characterized by breathing irregularities and associated unpleasant sensations in the heart. Appointment of the drug MagneB6 even as a monotherapy leads to a significant reduction in the intensity of the clinical manifestations (Filatov, 2007). An integrated therapy of the non-attack period of bronchial asthma and of chronic bronchitis should include, among other things, regular rational use of magnesium drugs. The table 4-6 represents effects of magnesium on the immune response, and the participation of magnesium in allergic inflammation of the airways in patients with bronchial asthma.

Table 4-6. The effects of magnesium and immune response (Skotnicki, 1993)

Activation of the alternative activation pathway of complement
Cytotoxicity of T-cells, lower
Intracellular cAMP, lower Intracellular Ca^{2+}, lower
Inhibition of the cellular degranulation of the anti-immune response Allergic response, lower
Leukotriene B4, lower Prostaglandin E2, lower Histamine, lower

Bronchial asthma attacks mostly during the late night. The balance of magnesium recognized as being one of the leading factors of normal work of the biological clocks (Durlach, 2002). The daily balance of magnesium and calcium in patients with bronchial asthma differs by a more pronounced rate of fluctuations (daily deviations >20%), with a fall in magnesium at night. The magnesium fall can precipitate bronchial spasm if left unattended. It is possible to reduce the incidence of the asthma attacks during night by using magnesium preparations of 2nd generation (magnesium asparaginate, magnesium citrate, magnesium orotate) which are safe and do not interact with steroids and/or beta-receptor agonists. Magnesium preparations can also be used to augment the positive effects of the bronchospasmolytics and to stabilize remission in patients with bronchial asthma.

4.9. MAGNESIUM AND PYRIDOXINE: IMMUNITY, ONCOLOGY AND TRANSPLANTATION

Magnesium levels affects both innate and acquired immune response through inflammation (Mazur, 2007), apoptosis, and alterations in number and function of innate immune cell populations (Tam, 2003). The magnesium deficit increases body's sensitivity to infection. Magnesium ions are involved in regulation of the phagocytosis of macrophages which digest malign or otherwise abnormal cells. Against the background of magnesium deficiency, phagocytosis cannot complete and phagocytic index lowers. With the shortage of magnesium also drop the blood levels of neutrophils and monocytes.

Phagocytosis is regulated by complement system. Magnesium ions are involved in proteolytic cleavage of the complement component C3 that has a significant role in antimicrobial and antiviral protection. It was found that magnesium is also necessary to prevent thymic involution. Magnesium is needed for the implementation of intercellular communication (in particular, through integrins). The interactions of T-and B-lymphocytes also require the presence of magnesium ions. Magnesium ions needed for full functional operation of the T-helpers and stimulates synthesis/secretion of the cytokines IL2, IL6, IL8, IL12 etc.

At magnesium deficiency, *Staphylococcus* strenuously produces toxin-1, responsible for toxic shock syndrome. Magnesium-based drugs do not only reduce the risk of side effects from the use of gentamicin, but also increase antimicrobial activity of penicillin. Early inclusion of drinking forms of magnesium and pyridoxine in a comprehensive neonatal sepsis therapy potentiates antibacterial therapy and results in significantly reduced mortality (Rayushkin, 1999).

The effect magnesium deficiency has on the immune system is also important for the development of the oncologies (Skotnicki, 1993). A magnesium deficiency leads to impaired immune response against true pathogens and, at the same time, to an increase in autoimmunity and inflammation. Both of these influences can contribute to oncologies. The table 4-7 summarizes some of the effects magnesium deficiency has on both immunity and oncology.

Table 4-7. Magnesium deficiency and carcinogenesis in experimental animals (Skotnicki, 1993)

Acute deficiency: death of animals within 6 weeks
Sub-acute deficiency: 20% of animals shown tumors of thymus in 8-24 weeks of the deficiency
Chronic deficiency: hyperleukocytosis, 10% of animals shown sings of myeloid leukaemia
Greater number of the chromosome defects in mother and in fetus
Decreased inhibition of growth of the transplant tumors
Breast adenocarcinoma in 46% of experimental animals for 52 days

Epidemiological studies and data of the evidence-based medicine suggest that:

- Chronic shortages due to lack of magnesium in the diet (or nutritional content less than 60 mg/kg of body weight) causes the development of malignant lymphomas of thymus and lymph (Molchanov, 2002)
- Low levels of magnesium and calcium in drinking water were associated with increased risk of death from esophageal cancer (Yang, 2002)
- Low levels of magnesium in drinking water associated with increased risk of death from liver cancer (Yang, 2002)
- Epidemiological surveillance revealed a correlation between the level of incidence of cancers and low consumption of magnesium in drinking water in a large-scale sponsored by WHO and conducted over 19 years (Foster, 1990)
- Magnesium deficiency has a role in development of skin cancer (Keith, 1991)
- There is a correlation between low levels of magnesium in the water/food and the incidence of the cervical cancer (Keith, 1991).
- Vitamin B_6 reduces the number of micronuclies in mice with reticuloblast mutagenic (Akaiwa, 1992).
- Vitamin B_6 has a protective effect against colorectal cancer as suggested by 11-year study of >32,000 female participants of which 604 developed cancer patients (Wei, 2005).

Table 4-8. Mg-dependent proteins of DNA repair

Gene	Protein	Function
APEX1	**DNA-lyase**	Repairs oxidative DNA damage
ERCC2	**TFIIH basal transcription factor complex helicase**	Nucleotide excision repair of DNA, also involved in the regulation of vitamin-D receptor activity
G3BP1	**GTPase-activating protein-binding protein 1**	Helicase activity (unwinds DNA double helix)
NUDT15	**oxoguanine triphosphatase**	Removes an oxidatively damaged form of guanine from DNA
POLK	**DNA polymerase kappa**	DNA polymerase specifically involved in DNA repair
RECQL	**DNA helicase Q1**	Unwinds single- and double-stranded DNA in 3'-5' direction, interacts with EXO1 and MLH1.
REV1	DNA repair protein REV1	Transfers a dCMP residue during post-replication DNA repair
TLK1	Ser/thr kinase tousled-like 1	Facilitates repair of double strand breaks
TLK2	Protein kinase tousled-like 2	Regulates processes involved in chromatin assembly
TREX1	3’ repair exonuclease 1	Repair of double stranded DNA with mismatched 3' termini
TREX2	3’ repair exonuclease 2	DNA repair

The possible molecular mechanisms that mediate relationship between the magnesium deficiency and the higher incidence of the oncological formations include, most likely, the impaired DNA repair and disbalance in apoptosis. The weakened DNA repair (because a numberof DNA-repairing enzymes depend on magnesium as the cofactor, see table 4-8) corresponds to a greater DNA damage. Normally, DNA damage induces apoptosis of the damaged cell. At magnesium deficiency, however, apoptotic processes are also inhibited (since a number of proapoptotic proteins, such as MAP kinases, are also magnesium-dependent, see table 4-9). Inhibition of apoptosis results in hyperproliferation of the cells with damaged DNA contributing to the formation and growth of tumors.

In treatment of certain oncological diseases (lymphosarcoma, leukemia), the introduction of anti-vitamin B6 (desoxypyridoxine) is used. For the treatment of many kinds of the tumors, radioisotope irradiation methods are applied. In both cases, a pronounced pyridoxine deficiency arises in the body of a patient. Massive antibiotic therapy in oncology leads to shortages of pyridoxine, sometimes in combination with a deficit of magnesium. Therefore, the patients should be supplemented with pyridoxine and magnesium.

Table 4-9. Mg-dependent proteins implicated in apoptosis

Gene	Protein	Function
ACVR1B	Activin receptor type-1B	activates SMAD transcriptional regulators, induction of apoptosis
ACVR1C	Activin receptor type-1C	Receptor for activin, activates SMAD2 and SMAD3
CERK	Ceramide kinase	phosphorylation of ceramide, implicated in proliferation, apoptosis, phagocytosis, and inflammation
DAPK2	Death-associated protein kinase 2	Calcium/calmodulin-dependent serine/threonine kinase which acts as a positive regulator of apoptosis.
ITPK1	Inositol-tetrakisphosphate kinase	phosphorylates inositol, modifies TNF-alpha-induced apoptosis
MAP3K5	Mitogen-activated protein kinase kinase kinase 5	signal transduction, activates MAP2K4 and MAP2K6, which in turn activate the JNK and p38 MAP kinases. Overexpression induces apoptosis, induced by TNF-alpha
MLTK	Mitogen-activated protein kinase MLT	Regulates the JNK and p38 pathways. cell cycle arrest, positive regulation of apoptosis
MYLK	Myosin light chain kinase	Critical participant in signaling sequences that result in fibroblast apoptosis.
NEK4	protein kinase Nek4	Required for mitotic progression. Inhibition results in apoptosis.
NEK6	protein kinase Nek6	Activated during M phase, required for chromosome segregation
PTEN	Phosphatidylinositoltrisphosphate phosphatase	inhibits cell migration and integrin-mediated cell spreading, induces apoptosis
STK3	Serine/threonine-protein kinase 3	Stress-activated, pro-apoptotic kinase
WNK2	“No lysine” 2 protein kinase	Apoptosis

The deficit of magnesium significantly complicates the rehabilitation of patients after transplantation of organs and tissues (Choi, 2005; Yuan, 2005). Although transplantation-related trauma is the major source of the magnesium deficiency, the aggressive therapy with cytostatics (cyclosporin, used in kidney transplants and other organs) leads to irreversible loss of magnesium. The deficit of magnesium in transplantology is so important for survival and quality of life of the patient that ignoring this problem is a grave medical error. Compensatory introduction of magnesium is mandatory in transplantology protocol of the treatment of patients. Given the inevitable loss of magnesium during the operation and then during the cytostatic period, it does seem more desirable to carry out preventive therapy with magnesium drugs in physiological dosages of magnesium (400 mg/day).

4.10. MAGNESIUM AND PROFESSIONAL PATHOLOGIES

Different elements have very different therapeutic ranges and safety limits. The elements also differ in respect to the range of physiological effects and, for instance, macronutrients such as sodium, potassium, calcium, magnesium have a wider range of physiological effects than many of the essential trace elements. In very small quantities, even trace elements hold to be "toxic" (Ni, Co, Mn, Cu, Pb *etc*) appear to be important for the normal body homeostasis. However, their positive therapeutic window is very narrow and greater concentrations of these elements lead to chronic intoxication. In particular, excess of the toxic trace elements such as lead, nickel, beryllium, barium, aluminum and cadmium leads to washing of magnesium out of the body. The essential trace elements are Fe, I, Cu, Zn, Co, Cr, Mo, Ni, V, Se, Mn, As, F, Si, Li and 6 out of them (Cu, Zn, Co, Ni, Mn, Li) are known to interact with magnesium. Conventionally essential boron demonstrates a synergy with magnesium functions in bone tissue.

Many of the elements we listed above are widely used in various industries. In particular, the current industrial methods of cleaning nickel, cobalt, copper and platinum group metals do not always provide an adequate safety for the workers who are at a higher risk of acute and chronic diseases. Indeed, individuals working in these industries often show elevated incidence of disease and mortality. For example, in the case of nickel- or cobalt-operating industry there are higher rates of profession-related diseases (5 per 100 employees). These diseases result from the chronic Ni/Co intoxication and show great diversity of clinical manifestations including chronic bronchitis, exogenous fibrotic alveolitis, dermatitis, toxic myocard dystrophy, malignant neoplasms of lungs, nasal cavities, pharynx, larynx and stomach. The link between magnesium deficiency and living in industrial regions dealing with nickel, aluminum, beryllium, lead, cadmium, strontium, barium, and radium is well-known (Izmerov, 1996; Soldatovic, 2002; Suslikov 2002).

4.10.1. Cadmium and Magnesium

Cadmium is one of the most common industrial toxicants (steel industry, tobacco smoke, smoke from waste incinerators, artificial soil fertilizers). In superphosphate fertilizers, there are 15-21 micrograms of Cd per gram of fertilizer; 1-2 microgram of the air-born form of Cd

is formed during smoking of one pack of cigarettes. Cadmium metal and cadmium oxide are used for the manufacture of special steel alloys, in the production of the light bulbs, batteries and accumulators, photocells, enamels, and paints. Inhalation is the major route through which the toxic forms of cadmium come into the organism. The body can assimilate 20-50% of cadmium from the inhalation flow (including cigarette smoke). Cadmium has been included on the list of carcinogens since 1993, both as soluble ($CdCl_2$, $CdSO_4$, etc) and as insoluble (CdS, CdO, etc) compounds. The vocational cadmium intoxication is, most often, linked to lung cancer, prostate cancer, infertility and renal insufficiency.

Cadmium affects a number of physiological and molecular systems. Cadmium is known to inhibit succinate dehydrogenases, glutathione dehydrogenases, glutathione transferases, oxidoreductases, catalase, carbonic anhydrases, alcohol dehydrogenases, acidic and alkaline phosphatases and also impairs vitamin D adsorption, insulin function, cellular immunity and synaptic transmission. Elevated concentrations of cadmium inside the cells inhibit biosynthesis of DNA, RNA and proteins, induce lipid peroxidation, inhibit DNA repair and thus promote chromosomal aberrations.

The primary physiological targets of cadmium toxicity are the reproductive system and the kidneys. Women accumulate cadmium 4-6 times faster than men and the rates of the cadmium adsorption are much higher in fetuses. The higher rate of cadmium absorption can result in the development of teratogenies in newborn (impairments of the kidneys and nervous system). Parenteral introduction of cadmium to the rat models of acute intoxication (0.5-1 mg/kg) disorganized hypothalamus-pituitary regulation of testicles. We have observed that testicular dysfunction occurred in different ways in adults and young rats. This dysfunction appears to be associated with an impaired synthesis or secretion of the follicle stimulating hormone, serotonin, ACTH and TTH. The excess cadmium appears to penetrate sperm cells through calcium channels and high concentrations of cadmium were found to be associated with varicocele-associated male infertility (Benoff, 2000). Excess cadmium against the background of magnesium deficiency often leads to the formation of cadmium-dependent kidney stones (Hering, 1987; Kuznetsova, 2006). Prescription of magnesium during cadmium intoxication has urolytic effect and opposes accumulation of cadmium.

4.10.2. Beryllium and Magnesium

Beryllium does not have any known role in human physiology and appears to be a purely toxic element. In industry, it is primarily used as a hardening agent in alloys. Beryllium is considered to have mutagenic and carcinogenic effects. Beryllium affects the enzyme activity throughout the body and inhibits, for instance, alkaline phosphatase activity already at 1 micromol/L. Beryllium induces lung tumors and other cancers possibly through chromosomal aberrations caused by destabilization of the native conformation of the DNA polymerase (Rossman, 1981). Industrial intoxication is more likely in instrument making, aviation and space industries; in the production and disposal of fluorescent tubes; waste incineration, and in coal industry. Children can get toxic dose of beryllium when playing with broken fluorescent lamps. The disease known as "beryllium rickets" often occurs against the background of magnesium deficiency. Chronic magnesium deficiency can occur as the result of the biological antagonism of Mg and Be (Yershov, 2000). At the same time, magnesium is the primary counterweight against accumulation of beryllium in organism.

4.10.3. Aluminium and Magnesium

Aluminum is an essential trace element which is found in extremely small concentrations in living organisms (30-50 mg per 70 kg adult) and is present in the cells in the form of Al^{3+} ions. The liver contains about 4 mg/kg, the spleen - 2.6 mg/kg, the bones - 3 mg/kg, the heart - 1 mg/kg, muscles - 1.2 mg/kg, the powdered skimmed brain tissue - 2.4 mg/kg, lungs - 43 mg / kg. With age, the aluminum content in the lungs and the brain tends to increase.

Minor doses of aluminum can be adsorbed through skin from paper towels, foil, napkins, disposable sheets, deodorants, perfume, talk, lipstick, as well as through the gastrointestinal tract with food while using aluminum utensils and aluminum foil. The products with high content of aluminum include tap water in certain regions, artificial food dyes (such as E541, E554, E555, E556, E559 *etc*), sausages and baker's yeast. Adsorption of aluminum increases at high temperatures and acidic pH: for example, when drinking hot tea with artificial food additives and with lemon. The accumulation of the metal is especially pronounced in patients who use medicines containing aluminum such as antacids (Almagel, Almagel Neo, Alfogel, Alugastrin, Alugel forte, Alumag, Aluminum hydroxide-Rivofarm) and some vitamin-mineral complexes (Multi-tabs perinatal). Newer antacids usually contain phosphorus and magnesium along with aluminum (Maolox, Phosphalugel). Patients can get an excess of aluminum during dialysis or when on parenteral nutrition.

Industry, however, is the major source of biotoxic aluminum. Aluminum intoxication happens, in particular, through the air of industrial cities. The most frequent excess accumulation of aluminum is observed in people who work in aviation, paint, mining, paper, and textile industries, on factories that produce or process aluminum, as well as in people working at the garbage dumps.

In aluminum production, the highly dispersed alumina clay, fluorine compounds, carbon monooxide and dioxide, as well as resinous aerosols represent significant health risks. In nickel production, the dust contains silicon, iron, aluminum, sulphur, nickel, cobalt, copper, as well as carbon monoxide, sulphur dioxide and other substances. Employment at the production of aluminum, nickel, copper and some other metals, especially when the production violates ecological standards, is a risk factor for oncologies.

Excess aluminum provokes disorders of the bone metabolism (including the development of vitamin D-resistant rickets), microcyte anemia, neurotoxicity. Aluminum excess also damages nervous system which leads to poorer memory operation, worsened ability to focus, reduced control of behavior, suicidal tendencies, encephalopathy, dementia (including Alzheimers's) and stroke. Aluminum contributes to removal from the body of magnesium, phosphorus, calcium while increasing the toxic effects of lead, nickel, iron, zinc and scandium. Aluminum excess is eliminated from body for at least 6 months and magnesium appears to assist removal of aluminum from the body.

4.10.4. Lead and Magnesium

Lead is recognized as a toxic element although the trace amounts of lead can be important for biological function. In nature, the lead occurs as five stable isotopes: ^{202}Pb, ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. The last three isotopes result from radioactive decay of ^{238}U, ^{235}U, and ^{232}Th. The use of lead in the production of lead-based paints and, still, as a gasoline

stabilizer (tetraethyl-lead) leads to a significant accumulation of lead in the environment. The easier absorbed (and, therefore, the most dangerous) lead substances are acetate, chloride, oxide and tetraethyl-lead while chromate, sulfide, sulphate, and carbonate lead are less soluble and somewhat less dangerous (Suslikov, 2000).

In USA, for example, the total economic losses which were the result of the accumulation of the lead in blood (up to 1-2 mg/L) were estimated to reach 5..8 billion dollars a year. Lead intoxication is likely to cause aberrant behavior, increased aggression, more negative emotional background and social maladjustment of children. The neurotoxic effects of lead can also cause long-term impairment of the intellectual ability. Lead also leads to hypertension and, as the result, to a higher risk of cardiovascular disease.

Magnesium is eliminated more intensely at higher concentrations of lead and vice versa. Even with a slight rise concentration of lead in blood, the levels of magnesium fall (Karczewski, 1987). The ratio of Mg/Pb below 25:1 in hair leads to more intense accumulation of lead (Oleszkewicz, 1998). Contrariwise, magnesium is one of the most important factors that limit accumulation of the toxic elements including lead. Upon lead poisoning, intake of magnesium increases the activity of erythrocytic delta-aminolevulinate dehydrase which is involved in the hem biosynthesis and also activates enzyme protection (the AST and ALT enzymes) in tissues (Todorovic, 2002). Magnesium also inhibits carcinogenic effects of lead.

4.10.5. Nickel and Magnesium

Nickel is used as an alloy component for the manufacture of some steels, galvanized metal products, and also as a catalyst in chemical industry. The most frequent industrial forms of nickel are aerosols of the metallic nickel as well as nickel oxide, nickel sulphate and nickel carbonyls. Acute intoxication is characteristic for nickel carbonyl rather than any other form. Adsorption of biotoxic nickel occurs through active transport and diffusion, with the excess flow of nickel into the bowel accompanied by saturation of the transporting channels which results in lowering the adsorption rate. In general, ~1% of the excess quantities of nickel is adsorbed by the body. Magnesium inhibits adsorption of nickel and, with sufficient level of magnesium intake with the food, the nickel adsorption can be significantly reduced. In humans, up to 45% of the total serum nickel contains nickel-plasmin protein (α2-macroglobulin). This protein has a molecular weight of about 700 KDa and binds nickel in 1:1 ratio (1 nickel ion per 1 globule of the protein).

Nickel and other industrial polluters can substitute magnesium in proteins (including the DNA-binding proteins, magnesium-containing enzymes, neuropeptides, etc) and energy molecules (ATP). This leads to an increased removal of magnesium from the body, to pathological inhibition of the downstream signaling from the Mg-containing proteins and to accumulation of toxic metals in tissues and organs. Nickel excess is especially toxic when it occurs on the background of the deficit of magnesium and zinc.

Nickel accumulates in kidneys, skin, lung tissue, nasopharyngeal tissues, the central nervous system and bones. In experimental model of 13-week chronic intoxication with nickel sulphate, the accumulation of excess nickel was greater in the kidney and lower in the liver (kidney> testes> lungs=brain> spleen> heart= liver) indicating that kidneys are most susceptible to the toxic effects of nickel (Avtsyn, 1991). The target of carcinogenic nickel

action is chromatin. Nickel does not only stimulate excess of DNA methylation, but also turns off anti-tumor protection by reducing the expression of a number of the tumor suppressor genes. Magnesium, zinc, calcium in sufficient quantities can protect the organism from the nickel poisoning.

Gradual displacement of magnesium under the influence of nickel has very serious consequences for health as it results in a chronic suppression of the immune function which leads to acute and chronic diseases. Excess of nickel combined with magnesium deficiency leads to cancers and produces teratogenic effects in the developing fetus. Unfortunately, the effects of nickel do not always lead to systematic disturbances of metabolism which makes the early diagnostics difficult (unless, of course, the levels of nickel and other trace elements are directly determined in tissues of the patient). The final phase of nickel intoxication is nickel-dependent chronic diseases such as allergies, chronic lung disease, impaired olfaction, myocardial dystrophy, lung cancer, skin cancer, abnormalities of nasal sinuses, larynx, esophagus, stomach, CNS, especially during fetal development. Nickel is also known to cause hyperglycemia through activating the production of cGMP and NO-synthases of the brain, adrenal and pancreas thus predisposing to diabetes. In low doses (0.02 mg/kg), exogenous nickel produces vasoconstriction (Liu, 2002). At the same time, myocardium undergoing ischemia releases endogenous nickel. Nickel also stimulates the influx of excess calcium in the cell through Ni-sensitive calcium channels.

These negative effects of the nickel intoxication can be partially avoided using magnesium preparations as antidotes and can be considerably reduced in magnitude when magnesium is used in a preventive treatment course. At the same time, using chelating agents against nickel intoxication was unsuccessful (Goyer, 1995; Luzhnikov, 1998) and, moreover, caused considerable side effects through excessive depletion of the physiological pool of nickel and of the other trace elements. The modern trend of disease prevention in nickel-cobalt industry is biological detoxification based on the principle of the natural antagonism among the trace elements, such as the antagonism between nickel and magnesium.

Magnesium is the most important antagonist of nickel and of other toxic trace elements. The magnesium drugs that can be efficiently used to alleviate and to prevent excessive accumulation of the toxic elements such as Ni, Pb, Be, Al, Sb contain organic salts of magnesium (lactate, pidolate, citrate, asparaginate, orotate *etc*). Calcium alginate, pectins, artichoke extracts also delay the accumulation of toxic metals in the body through chelating effect and formation of insoluble salts of the toxic metals which are then excreted through gastrointestinal tract.

4.10.6. Radiation and Magnesium

The planet Earth is plunged an ocean of the high energy particles coming from the Sun, cosmic rays as well as from the interaction of cosmic rays with the upper atmosphere. Although the Earth's magnetic shield, the Van Allen belt, does a very good job of protecting the Earth's inhabitants, the magnetosphere is in constant state of flux which is dependent, in particular, on the cycles of solar activity. When man approaches the space either during spaceflight or simply during high-altitude flight, the high energy particles interact with the body and its elemental composition.

During high-altitude flights (>10000 meters above the sea level), especially in the vicinity of the polar regions, the radiation exposure is much greater than during low-altitude flight. During the solar flares, solar protons, nuclei of helium and of heavier atoms accelerate in the interplanetary space and reach high levels of kinetic energy. When the Earth intersects with these intensified flows of the charged particles, the levels of radiation in the Earth's atmosphere can substantially increase. For example, in February 1956 the Ground Level Enhancements (GLE) of the secondary radiation reached to over 30 times (the GLE5 event). The ACREM project (Air Crew Radiation Exposure Monitoring) focused on local methods for calculating flows of high-protons, using the data network from the ground neutron monitors (Schrewe, 1999). This approach allowed to estimate the dose of radiation as a function of height and time throughout the entire globe, regardless of the degree of anisotropy or perturbations of the geomagnetic field (table 4-10).

Table 4-10. Estimates of the radiation exposure in subsonic aircraft

Stage of flight	Unit	Radiation doses	
		Flight altitude (m)	
		<6000	<12000
Takeoff site	microR · h-1	12,6±0,3	13.3±0,6
Takeoff	microR	17,4±0,5	91,9±8,3
Flight altitude	microR	39,4±2,1	278,9±32,8
Landing	microR	16,8±0,6	38,5±2,0
Landing site	microR · h-1	13,3±0,4	16,1±0,7
Average per flight	microR	25,5±1,2	222,9±22,5

In fact, direct measurements of the radiation dose on board of the charter flights of commercial aircraft has shown that that the dose ranges from 4 to 10 mZivert/year and is comparable with dosimetry data from nuclear power stations. One of the main mechanisms through which radiation damages living systems is the direct DNA damage. Against the background of calcium and magnesium deficiency (which also blocks DNA repair mechanisms), cumulative effect of the radiation load quickly develops into a variety of pathologies. In comparison to other kinds of human cells, leukocytes are the most sensitive to radiation so the higher radiation doses lower immunity. The preventive role of physiological doses of calcium, magnesium, vitamin C, folic acid, pectins, in the prevention of long-term adverse effects of radiation on humans are known (Izmerov, 1996).

4.11. MAGNESIUM AND SPORTS

Under physical stress, the muscle tissue exhibits an increased demand for basic nutrients and minerals, including magnesium. A lack of sufficient magnesium in the diet of athletes can result in a palette of magnesium-dependent pathologies. Insufficiency of magnesium in athletes appears to be an extremely common condition and is certainly related to underachievement in sports. Studies of the diagnosis and treatment of magnesium deficiency among athletes and among heavy physical workers are actively done in European countries (Germany, Austria, France, Hungary and others) and also USA. Awareness of magnesium

deficiency being persistent among athletes led to the inclusion of magnesium preparations into individual courses of correction of the mineral and energy status of the athletes. *The primitive one-sided view that strong will has the crucial impact on athletic performance seems to slowly die away giving place to a complex program of the development and self-development* of athletes. In this paradigm, the material basis for achievement in sports is the harmonious biochemical constitution of all nutrients, minerals included.

The following physiological effects of magnesium, discussed earlier, are especially important for an athlete: energy metabolism, neuromuscular transmission and muscle contraction, conductivity and excitability of the nerves which grows with the shortage of magnesium. Causes of magnesium deficiency among athletes include disproportionate diet, alcohol and imbalanced vitamin/mineral regimens. The *disproportionate diet* is characterized by an increased proportion of animal products over the vegetables and a higher consumption of protein and fat foods. Such a diet increases the need for magnesium not only because it is poor in magnesium (in particular, because of the food processing and refining) but also because magnesium tends to form insoluble precipitates when reacting with dietary protein and fats. Chocolate, cocoa-rich foods, almonds, forest nuts, beans are rich in magnesium and can be used for correction of chronic magnesium deficiency. However, these products are virtually excluded from the low-calorie diets for weight control prevalent among the athletes. Special food salts with low sodium chloride content and fortified with magnesium and potassium are also rarely used in sports medicine. The problem of *alcoholism in sports* (especially after the sporting career, during the retirement) does certainly exist. Alcohol, on the background of a high physical activity, contributes to an increased elution of magnesium from the body thus aggravating the magnesium deficiency. In sports, *special diets and weight control systems* are very important. It should be noted that the food standard for people with normal physical loads, currently adopted in Europe and USA, contains too low daily requirements of vitamins and minerals and does require a major revision. Commonly adopted nutritional standards for athletes in respect to vitamins and minerals were not even formulated. A New York study of 358 young athletes (children 10-15 years old) indicated that magnesium deficiency is not infrequent in this population (Bollella, 1999).

Apart from insufficient magnesium intake, athletes, compared to the rest, are characterized by an increased demand for this element because of the prolonged physical exertion, stress and significant loss of magnesium with sweat (especially in the hot weather and during regular visits to sauna). Studies have shown that magnesium plasma levels and erythrocyte magnesium in athletes are often below the normal before and after sport events (Rayssiguier, 1990; Skalniy, 1999). The lowest rates of magnesium were recorded in student athletes and among marathoners, oarsmen, and football players. In professional sport, the incidence and the extent of magnesium deficiency depend on the length of the career and professionals older than 30-35 often develop a deep deficit of magnesium.

The content of magnesium in the body of an athlete can not be viewed in isolation from the rest of the mineral homeostasis. An examination of the magnesium content in the context of elementary homeostasis (Mg, K, Ca, Na, P, Se, Zn, Co, Cr, Cu, Fe, Mn, Mo, Si, Li, Ni, V, Pb, Sn, Cd, Al , As, Be, Bi and Ti) in 24 children athletes (8-12 years old) revealed that magnesium deficiency has the leading place among the 25 elements studied (Gromova, 1998-2000). However, in none of the examined volunteers was magnesium deficiency the only abnormality of the element status. Deficiency of magnesium correlated with excess of lead and, at the same time, with the deficiency of the calcium and manganese. More

comprehensively the biochemical relationships between magnesium, calcium, manganese and lead were analyzed in (Goyer, 1995). It is known that during magnesium deficiency manganese assumes some of the biochemical functions of magnesium while calcium is held by the bone only poorly when at magnesium deficiency.

The most important points of application of the pharmacodynamic properties of magnesium in respect to sports and athletes are:

- Magnesium ion plays an important role in metabolism and is a cofactor of many enzymes (such as creatine kinase, hexokinase, adenylyl cyclases, guanilate cyclases, K+/Na+ ATPases, *etc*) the activity of which is important for optimal neuromuscular function (which manifests as the reaction speed) and, therefore, for sports achievements.
- Magnesium is involved in reactions of oxidative phosphorylation, the synthesis of proteins, nucleic acids and lipid metabolism, secretion, and insulin activation. These functions of magnesium are important for stability of the muscle mass.
- Magnesium is a natural physiological antagonist of calcium that competes with calcium at all levels of cellular function (transmembrane calcium current, calcium release from sarcoplasmic reticulum, mechanisms that regulate calcium intake by mitochondria). Formation of the actin-myosin bridges occurs only in the presence of the Mg^{2+}·ATP. These functions determine, in particular, higher tolerance of prolonged physical effort characteristic for cycling, marathon, and swimming over long distances.
- Magnesium has inhibitory effect on the blood coagulation and stabilizes platelet membranes. Both effects are important for prevention of the thrombotic tendencies in various sports (marathon running, heavy athletics *etc*).

MagneB6 represents a new generation drug for magnesium correction which contains magnesium in bioorganic form which is easily adsorbed. The high degree of purification of the drug's components ensures both high drug safety and efficiency. While the fight against doping in sport, along with realization of the strongly negative effects of hormonal drugs, certainly reduces the interest in the conventional (but illegal) doping, magnesium drugs represent important means of legitimate pharmacologic corrections of the mineral imbalances so characteristic for sportsmen, both professional and semi-professional. Restitution of the magnesium balance is a natural way to improve physical performance without producing any of the side-effects which characterize usual pharmacopoeia of the sports medicine (Golf, 1998).

Chapter 5

5. CORRECTION OF THE MAGNESIUM DEFICIT

5.1. MAGNESIUM DIET

Correction of magnesium deficiency includes dietary and pharmacological components. For the selection of the right diet, one should take into account not only the quantitative content of magnesium in food, but also its bioavailability. Thus, fresh vegetables, fruits, fresh herbs (parsley, dill, green onions, etc), and nuts have maximum concentration and bioavailability of magnesium. When products are processed for long-term storage (drying, canning, *etc*), concentration of magnesium decreased only slightly, but its bioavailability falls down sharply. That is why in summer, when there is a lot of fresh fruits, vegetables and greens on the menu, both the extent and the incidence of the magnesium deficit is reduced (Fedotova, 2003). This is important to keep in mind in the case of children with ADHD who appear to have a deeper deficit of magnesium during the school classes (from September to May). In summer, ADHD children and their parents display fewer complaints than in autumn, winter and spring.

Depending on geographic zone, the content of magnesium and of other minerals in one and the same product can fluctuate significantly. For example, in wheat bran grown on Russian soil the average levels of magnesium (448 mg/100g; Skurihin, 2002) are lower than those in the wheat bran grown in western Europe (590 mg/100g; Murrau, 1999). The table 5-1 details the average magnesium contents of various foods.

Table 5-1. The content of magnesium in different food products. "*" marks the products particularly rich in magnesium (Murrau, 1999)

Product	Mg-content, mg/100g
Brown algae, kelp	760*
Wheat bran	590*
Sesame	540*
Pumpkin seeds	535*
Sunflower seeds	420*
Red wine	258*
Germinated wheat grain	250*
Soybean	247*
Brewer's yeast	231*

Table 5-1. (Continued).

Product	Mg-content, mg/100g
Watermelon	224*
Nuts, almond	267*
Nuts, different	158-267*
Hazelnut	184
Peanuts	175
Walnuts	131
Dry milk serum	180
Greens	170
Oatmeal flakes	142
Beans	130
Brown rice	130
Pea	107
Coconut chips	90
Dried pitted apricots	105
Prunes, Dried	102
Rough bread	90
Apricots, raisins	60
Dates	58
Shrimps	51
Avocados	45
Parsley	41
Garlic	36
Dandelion leaves	36
Bananas	35
Cheese	30
Marine fish	24-73
White rice	27
Aubergines	16
Meat (beef)	20
Meat (chicken)	19
Milk	13

5.2. SOURCES OF MAGNESIUM IN THE ENVIRONMENT

Magnesium is present as a major component in nearly 200 different minerals. Magnesium chloride and sulphate are also the major components of the dried residue of the sea water and sea bathing is often recommended as a supplementary procedure for correction of magnesium deficiency. Normally, absorption of magnesium, iodine, calcium and other minerals from seawater through skin and mucus is insignificant but it grows observably when the patient has deficiency of magnesium. Therefore, sea bathing and mud bathing, along with inhalations of the sea water, somewhat help in restoration of the mineral balance in the course of treatment of cervical erosion, chronic tonsillitis, bronchial asthma and other diseases. The content of the soluble salts of magnesium and calcium determine the hardness of the drinking water of a particular region. Magnesium is also present in the crude salt as well as in salts from specific natural deposits: Black Indian salt, Salzburg salt, Bishofit from Ural, Hungarian salt, Saxon salt, Irish salt (of the Saga type), Greek salt *etc*.

Of great importance for the magnesium correction is the treatment with mineral water that contains adequate supply of magnesium. Since ancient times it was noticed that the incidence of cardiovascular disease and of many others diseases tends to be higher in certain regions which were later found to be impoverished in minerals and trace elements. The residents of mega-cities often receive water with the addition of chlorine, fluorine and other special components from the water-cleansing columns. Many of these chemical components adversely affect the balance of magnesium, potassium and calcium. It should be noted, however, that most of the commercially available mineral waters are not very high in magnesium and mineral waters naturally high in magnesium (such as Slovenian "Donat") are not very numerous.

5.3. Pharmacological Correction of Magnesium Deficiency

Pharmacological correction of magnesium deficiency is based on regular intake *per os* of 5-15 mg/kg of magnesium salts for several months and in accordance with age and gender requirements (see tables in Chapter 2). For the correction of magnesium, as it is the case of correction of other mineral deficiencies, bioorganic drugs of different generations can be used. It is known for more than half a century that low adsorption, low assimilation and considerable side effects (metal taste in the mouth, nausea, vomiting) are essential drawbacks of the 1st generation of the magnesium drugs. During the two last decades, progressive pharmaceutical companies actively elaborate second and subsequent generations of bioorganic drugs and supplements which contain minerals in the form of organic salts, complexes with amino acids and other organic ligands (table 5-2).

Table 5-2. Classification of the drugs for the correction of mineral and trace element deficiencies (Gromova, 2003)

Generation	Composition	Examples
I	Inorganic compositions	Magnesium oxide, magnesium sulphate, zinc oxide, potassium chloride, sodium selenite
II	Organic compositions	Magnesium lactate, magnesium pidolate, zinc asparaginate, chromium picolinate, chromium nicotinate
III	Minerals in combination with biological ligands exogenous natural (plant and animal) and synthetic origin	Organic salts plus vitamins (magnesium lactate together with pyridoxine), amino acids, alkaloids, bioflavonoids, enzymes, natural pigments like chlorophyll, plant extracts
IV	Minerals in conjunction with exoligands, complete analogs of endogenous ligands, "orthomolecular" complexes with neuropeptides, amino acids, enzymes, polysaccharides	Extract of Ginkgo Biloba, Se-methionine, Se-cysteine, Zn-carnosine, Mg-creatinine kinase, Cu -ceruloplasmin, Se-protein,Zn-metallotionein, Mn-containing superoxide dismutase

Organic magnesium salts are better adsorbed, tolerated better by patients, produce less side effects and restitute magnesium deficiency more efficiently (table 5-3, figure 5-1).

Table 5-3. Magnesium forms and their bioavailability (NB: during magnesium deficiency, bioavailability of all the forms slightly increases)

Magnesium salt	Brutto formula	Bioavailability	Generation	Side effects
Magnesium oxide	MgO	4,7%	I	Dyspepsia
Magnesium hydroxide	$Mg(OH)_2$	5%		Dyspepsia, diarrhea Диспепсия, diarrhea
Magnesium carbonate	$MgCO_3$	3%	I	Dyspepsia, diarrhea
Magnesium peroxide	MgO_2	6%	I	Dyspepsia, diarrhea
Magnesium sulfate	$MgSO_4$	5%	I	Dyspepsia, acute inflammation of gastrointestinal tract
Magnesium citrate	$C_{12}H_{10}Mg_2O_{14}$	37%	II	N/A
Magnesium asparaginate	$C_4H_8MgN_2O_3$	32%	II	N/A
Magnesium orotate	$C_{10}H_6MgN_4O_8$	38%	II	N/A
Magnesium lactate	$C_6H_{10}MgO_6$	38%	II	N/A
Magnesium pidolate	$C_{10}H_{12}MgN_2O_6$	43%	II	N/A

Ranade, Somberg (2001) presented the comparative analysis of bioavailability of various salts of magnesium. Therapeutically, the magnesium salts constitute a specific class of drugs with quite different pharmacological applications. For example, magnesium citrate is used in nephrolithiasis, magnesium hydroxide as an antacid. There are several well absorbed galenical forms of magnesium drugs: magnesium citrate, magnesium gluconate, magnesium orotate, magnesium thiosulfate, magnesium lactate (MagneB6 tablets), magnesium pidolate (MagneB6 solution to drink). The contents of elemental magnesium in various forms do vary. For example, magnesium hydroxide, chewing tablet - 130 mg of elemebtary magnesium; magnesium gluconate, tablet 0.5 g - 27 mg of magnesium; magnesium citrate sparkling tablet 0,15 g - 24,3 mg; magnesium orotate, tablet 0,5 g - 32,8 mg; magnesium thiosulfate, tablet 0,5 g - 49,7 mg; magnesium lactate (Magne B6 tablets, 470 mg) - 48 mg (Ogunyemi, 2007).

For magnesium correction, different generations of drugs can be used. The first generation include inorganic compositions: magnesium oxide, sulfate, chloride, etc; the the second - organic compounds: magnesium lactate, orotat, pidolat, glitsinat, asparaginate, citrate, ascorbate. Pidolate, citrate, gluconate, aspartate are characterized by a higher excretion with urine than inorganic salts (Coudray et al. 2006). At the same time, inorganic salts of magnesium are poorly tolerated and more often produce dyspeptic complications such as diarrhea, vomiting, stomach pains (Grimes, Nanda, 2006).

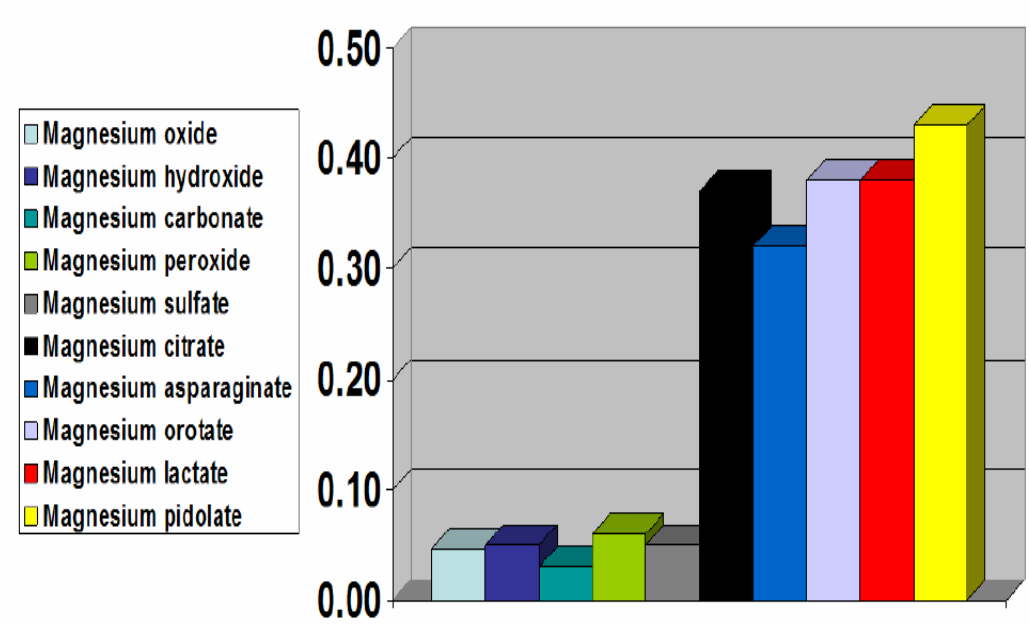


Figure 5-1. Magnesium bioavailability of inorganic and organic salts.

Recently proposed "natural" drugs made from crushed animal bone, dolomite, egg shells, oyster shells contain too much harmful impurities and, in particular, lead (figure 5-2).

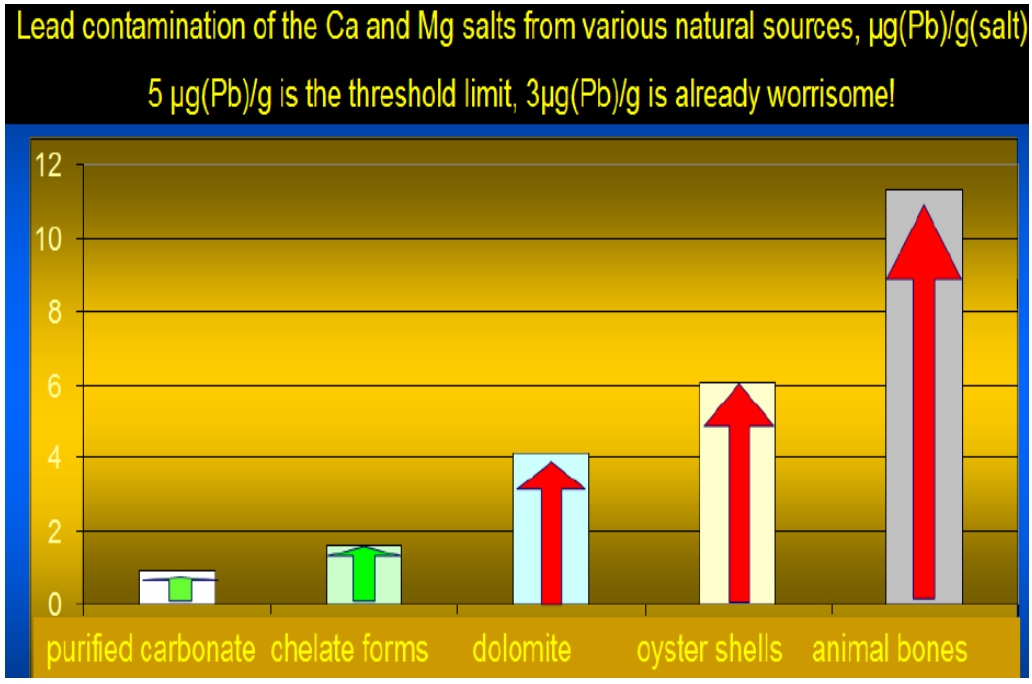


Figure 5-2. Lead impurities in the "natural" magnesium preparations (Blumberg, 2004).

5.4. PARENTERAL MAGNESIUM THERAPY

Parenteral (especially intravenous) therapy with magnesium is indicated in urgent cases of magnesium deficiency as well as in the case when previously used therapy was ineffective. The therapeutic forms for the parenteral therapy differ in their efficiency, magnesium content and bioavailability (Durlach, 2004). A comparison of the magnesium gluconate, fumarate and chloride indicated that parenteral infusion of magnesium at concentrations 5 mmol/L would be most optimal from the point of view efficiency and safety (Durlach, 2002).

Parenteral magnesiotherapy normalizes the absorption of magnesium. Treatment is more efficient if magnesium is introduced along with magnesium fixator such as vitamin B6 or insulin. Parenteral therapy must be done in stationary conditions and the usual dosage is 100 mg/hour during the 4-6 hours a day (table 5-4).

Table 5-4. Contents of elemental magnesium in pharmaceutical forms for parenteral introduction

Preparation	Solution	Elemental magnesium, (mg/ml of solution)
Magnesium ascorbate	5% injection solution	6,1
Magnesium glutamate	10 % injection solution	7,6
Magnesium sulfate	10% intravenous solution	9,9
Magnesium ascorbate	10 % injection solution	12,2
Magnesium chloride	20% intravenous solution	24
Magnesium sulfate	25% intravenous solution	24,75
Magnesium sulfate	50% intravenous solution	49,5
Magnesium diasporal forte	injection solution, 2ml	320

Before any treatment course of parenteral magnesium therapy, it is necessary to determine the levels of magnesium in plasma and erythrocytes. Contraindications for parenteral magnesium therapy include:

- severe renal failure;
- miastenia gravis;
- malignant neoplasms;
- urinary tract infection (which accelerates precipitation of the magnesium ammonium phosphates)

5.5. MAGNESIUM-PRESERVING DIURETICS

Common diuretics such as furosemide (and, to some extent, indapamide and hypothiazid) accelerate elimination not only of sodium, calcium, potassium and chlorine, but also of a number of important minerals: Se, V, Zn, Ni, Li, as well as Mg (Gromova, Grishina, 2005). This should be taken into account when planning the course of magnesium therapy or when prescribing diuretics. Mg-preserving diuretics such as amiloride or aldacton are especially recommended when more than 6mmol of magnesium is excreted in two hours.

5.6. MAGNESIUM FIXATION

Vitamin B6, vitamin D and vitamin B1 are the most important fixators of magnesium in the body. These fixators can be used immediately upon diagnosis of primary magnesium deficiency and also in the case of ineffective treatment by other drugs.

- In pharmacological doses (in the form of pyridoxine hydrochloride), vitamin B6 increases the magnesium in plasma and erythrocytes and reduces magnesium elimination when applied along with a a dose of magnesium.
- Vitamin D in pharmacological doses, either natural (D3 or cholecalcipherol) or synthetic (D2 or ergocaciferol) is used to reduce the risk of acute or chronic hypercalcemia. Vitamin D-based therapy in combination with magnesium therapy should take into account three points:

    - Calcium therapy and phosphate therapy cannot be done simultaneously;
    - In conjunction with magnesium therapy, not pharmacological but physiological does of vitamin D have to be used (200-400 IU/day);
    - Systematic monthly control of calciemia (<105 mg / l) and 24h calciuria (<4 mg/kg/day) is essential.

- Vitamin B1. Vitamin B1 in physiological doses (1-1,5 mg / day) improves the metabolism of magnesium. Magnesium is cofactor in many thiamine-dependent enzymes.

Chapter 6

6. EFFECTS OF VARIOUS DRUGS ON MAGNESIUM HOMEOSTASIS

6.1. PARTIAL MAGNESIUM ANALOGUES

Partial analogues of magnesium are chemical compounds capable of reproducing some effects of magnesium. They are usually recommended when previous attempts of using magnesium as monotherapy were not successful in respect to the condition in question. These compounds might have very different chemical formulas but share with magnesium at least some of the molecular pathways involved. For example, beta-blockers act upon the beta-adrenoceptors while magnesium is important for the signal transduction downstream from the adrenoceptors (adenylate cyclases, in particular). Another example: many anticonvulsants, like magnesium, are antagonists of the NMDA channels.

- propranolol (avlokardil, inderal, obzidan) in high doses (30-120 mg) limits heart rate to 60 strokes per minute. Maximum efficiency is achieved with the relatively new forms of the drug (propranobene capsules 80 and 160 mg, propranolol tablets 10, 40, 80,160 mg). Therapeutic effects of isoproterenol apparently increase when serum and erythrocyte magnesium is in the normal range.
- verapamil (izoptin) is one of the most effective calcium antagonists and has beneficial effects on myocardial function. Use of the drug in the case of inborn cardiovascular pathology (mitral valve prolapse, in particular) appears to be much less efficient.
- anticonvulsants markedly reduce signs of the hyperexcitability of the central and/or peripheral nervous system

    - phenytoin (150-300 mg/day) recommended for mitral valve prolapse (Pvm) and/or hypoglycemia;
    - baclofen (Liorezal) in convulsions of extremities;
    - carbamazepine (0.75-1.5 mg/day) prescribed for paresthesias;
    - clonazepam (Rivotril) (300-600 mg/day), headaches and convulsions;
    - phenobarbital (Gardenal) (3-6 mg/day) is sometimes combined with belladonna alkaloids to be used in neuroses. With long-term usage, it is

necessary to monitor the level of 25-OH-D3 (25-hydroxycholecalciferol) hormone so that deficiency of this hormone could be compensated (4 mg/day 25-OH-D3).

6.2. MAGNESIUM PROTECTORS

Magnesium protectors are chemical compounds that reinforce adsorption of magnesium into the cells and reduce its elimination through gastrointestinal tract.

- Vitamin B6 (pyridoxine hydrochloride) strengthens the effects of magnesium. This vitamin is not synthesized in the body and should come with food. The need for vitamin B6 is enhanced when under stress, in patients with heart disease, hyperhomocysteinemia, atherosclerosis, nephropathy.
- Vitamin antioxidants (vitamin A, C and E) increase cellular Mg content
- Vitamin D and its metabolites increase the absorption of magnesium
- Riboxin, carnitine, taurine increase cellular Mg
- Orotic acid increases cellular Mg content. Orotic acid is a growth factor of the endogenous bacteria which unable to synthesize it. Orotic acid is synthesized in the human body from the L-aspartic acid and carbamoylphosphate and its synthesis is affected in heart disease, blood loss, and post-surgery. After surgeries, orotic acid supplements (3 weeks-2 months) are recommended.
- Calcium drugs increase cellular Mg (but excess calcium has the opposite effect).
- Adrenaline and glucocorticoids increase Mg content in the cells. Excess adrenaline has the opposite effect.

6.3. MAGNESIUM-DEPLETING DRUGS

- Estrogen-based drugs, as well as endogenous estrogens, reduce circulating magnesium. In addition, estrogens antagonize pyridoxine which assists magnesium transport into the cells, especially in gastrointestinal tract. Oral contraceptives or hormonal replacement therapy stimulate magnesium deficiency.
- ß-adrenoblockers in excess inhibit Mg adsorption.
- Insulin, caffeine, aminophylline, effedrin stimulate loss of intracellular Mg.
- Furosemide, hypothiazid increase excretion Mg through kidneys. At the same time, calcium-preserving diuretics (amiloride, spironolactone, arifon) are also magnesium-preserving.
- Aminoglycosides increase the excretion of Mg. Increased magnesium loss from the epithelium of the inner ear is one of the main reasons of the ototoxicity and neurotoxicity of the aminoglycoside antibiotics.
- Cyclosporin is nephrotoxic and increases Mg elimination with urine. Nephrotoxicity of cyclosporine A is based on gross interference of the drug in magnesium homeostasis.
- Cisplatin leads to hypomagnesemia by disrupting Mg resorption in glomeruli.

Chapter 7

7. TOXICOLOGY OF MAGNESIUM: HYPERMAGNESEMIA

The cells of the medulla oblongata are especially sensitive to the magnesium ions and hypermagnesemia, acting on the medulla, can cause several complications including: suppression of the central nervous system (which manifests as apathy, drowsiness); paralysis of breathing and loss of consciousness; dysfunction of the peripheral conductivity which results in suppression or even disappearance of reflexes; dysfunction of cardiovascular system manifesting feeling of heat, sweating and arterial hypertension. Excess of magnesium in the body usually arise because of

- excess of Mg-based infusions (such as using $MgSO_4$ for eclampsia treatment);
- excess use of Mg-based antacids;
- chronic renal insufficiency;
- hypothyroidism;
- dehydration;
- adrenal failure.

Especially the excessive infusion of the magnesium sulfate could lead to dangerous hypermagnesemia, characterized by specific clinical course and which can be reliably detected by using measurements of magnesium in plasma and erythrocytes. Hypermagnesemia usually arises because of the long-term intravenous infusions which aimed at maintaining the pregnancy. A typical administering schedule (200..300mg infusion everyday, 20-30 days, 2-3 courses during pregnancy) can easily cause hypermagnesemia unless magnesium levels are controlled.

Excess of Mg negatively impacts not only the mother but also the fetus. The $MgSO_4$ drug freely passes through the placenta and can lead to hypotonia, hyporeflexia and depression of breathing in newborn. With the exception of severe forms of eclampsia, magnesium infusions are strictly prohibited 2 hours prior to childbirth so that the breathing of the newborn won't be suppressed. Suppressed breathing due to hypermagnesemia is eliminated through the infusion of calcium chloride in the umbilical. $MgSO_4$ is also categorically contraindicated during oligouria and chronic renal insufficiency (creatinine clearance <20 ml/min). Excess of

magnesium sulfate provokes periventricular leukomalatia and intraventricular hemorrhages which subsequently result in gross neurological pathology (Canterino, 1999; Grether, 2000).

Application of excess $MgSO_4$ in pregnant rats leads to severe ischemic changes in the brain of the offspring (Sameshima, 1999). Long-term use of $MgSO_4$ in pregnant in the absence of monitoring the level of magnesium in blood confers a fourfold risk of giving birth to children with infant cerebral paralysis and the use of $MgSO_4$ combined with urogenital infection among pregnant increases the risk of ICP in newborns even further (Matsuda, 2000). If everyday $MgSO_4$ infusions last continuously for > 4 weeks, bone defects and congenital rickets become possible (Mashkovsky, 2003).

Abnormally high levels of magnesium ions in plasma comprise the major diagnostic criterion of hypermagnesemia. The lower border for diagnosing hypermagnesemia corresponds to level of magnesium in plasma being greater than 1.26 mmol/L (figure 7-1). At these levels, the hypermagnesemia is weakly expressed. When concentration of magnesium in plasma reaches 1.55-2.5 mmol/L, nausea, vomiting, bradikardiya, atrioventricular blockade, and acute feeling of thirst and heat can be observed as side effects. At this point, hypermagnesemia has a clearly observed depressing effect on the central nervous system causing ataxia, weakness, stupor, respiratory depression, and hypotension. Levels of magnesium higher than 7.5 mmol/L (children, 5.5 mmol/L) can lead to a transient cardiac arrest.

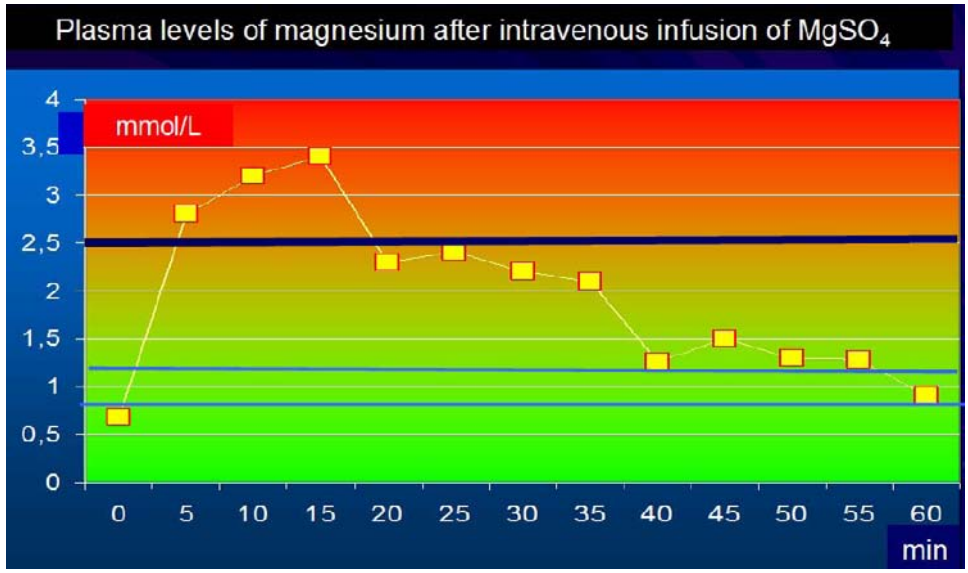


Figure 7-1. The concentration of plasma magnesium (mmol/L) during intravenous infusion of MgSO4. Green band marks the region of the safe concentrations; concentrations over 2.5 mmol/L can have toxic effects.

The criteria for safe magnesium levels during $MgSO_4$ therapy vary by country. In Russia, for example, the safe levels are held to be 2.5-3.75 mmol/L. Pronounced anticonvulsive effect are achieved at 2.5 mmol/L; knee reflexes disappear at 5 mmol/L; suppression of breath occurs at 6-7.5 mmol/L. The French data indicate that hypotension develops at 2.5-3.2 mmol/L, drowsiness at 2.5-3 mmol/L, weakness and ataxia at 3.5-5 mmol/L, suppression of breath at 5 mmol/L, coma- at 6-7 mmol/L (Dinsdal, 1988). According to the Japanese

Association of Obstetricians and Gynaecologists (2000), Mg levels during $MgSO_4$ course of infusions should not exceed 1,8-3 mmol/L because already at these levels mothers develop transient disorders of the brain function and the fetus can suffer irreversible brain lesions as a result of microhemorrages (mostly intraventricular) and mosaic leukomalacia. The levels of 3,5-5 mmol /L considerably increase the chances of the cardiac arrests of the fetus whereas 5-6,5 mmol/L induces breathing paralysis and death of the fetus *in utero*.

In conducting the course of infusions of $MgSO_4$ the following should be evaluated:

1) Urine excretion: not be less than 30 ml/h;
2) Breathing frequency: at least 15-16 per minute;
3) The pronounced and acute knee reflex (suppression of the knee reflex comes much earlier than suppression of breath).

Hypermagnesemia manifests as a characteristic set of complaints which include:

- double vision,
- heat waves over the face,
- headache,
- nausea,
- indistinct talk, as if in stupor,
- hypotension,
- fainting,
- ECG: during hypermagnesemia lengthening of QT is observed along with extending of the QRS complex (at concentration of magnesium being at 2.5-5.5 mmol/L).

$MgSO_4$ infusions are contraindicated when the patient has

- heart block,
- miastenia,
- progressive muscular dystrophy,
- thrombophilia or thrombocytopenia,
- latent tetanus,
- respiratory alkalosis in children,
- aminoglycoside therapy.

Parenteral use of magnesium salts other than $MgCl_2$ can lead to hypochloremic alkalosis and loss of potassium (hypokaliemia). In this case, a chloride-containing magnesium salt is used: magnesium chloride or magnesium asparaginate hydrochloride. $MgSO_4$, apparently, increases hypochloremic alkalosis. When the plasma magnesium levels approach 5 mmol/L, it is necessary to quickly introduce intravenously 10% solution of calcium gluconate (dose of 10-30 ml).

Chapter 8

8. PHYSIOLOGICAL IMPORTANCE OF PYRIDOXINE

In the human body, the highest levels of the pyridoxine (vitamin B6) are found in liver, myocardium and kidneys. Pyridoxine improves the use of unsaturated fatty acids by the body and also has beneficial effects on the functions of the nervous system, liver, blood. There are three derivatives in the form of pyridoxine, pyridoxal and pyridoxamine and the term "pyridoxine" often denotes all the three (figure 8-1).

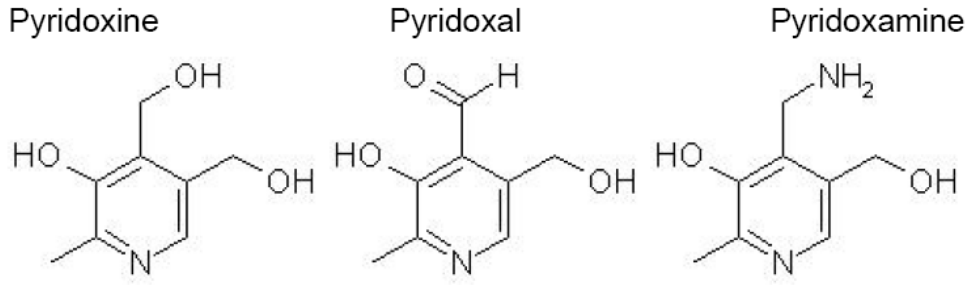


Figure 8-1. The three forms of the vitamin B6.

Derivatives of pyridoxine are bound to ~100 enzymes either as cofactors or as substrates. Most of these enzymes require pyridoxal phosphate as a cofactor. These enzymes support fat metabolism, amino acid metabolism (transamination, deamination and decarboxylation of amino acids; tryptophan turnover, turnover of sulphur-containing amino acids), and intracellular signalling. Some of the proteins are involved in energy metabolism (glycogen phosphorylase) and biosynthesis of another important cofactor, NAD (kynureninase). At least four enzymes are involved in biotransformations of pyridoxine (two pyridoxal phosphate phosphatases, pyridoxamine 5'-phosphate oxidase, and pyridoxal kinase).

8.1. METABOLISM, ABSORPTION AND ELIMINATION OF PYRIDOXINE

In food stuffs, pyridoxine and its derivatives are bound to the proteins. In the process of digestion in the small intestine, they are released and absorbed through diffusion. Firstly, pyridoxine forms are dephosphorylated and then are phosphorylated again after being

transported with the blood. In blood, pyridoxine transforms into pyridoxamine and pyridoxic acid. In tissues, pyridoxine is converted into pyridoxine phosphate, pyridoxal and pyridoxamine phosphate. Pyridoxal is converted into 4-pyridoxic acid and 5-phosphopyridoxic acid, both acids are excreted with urine.

8.2. PYRIDOXINE DEFICIENCY

Pyridoxine deficiency (ICD-10 diagnosis E53.1) arises as the result of insufficient intake or as the result of an increased demand of the organism. For example, high levels of physical activity, pregnancy, protein diet rich in tryptophan/methionine/cysteine, artificial nursing or taking medications which suppress the exchange of pyridoxine in the body (cycloserine, isoniazid etc) as well as intestinal infections, hepatitis, radiation sickness - all of these factors increase the biological need of the organism in pyridoxine and can lead to pyridoxine deficiency.

Pyridoxine deficiency is often accompanied by higher irritability or, on the contrary, stupor, decreased appetite, frequent nausea, and magnesium deficiency/hypomagnesemia. Pyridoxine deficiency is often characterized by dry skin and dermatitis of the neck, nasolabial area, above the eyebrows, and around the eyes while vertical fissures of the lips, stomatitis, and glossitis are rarer. Not uncommon are conjunctivitis and polyneuritis of the upper and lower limbs. Pregnant women with pyridoxine deficiency often complain of nausea, vomiting, declined appetite, irritability and insomnia.

Table 8-1. Clinical manifestations, pathogenetic and clinical factors that underly pyridoxine deficiency (after "Questionnaire for detection of micronutrient deficiencies", Gromova, 2001)

Clinical signs of pyridoxine deficiency
Seboreia-like facial dermatitis, dry dermatitis in nasolabial folds, over the eyebrows, around the eye, sometimes on the neck and under hair
cheilosis (angular stomatitis) with vertical fissures of the lips
Glossitis, atrophy of the papillae
Conjunctivitis
Polyneuritis of the upper and lower limbs
Zoster
exudative diathesis
chronic gastritis with achlorhydria
chronic enteritis, maladsorption syndrome, enteropathiya, Whipple disease, Crone disease, chronic pancreatitis with secretory deficiency
Atherosclerosis
Disbacteriosis
Hypochromic anemia
Leukopenia

Poikilocytosis
Sclerotic vascular changes (retina test)
Dental caries, especially in pregnant
irritability, stupor
cramps, seizures, spasmodic epileptiform seizures
high meteosensitivity
Causes of pyridoxine deficiency
High levels of physical load
Diet deficient in pyridoxine-containing products
Protein diet with high content of tryptophan, methionine, cysteine
Pregnancy
Too cold climate
Too hot climate
Work with chemical poisons and harmful substances
Artificial nursing with cow milk or with low-vitamin mixtures
Rare genetic defects in genes involved in metabolism of pyridoxine
Drugs that suppress pyridoxine metabolism
amiodaron (antiarrhythmic)
Chemical analogues of vitamin B6
vitamin D (high doses, long-term)
Hydralazine
Massive therapy with antibiotics
methylxanthines (theophylline, teobromin, caffeine, etc)
Estrogen-based drugs such as oral contraceptives
penicillamine
drugs containing tryptophan, methionine, cysteine
Anticonvulsants (with exception of magnesium and calcium drugs)
Tuberculosis drugs (ftivazide, cycloserin, isoniazid)
Antiepileptics (levodopa)
Ethanols and narcotics
Diseases accompanied by pyridoxine deficiency
intestinal infections
Hepatitis
radiation sickness
diabetes mellitus
Radiculitis
Meniere disease, sea and air sickness
neuritis, neuralgia
Little disease
Parkinsonism
Sideroblastic anemia
Toxicosis in pregnant

The weeks 12-14 of pregnancy correspond to the peak of pyridoxine deficiency. When combined with magnesium deficiency, clinical manifestations of the pyridoxine deficiency aggravate and manifest as epileptic-like seizures, leukopenia, hypochromic and degradation of the connective tissue in vessels and other organs. In particular, dental caries during pregnancy is often a sign of pyridoxine deficiency. These and other features of the clinical manifestations of pyridoxine deficiencies are summarized in the table 8-1.

To diagnose hypovitaminosis B6, the test used most often is evaluation of pyridoxine concentrations in blood plasma. The values of 5-30 ng/ ml are considered normal (conversion factor to nmol/L is 4.046, ie, normal values are 20-121 nmol/L). A pronounced deficit corresponds to values lower than 5 ng/ml. Additional diagnostic tests include lower excretion of 4-pyridoxinic acid in urine and the tryptohpan load test.

Antistress effects of pyridoxine have been shown both experimentally (Henrotte, 1992) and clinically (Bell, 1992). Low levels of pyridoxine in plasma are associated with symptoms of depression (Hvas, 2004). Elevated blood pressure is one of the principal components of stress and dietary vitamin B6 deficiency is also linked to hightened blood pressure (Lal, 1995). Treatment of hypertensives with pyridoxine significantly reduced systolic and diastolic blood pressure, levels of adrenaline and noradrenaline in plasma (Aybak, 1995, van Dijk, 2001). Our analysis of the functional linkages between pyridoxine and neural function (Torshin, Gromova, 2008) pointed to a number of possible molecular mechanisms through which pyridoxine exerts its antisressory and antidepressant effects. These mechanisms include effects on the metabolism of GABA and catecholamine metabolism.

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter. Lower levels of GABA result in increased excitability of the nerve centers. Two pyridoxal-dependent enzymes affect the metabolism of GABA: glutamate decarboxylase 1, involved in the synthesis of GABA and aminobutirate aminotransferase, involved in the inactivation of GABA. Glutamate decarboxylase 1 (gene GAD1) is involved in the synthesis of GABA from L-glutamate. The deficit of this enzyme activity leads to pyridoxine-dependent seizures. Aminobutirate aminotransferase (gene ABAT) converts GABA to succinic semialdehyde. Deficit of ABAT activity leads to psychomotor retardation, hypotonia, lethargy, and abnormal EEG. The low levels of pyridoxine lead to lowered activity of both enzymes. Since, however, both the enzyme act in opposite directions relative to the levels of GABA, a lack of pyridoxine will have a mixed impact on the levels of GABA and modulate production of GABA. This mixed molecular effect partly explains the opposing clinical manifestations of the pyridoxine deficiency: higher irritability or, on the contrary, stupor.

The effect of pyridoxine on the metabolism of catecholamines is also two-sided. On one hand, pyridoxine deficiency leads to a lower activity of dihydrophenylalanine (DOPA) decarboxylase (gene DDC, figure 8-2) which synthesizes dopamine – precursor of adrenaline and noradrenaline. This enzyme also converts 5-hydroxytryptophane to serotonin and lowered DOPA decarboxylase activity might lead to lower levels of serotonin and catecholamines. On the other hand, pyridoxine deficiency reduces the activity of cystathionine beta-synthase (CBS, figure 8-3, gen CBS), which leads to hyperhomocysteinemia and also activates serine hydroxymethyltransferase (figure 8-4, gene SHMT1), which leads to an increased level of S-adenosylmethionine (SAM). Both the high level of homocysteine and the high level of SAM are associated with an increased content of catecholamines in the blood due to lower activity of the enzyme catechol-O-methylransferse (COMT).

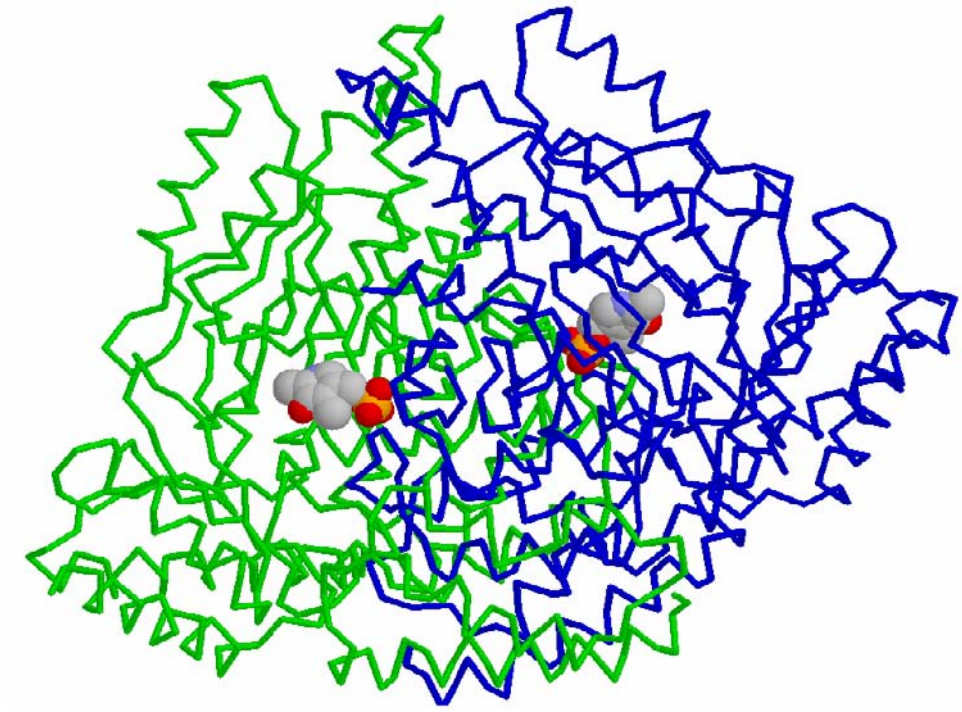


Figure 8-2. Structure of DOPA decarboxylase (PDB code 1js3), pyridoxal phosphate is located in the active center of each globule of the dimer.

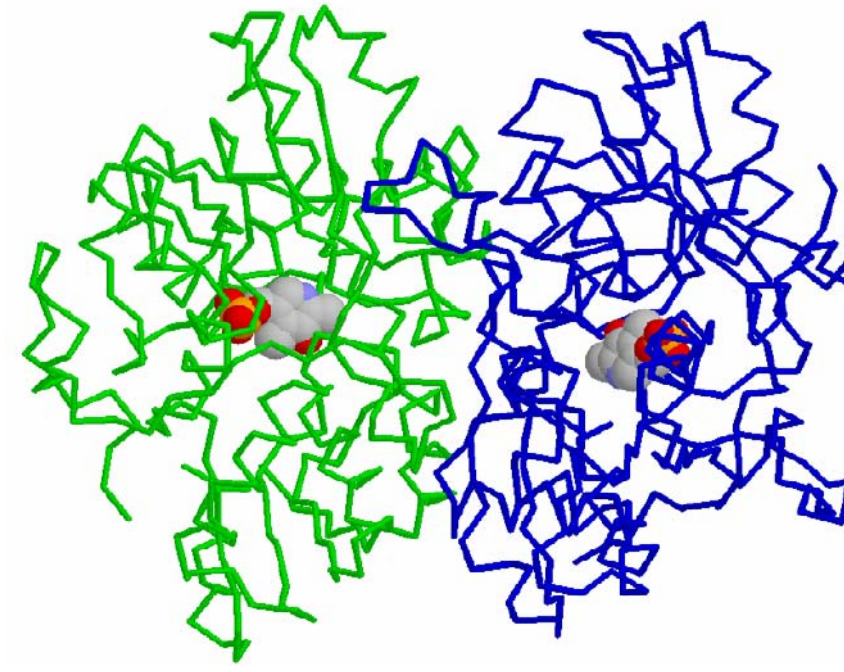


Figure 8-3. Dimer of the cystathionine beta synthetase (PDB code 1jbq).

Although the impacts of pyridoxine deficiency on the metabolism of GABA and catecholamines are two-sided, in the case of catecholamine metabolism pyridoxine deficiency is likely to lead to a stress-dependent increase in blood catecholamines. Although pyridoxine deficiency leads to a reduction in the synthesis of catecholamines (inactivation of DOPA decarboxylase), catecholamines will still be synthesized (albeit in smaller quantities) and then secreted in the bloodstream. However, the lack of pyridoxine also inactivates COMT (through the systemic increase in the levels of homocysteine and SAM). Inactivation of COMT corresponds to a chronically increased level of the catecholamines in the blood. A prolonged action of the catecholamines (albeit in smaller concentrations becase of inactivation of DOPA decarboxylase) will put additional stress on the physiological systems of the body. The compensation of pyridoxine deficiency will activate COMT enzyme which will intensify the removal of catecholamines from the bloodstream.

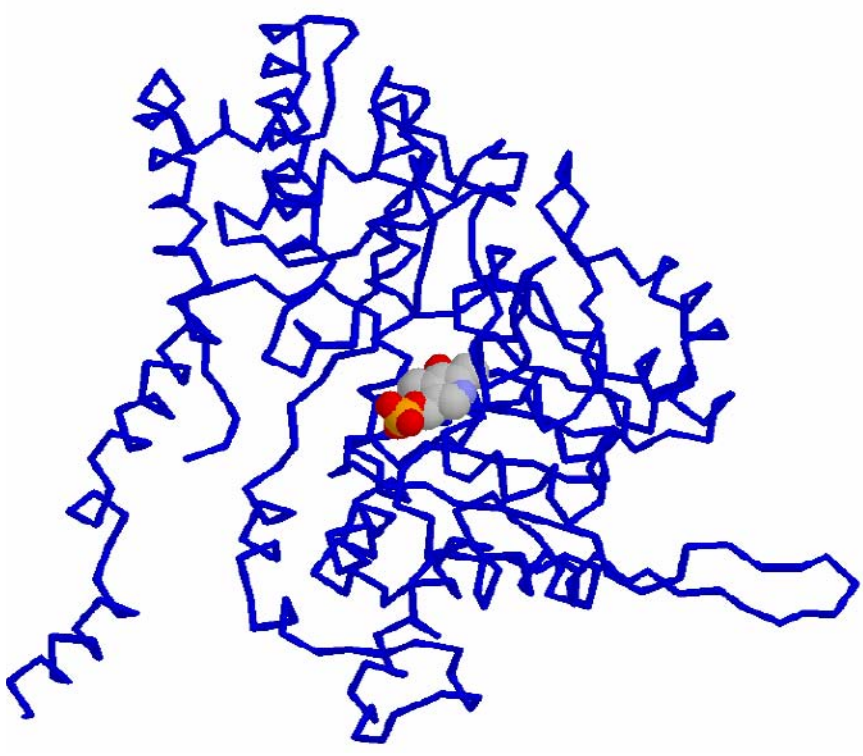


Figure 8-4. The spatial structure of serine hydroxymethyltransferase (PDB code 1bj4).

8.3. Dietary Pyridoxine Requirements

Recommended daily allowances of vitamin B6 (in Russia) range 2-3 mg/day for men and 1.5-2.5 mg/day for women (pregnant, 2.3 mg/day, nursing - 2.5 mg/day). Pathologies increase the daily requirement of pyridoxine and require intake of special pyridoxine-containing drugs. Normally, sufficient amount of pyridoxine can be taken in with food (table 8-2).

Table 8-2. Amounts of various foods that supply daily requirement of pyridoxine (Kodentsova, 2002)

Product	Content, mg/100g	Amount of the product that provides RDA, g
Liver, kidney, poultry, meat	0,30—0,70	300—700
Fish	0,10—0,50	400—2000
Beans	0,9	200—250
Cereals, pepper, potatoes	0,30—0,54	400—700
Bread (rough flour)	0,3	700
Butter	0,4—0,5	200—250

8.4. TREATMENT OF PYRIDOXINE DEFICIENCY

When clinical signs of pyridoxine deficiency are present, pharmacological correction with pyridoxine-containing preparations is recommended. Pyridoxine is indicated in the case of:

- Hypovitaminosis
- Toxicosis in pregnancy
- Hyperhomocysteinemia
- Cyderoblastic anemia
- Parkinsonism, Little disease
- Radiculitis, neuritis, neuralgia
- Generalized anxiety, autism, neuroses
- Meniere disease, sea and air sickness
- Atherosclerosis
- Diabetes
- chronic gastritis, chronic enteritis, Whipple disease, Cron disease
- Seboreia-like dermatitis
- Aggressive therapy with antibiotics
- Profession hazards (toxic chemicals, radioactive substances)
- High levels of physical excercise, sports
- Lactation

Pyridoxine has many other medicinal uses. For example, pyridoxine in therapeutic doses is effective in counteracting ethylene glycol poisoning. Usage of pyridoxine and magnesium can reduce alcohol cravings (Aron, 2001). Drugs containing pyridoxine (such as phacovit, phakolen) also activate glutathione synthesis, increasing levels of SH-soluble protein groups thus stimulating antioxidant effects. Treatment of oxalaturia with pyridoxine drugs gives a positive effect in 50% of cases by reducing the amount of salts excreted with urine. Magnesium significantly potentiates antioxalate effect of vitamin B6. Large doses of pyridoxine (60-600 mg/day) are used for the treatment of homocystinuria, a rare congenital disease with abnormality in the methionine metabolism, addition of magnesium increases effectiveness of the treatment in terms of the normalized levels of the homocystine.

Table 8-3. Pharmacologic maximum admissible and toxic doses of pyridoxine. The doses vary from country to contry. In USA, for example, 500 mg/day is the upper safe limit of use of pyridoxine while doses > 100 mg/day require constant monitoring by neurologist *(Dietary Reference Intakes. Institute of Medicine, 2004, National Academy Press, Washington; Rebrov, 2006)***. Effective dose is selected through titration individually for each patient. The maximal dosages for each usage are given in parentheses**

Condition/age	Dose of pyridoxine (mg/day)
Homocystinuria (Cystathionine beta synthase deficiency)	50-600
Pyridoxine-dependent homocystinuria	100-500
Inborn defects of tryptophan metabolism	100-500
hypochromic anemia (preferably in combination with iron supplements)	100-200
Mitochondrial deficiency (preferably in combination with iron supplements)	100-200
Hypoplastic anemias (preferably in combination with iron supplements)	100-200
Skin burns (including solar burn)	5-15 (50)
Hepatitis (abnormal tryptophan, lower kynureninase)	5-15 (50)
Herpes, psoriasis	100-200
Diabetes (magnesium synergically lowers insulin resistance)	5-15 (30)
Oxalaturia (magnesium raises antioxalate effect twice)	5-15 (30)
Lymphosarcoma, leukemia	30-150 (200)
Radiation sickness	15-30 (200)
Gangrene (long-term usage)	100-150 (200)
Carpal tunnel syndrome (long-term usage)	100-150
Thyreotoxicosis	5-15 (30)
Systemic lupus erythematosus	50-200
Sensory neuropathia (long-term usage)	40-200
Premenstrual syndrome	50-200
Alcoholic syndrome	50-100 (200)
Anticonvulsant usage	2-15
Pregnancy, lactation (14-18 years). Doses below 25 are completely safe.	<80 (USA); <25 (Russia)
Pregnancy, lactation (19+ years). Doses below 25 are completely safe.	<100 (USA); <25 (Russia)

NB! Overdose and complications		
Condition	Dose (mg/day)	Complications
Gastritis, ulcers of stomach and duodenal, increased acidity	>30	Can stimulate further increase in acidity of the gastric juice
Lactation	200-600	Can suppress lactation, possible neurological complications
Adult healthy volunteers (2-40 months)	2000-6000	Sensory neuropathia, convulsions, disturbances in gait. Such doses are categorically forbidden to use in treatment.

Therapy of the pyridoxine-dependent convulsions uses both magnesium and vitamin B6. An efficient therapeutic dose of the vitamin for treating the convulsions usually ranges from 2 to 15 mg/day. Intake of 5 mg/day pyridoxine for several weeks lowers blood pressure by increasing diuresis and decreasing vascular tone. Regular intake of pyridoxine is known to reduce the risk of cardiovascular disease (Cameron, 2002) as well as of the bowel and rectum cancer (Wei, 2005). The treatment of these and other disease is done by administering various

doses of pharmacological forms of pyridoxine, depending on the disease, age and responsiveness of each particular patient to the therapy (table 8-3).

Forms of the vitamin B6 are non-toxic. Nevertheless, serious overdose can induce a number of side effects (lower part of the table 8-3). Sometimes, overdose of vitamin B6 results in allergic reactions such as skin rush (Murata, 1998). Vitamin B6 may increase acidity of the gastric juice (Kukes, 1999). A very large dose pyridoxine of up to 6000 mg daily was shown to cause sensory neuropathia (Toussaint, 2004, 1998). Doses of 200, 2000, 5000 mg can cause numbness and tingling sensation in hands and feet, as well as loss of sensitivity in the same areas (Den, 2003). Ultra-high doses of vitamin B6 (500 mg/kg , parenteral, 8 days) in the experiment in rats caused a dramatic change in gait and possible disruption of the sensory pathways. Symptoms quickly disappear by stopping intake of pyridoxine or by lowering the dose.

Chapter 9

9. DETERMINATION OF THE MAGNESIUM AND PYRIDOXINE LEVELS

9.1. THE MAJOR METHODS FOR DETERMINATION OF THE MAGNESIUM DEFICIENCY

Determination of the magnesium levels in plasma can be crucial for correct diagnostics when patient manifests:

- Neurological pathology (tetany, hyperexcitability, tremor, convulsions, muscle hypotonia);
- Renal failure;
- Cardiac arrhythmia;
- Hypothyroidism;
- Adrenal failure
- Mg levels are decreased in CVD, anemia, diabetes
- Other conditions and states described earlier (chapters 3&4).

It should be noted that the level of plasma magnesium may be retained within its normal limits even when the total amount of magnesium in the body is depleted by 80%. Therefore, the reduction of magnesium in plasma is a sign of *severe* magnesium deficiency. Normal physiological level of magnesium is 0,75-1,26 mmol/liter. When plasma magnesium is less than 0.75 mmol/L, diagnosis hypomagnesemia is made. However, it's not to be forgotten that plasma retains only 1% of the total amount of magnesium in the body, so fluctuations of the plasma levels do not reflect well the organismal state. Levels of magnesium in cerebrospinal fluid and erythrocytes often parallel the concentration in the plasma.

Normal level ranges of plasma magnesium:

- adults (men, women): 0.75-1.26 mmol/L
- pregnancy: 0.8-1.05 mmol/L
- children: 0.74-1.15 mmol/L
- The magnesium norms are usually adjusted by age (table 9-1).

Table 9-1. Age-adjusted norms (mmol/L) of magnesium in plasma (Tits, 2001). Methods: atomic adsorption spectrophotometry (AASPH), titan yellow photometry (TYPH)

Age/substrate	Norm	Units	Method
Newborns	0,62–0,91	mmol/L	AASPH
5 months-6years	0,70–0,95	mmol/L	
6–12 years	0,70–0,86	mmol/L	
12–20 years	0,70–0,91	mmol/L	
20–60 years	0,66–1,07	mmol/L	
60–90 years	0,66–0,99	mmol/L	
>90 years	0,70–0,99	mmol/L	
Erythrocytes	1,65–2,65	mmol/L	
24h urine	3,0–50	mmol/24h	TYPH
Cerebrospinal fluid	1,1–1,5	mmol/L	AASPH

Average levels of magnesium and calcium (adults):

- Plasma magnesium: 0.82±0.09 mmol/L
- Plasma calcium: 2.43±0.09 mmol/L
- Erythrocyte magnesium: 2.31±0.08 mmol/L
- Erythrocyte calcium: 1,30±0.04 mmol/L

Clinical evaluation of hypomagnesemia in adults:

- 12-17 mg/L (1-1.4 mEq/L, 0.5-0.7 mmol/L) - moderate deficiency of magnesium;
- Below 12 mg/L (<1 mEq/L, <0.5 mmol/L) – severe magnesium deficiency;
- Severe hypomagnesemia (<0.45 mmol/L) is observed in acute MI and stroke.

Conversion of units: 1 mmol/L = 0.04114 mg/L; 1 mEq/L = 2 mmol/L

Determination of magnesium in blood plasma can be done by method of xilidil-blue adsorption using autoanalyzer like "UltraKone LabSystems" or any other analogous device. A blood sample of 3ml is collected from the ulnar vein. It is advisable not to use bandage because with strong squeeze of the arm the levels of magnesium and calcium can momentary increase because of vascular microtraumas. Blood is taken for analysis strictly on an empty stomach, from 8:00 to 10:00. According to circadian magnesium rhythm, taking blood at this time is likely to reflect the average concentration of magnesium in plasma per 24h. Extracted blood is put into centrifuge not later than 30-60 min after the sample collection in order to avoid loss of magnesium from blood cells into plasma.

Magnesium levels can be determined in different kinds of the blood cells. Cells differ in their capacity to concentrate magnesium (lymphocytes> phagocytes> platelets> erythrocytes). Measurements of changes in magnesium levels in different types of cells help more exact interpretation of the extent of the abnormality of the magnesium homeostasis. Lowered lymphocyte magnesium corresponds to hyperaldosteronism and impaired immunity. Low level of magnesium in phagocytes is found during infectious disease, immunodeficiency, and

tumors. Reduced level of magnesium in platelets indicates prothrombotic propensity. Erythrocytic magnesium decreases in heart insufficiency, ischemic heart disease (including myocardial infarction), anemia, and diabetes. At present, reference values for the normal and abnormal levels of magnesium in different kinds of cells are not commonly established and only general approximates are available (table 9-2).

Table 9-2. Reference values of ionized magnesium and magnesium in blood cells (Tsyganenko, 2002)

Cell type	Normal magnesium level
Erythrocytes	0.19-0.21 femtomol/cell
Lymphocytes	3.50-5.70 femtomol/cell
Platelets	0.07-0.12 femtomol/cell

9.2. Additional Methods for Determining the Magnesium Deficiency

Magnesium load test is, perhaps, one of the most reliable methods to assess the state of magnesium in the patient and to determine the extent of magnesium deficiency. To this end, the urine samples are collected from patients for one day and the steady levels of magnesium are determined. Then, the patients are infused intravenously with 30 mmol of magnesium sulfate in 0.5L of physiological solution or 5% dextrose during 8-12 hours (newborns are infused with 0.12 mg/kg of 25% solution of magnesium sulfate). The urine samples are collected for 24 ours after beginning of infusion and the changes in the urine content of magnesium are determined. In the absence of considerable magnesium deficiency about 18-30 mmol of magnesium should be excreted with urine. Lower values suggest organism-wide depletion of magnesium. The diagnosis magnesium deficiency is made usually when the amount of excreted magnesium will be less than 18 mmol per day (i.e., 50% of the initial dose). Despite apparent difficulties with this methodology (it's applicable only to in-patients and is labour-costly), the levels of magnesium determined in this manner reproduce well and reflect the extent of the magnesium deficiency in the entire organism. Of course, the exact diagnosis requires not only magnesium load test but using the entire array of clinical symptoms which were outlined in Chapters 3&4.

9.3. Determination of Pyridoxine

The most frequently used way to assess the balance of vitamin B6 is determination of the pyridoxine in plasma using enzymatic or radiometric methodologies or high performance liquid chromatography (HPLC). Plasma is extracted from the blood sample taken from the ulnar vein, on an empty stomach in the morning and is mixed with EDTA, heparin or sodium citrate). The sample should be protected from light and is to be frozen at -80C. Repeating freezing/thawing should be avoided. The reference values of pyridoxine are 5-30 ng/ml (20-120 nmol/L), the deficiency is strongly indicated by levels lower than 5 ng/ml. The

pyridoxine levels, however, are affected by the drug intake and it is necessary to clarify with the patient whether he/she takes amiodaron, anticonvulsive medications, hydralazine, isoniazid, levodopa, penicillamine, theophylline, oral contraceptives or excessive ethanol.

Additional diagnostic tests include determination of the levels of the 4-pyridoxine acid in urine (lower when there is pyridoxine deficiency) and the tryptophan load test (Murray, 1999). In the latter case, level of xanthurenic acid in urine is determined after the tryptophan load (2g of Trp). The test is positive (that is, deficiency of vitamin B6 is present) when the change in the levels of urine excretion of xanthurenic acid exceeds 50 mg/24h. The test is most useful during pregnancy and indicates that 12-14 weeks of pregnancy correspond to maximum excretion of xanthurenci acid. In the case of pregnancy-related toxicosis during weeks 10-16 weeks test is often positive. Again, diagnosis of the pyridoxine deficiency is more complete when clinical manifestations (Chapter 8) are taken into account along with the results of the lab tests.

CONCLUSION

Most people think they know everything about nutrition. All have heard about the harmful effects of excessive consumption of salt and refined sugar, the importance of calcium for bone health, the role of iodine to the thyroid gland for hemoglobin. Up to date, however, more than 80 macro- and trace elements, no fewer than 100 natural forms of 15 essential vitamins and a large amount of vitamin-like substances were discovered to be essential for the human body. Homeostasis of these micronutrients has a fundamental role for human health, not less important role than the intake of the bulk nutrients like fats, carbohydrates and proteins. In particular, the fundamental importance of magnesium in maintaining the homeostasis of other elements and vitamins has long been underestimated.

Meanwhile, the loss of traditional healthy diet, increasing stress and the environment pollution lead to a wide spread of chronic magnesium deficiency. The desire to produce an increasing number of food products and to ensure their ever-growing sales lead to considerably lowered nutritional quality of the food stuffs. *People are, literally, starving in the midst of plenty*. Despite the fact that people eat a lot, the food they consume often misses a whole range of essential micronutrients.

As a consequence, the pressure «diseases of civilization» increases more and more.

The reasons for these diseases of civilization are, seemingly, well-known among progressive medical community. At the same time, most of the people and, alas, many medical practitioners, seem to treat the fundamental knowledge in the area of healthy nutrition with extremist skepticism. This sort of skepticism, however, is based on ignorance and reckless disregard for life and the life of the patient.

The results of numerous studies in biochemistry, pharmacology, epidemiology, and evidence-based medicine point to the fundamental importance of magnesium for human health at any age. For emergency conditions, magnesium is used more than a century. Since 1930s, magnesium has been gradually introduced to vitamin-mineral complexes and is used for the enrichment of the diet. The ongoing research indicates that the most easily absorbable forms of magnesium are similar to the natural magnesium forms.

Because of the widespread prevalence of magnesium deficiency, pharmacological correction with high-quality magnesium-containing drugs is of particular importance. There are many commercially available magnesium preparations and quite a number of them cannot even be recommended for compensation of the magnesium deficiency. This book summarizes the data of numerous studies, which allowed to formulate reasonable criteria for selecting drugs for the prevention and treatment of magnesium deficiency. The systematized data on the physiological importance of the balance of magnesium, presented in this book, can be of great help both in clinical practice and fundamental research.

APPENDIX I. THE CONTENTS OF MINERAL SUBSTANCES AND PYRIDOXINE IN DIFFERENT FOODS

The mineral content of various food products (Tutelyan, 2004).

Products	Mineral content, mg per 100 g					
	Na	K	Ca	Mg	P	Fe
Apples	26	248	16	9	11	2,2
Apricots	30	305	28	19	26	2,1
Barley	-	172	38	94	323	3,3
Beans	40	1100	150	103	541	12,4
Beets	86	228	37	43	43	1,4
Buckwheat	-	167	70	98	298	8,0
Cabbage	13	185	48	15	31	1,0
Carrot	21	200	51	38	55	1,2
Cherries	20	256	37	26	30	1,4
Cucumbers	8	141	23	14	42	1,4
Currant,black	32	372	36	35	33	1,3
Currant,red	21	275	36	17	33	0,9
Dill	43	335	223	70	93	1,6
Garlic	120	260	90	30	140	1,5
Gooseberry	23	260	22	9	28	1,6
Grapes	26	255	45	17	22	0,6
Horse-radish	140	579	119	36	130	2 0
Lemons	11	163	40	12	22	0,6
Melons	32	118	16	13	12	1,0
Millet	39	201	27	101	233	7,0
Onion	50	225	87	10	58	1,0
Oranges	13	197	34	13	23	0,3
Parsley	79	340	245	85	95	1,9
Pea	69	873	115	107	329	9,4
Pears	14	155	19	12	16	2,3
Pepper,red	19	163	8	11	16	-
Plums	18	214	28	17	27	2,1
Potato	28	568	10	23	58	0,9

Pumpkin	14	170	40	14	25	0,8
Radish	17	357	35	22	26	1,2
Radishes	10	255	39	13	44	1,0
Raspberry	19	224	40	22	37	1,6
Rice	26	54	24	27	97	1,8
Salad	8	220	77	40	34	0,6
Semolina	22	120	20	30	84	2,3
Products	Mineral content, mg per 100 g					
	Na	K	Ca	Mg	P	Fe
Sorrel	15	500	47	85	90	2,0
Tomatoes	40	290	41	20	26	1,4
watermelons	16	64	14	224	7	1,0
Wheat flour	12	176	24	44	115	2,1
White bread	495	180	33	54	130	2,4

The pyridoxine content of various food products (Liflyandsky, 1999). Contents in mg per 100g of the product are indicated. Products containing high doses of both magnesium and pyridoxine are marked.

APPENDIX II. REFERENCE VALUES OF MINERAL AND TRIGLYCERIDE LEVELS (GROMOVA, 2001)

Element	Age group		Common units	SI units
Aluminum	Adults		< 3 mcg/L	< 0,11 mcmol/L
Iron	Newborn		36–184 mcg/dL	6,4–3,3 mcmol/L
	< 6 months		36–156 mcg/dL	6,4–28 mcmol/L
	>7 months		43–184 mcg/dL	7,7–33 mcmol/L
	Adults	M	37–145 mcg/dL	6,6–26 mcmol/L
		F	59–158 mcg/dL	11–28 mcmol/L
		F	291–430 mcg/dL	52–77 mcmol/L
Potassium	Newborn		3,6–6,1 mEq/L	3,6–6,1 mmol/L
	2 - 12 months		3,6–5,8 mEq/L	3,6–5,8 mmol/L
	>1year		3,1–5,1 mEq/L	3,1–5,1 mmol/L
	Adults		3,5–5,1 mEq/L	3,5–5,1 mmol/L
	Erythrocytes		7,5–9,6 fmol/cell	7,5–9,6fmol/cell
	Leukocytes		39–64 fmol/cell	39–64 fmol/cell
	Thrombocytes		0,7–1,3 fmol/cell	0,7–1,3fmol/cell
Calcium	Total	Newborn	7,2–11,2 mg/dL	1,8–2,8 mmol/L
		2–12 months	8,4–10,8 mg/dL	2,1–2,7 mmol/L
		>1 year	8,4–10,4 mg/dL	2,1–2,6 mmol/L
		Adults	8,6–10,2 mg/dL	2,15–2,55 mmol/L
	Plasma		4,7–5,2 mg/dL	1,17–1,29 mmol/L
Magnesium	Newborn	F	1,7–2,5 mg/dL	0,7–1,03 mmol/L
		M	1,7–2,4 mg/dL	0,7–0,99 mmol/L
	<1 year	F	1,9–2,4 mg/dL	0,78–0,99 mmol/L
		M	1,6–2,5 mg/dL	0,66–1,03 mmol/L
	1 – 3 years		1,7–2,4 mg/dL	0,7–0,99 mmol/L
	4 – 6 years	F	1,7–2,2 mg/dL	0,7–0,9 mmol/L
		M	1,7–2,4 mg/dL	0,7–0,99 mmol/L
	7 – 9 years	F	1,6–2,3 mg/dL	0,66–0,95 mmol/L
		M	1,7–2,3 mg/dL	0,7–0,95 mmol/L
	10 – 12 years		1,6–2,2 mg/dL	0,66–0,9 mmol/L
	13 – 15 years		1,6–2,3 mg/dL	0,66–0,95 mmol/L
	16 – 18 years		1,5–2,2 mg/dL	0,62–0,9 mmol/L
	Adults		1,7–2,55 mg/dL	0,7–1,05 mmol/L
	Plasma		0,46–0,6 mmol/L	0,46– 0,6 mmol/L

(Continued).

	Erythrocytes		0,19–0,21 fmol/cell	0,19–0,21 fmol/cell
	Lymphocytes		3,5–5,7 fmol/cell	3,5–5,7 fmol/cell
	Thrombocytes		0,07–0,12 fmol/cell	0,07–0,12 fmol/cell
Element	Age group		Common units	SI units
Copper	Newborns		8,9–46 mcg/dL	1,4–7,2 mcmol/L
	4–6 months		25–108 mcg/dL	4–17 mcmol/L
	7–12 months		51–133 mcg/dL	8–21 mcmol/L
	1–5 years		83–152 mcg/dL	13–24 mcmol/L
	6–9 years		83–133 mcg/dL	13–21 mcmol/L
	10–13 years		83–121 mcg/dL	13–19 mcmol/L
	14–19 years	F	70–159 mcg/dL	11–25 mcmol/L
		M	64–114 mcg/dL	10–18 mcmol/L
	adults	F	76–152 mcg/dL	12–24 mcmol/L
		M	70–140 mcg/dL	11–22 mcmol/L
Sodium	Newborns		132–147 mEq/L	132–147 mmol/L
	2–12 months		129–143 mEq/L	129–143 mmol/L
	>1year		132–145 mEq/L	132–145 mmol/L
	Adults		135–145 mEq/L	135–145 mmol/L
Chloride	Newborns		95–116 mEq/L	95–116 mmol/L
	2–12 months		93–112 mEq/L	93–112 mg/dL
	>1year		96–111 mEq/L	96–111 mmol/L
	adults		98–106 mEq/L	98–106 mmol/L
Zinc	До 4 months		65–137 mcg/dL	10–21 mcmol/L
	4–12 months		65–130 mcg/dL	10–20 mcmol/L
	1–5 years		65–118 mcg/dL	10–18 mcmol/L
	6–9 years		78–105 mcg/dL	12–16 mcmol/L
	10–13 years	F	78–118 mcg/dL	12–18 mcmol/L
		M	78–98 mcg/dL	12–15 mcmol/L
	14–19 years	F	59–98 mcg/dL	9–15 mcmol/L
		M	65–118 mcg/dL	10–18 mcmol/L
	Adults	Plasma	46–150 mcg/dL	7–23 mcmol/L
		Whole blood	425–560 mcg/dL	65–86 mcmol/L
Phosphate	Newborns		5,0–9,6 mg/dL	1,6–3,1 mmol/L
	2–12 months		5,0–10,8 mg/dL	1,6–3,5 mmol/L
	>1year		3,4–6,2 mg/dL	1,1–2,0 mmol/L
	Adults		2,7–4,5 mg/dL	0,87–1,45 mmol/L
Selenium	Whole blood		67–105 mcg/L	0,85–1,33 mcmol/L
	Plasma		45–83 mcg/L	0,57–1,05 mcmol/L

Reference values of magnesium in urine

Common units	SI units	Remarks
60 – 210 mg/24h	2,5 – 8,5 mmol/24h	24h urine
4,1–13,8 mg/L	1,7–5,7 mmol/24h	One-time urination

Reference values of plasma triglycerides by age group. Increased triglycerides are often associated with magnesium deficiency.

Age	Gender	Triglyceride level, mmol/L
< 10 years	Boys	0,34 - 1,13
	Girls	0,40 - 1,24
10 - 15 years	M	0,36 - 1,41
Age	Gender	Triglyceride level, mmol/L
15 - 20 years	F	0,42 - 1,48
	M	0,45 - 1,81
20 - 25 years	F	0,40 - 1,53
	M	0,50 - 2,27
25 - 30 years	F	0,41 - 1,48
	M	0,52 - 2,81
30 - 35 years	F	0,42 - 1,63
	M	0,56 - 3,01
35 - 40 years	F	0,44 - 1,70
	M	0,61 - 3,62
40 - 45 years	F	0,45 - 1,99
	M	0,62 - 3,61
45 - 50 years	F	0,51 - 2,16
	M	0,65 - 3,70
50 - 55 years	F	0,52 - 2,42
	M	0,65 - 3,61
55 - 60 years	F	0,59 - 2,63
	M	0,65 - 3,23
60 - 65 years	F	0,62 -2,96
	M	0,65 - 3,29
65 - 70 years	F	0,63 - 2,70
	M	0,62 - 2,94
	F	0,68 - 2,71

APPENDIX III. TESTING GLYCOSYLATED HEMOGLOBIN-C (HBA1C)

Glycosylated hemoglobin allows to assess the level of glycemia which were 1 – 2 months before the test. This compound forms as a result of slow non-enzymatic reaction of hemoglobin A in erythrocytes with the blood glucose. The rate of this reaction depends on the average level of glucose during the life of erythrocyte. There are several forms of glycosylated hemoglobin: HbA1a, HbA1b, HbA1c, the last form is most prevalent and produces better correlation with the extent of diabetes. Glycosylated hemoglobin reflects levels of hyperglycemia that occurred during the span of the erythrocytes (up to 120 days) indicating, thus, what was the average concentration of glucose in the previous 4-8 weeks. Normalization of glycosylated hemoglobin in the blood occurs 4-6 week after normal levels of glucose are reached. In diabetics, the level of this compound can be 2-3 times higher the reference level. A 1% increase in glycosylated hemoglobin corresponds, on average, to an increase of about 2 mmol/L in plasma glucose. Lowering of the glycosylated hemoglobin by 10% corresponds to 45% decrease in the risk of progression of diabetic retinopathy.

Indications for the analysis of HbA1C:

- Diabetes diagnostics/screening;
- Long-term monitoring in treatment of diabetics;
- Determination of the compensation of diabetes
- Supplement to glucose tolerance test in the case of subclinical diabetes;
- Screening of pregnant for latent diabetes.

The levels of glycosylated hemoglobin do not depend on the time of day, physical exertion, or patient's emotional state. Conditions that cause shortening of the average life span of erythrocytes (severe blood loss, hemolytic anemia) may lead to falsely lowered results of the test. False increase in the result may be due to high concentration of fetal hemoglobin (HbF).

The blood is taken from vein, mixed with anticoagulant (EDTA) and processed through cation exchange low pressure chromatography (DiaSTAT). Measurement units are % of the total hemoglobin

Reference values: 4.5 - 6.5% of the total hemoglobin content.

Raised HbA1c corresponds to:

- Diabetes mellitus and other states with impaired glucose tolerance is;
- Determination of diabetes compensation:
    - 5.5-8% - well compensated diabetes;
    - 8-10% - well enough compensated diabetes;
    - 10-12% - partlially compensated diabetes;
    - 12% - diabetes is not compensated

Reduced HbA1C corresponds to:

- Hypoglycemia;
- Hemolytic anemia;
- Blood loss;
- Recent blood transfusions.

APPENDIX IV. GENES IMPLICATED IN MAGNESIUM HOMEOSTASIS

Bioavailability of the magnesium is regulated by a number of gene products of which **TRPM6 and TRPM7** are the most important. The transient receptor potential cation channel 6 (TRPM6) is an ion channel for the transport of divalent cations. TRPM6 specifically interacts with the $Mg(^{2+})$-permeable cation channel TRPM7 resulting in the assembly of functional TRPM6/TRPM7 complexes at the cell surface (Chubanov, 2004). Patients with hypomagnesemia and secondary hypocalcemia were found to carry mutations in TRPM6 (Schlingmann, 2002). TRPM7 might be involved in the stress-related magnesium deficiencies (Wang, 2006).

SLC41A1 cation transporter is upregulated under hypomagnesic conditions: in mice placed on a low magnesium diet, expression of Slc41a1 mRNA was upregulated in kidney, colon, and heart. In addition to Mg^{2+}, SLC41A1 can also transport Sr^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Ba^{2+} and Cd^{2+} (Goytain, 2005).

Members of the **FXYD** protein family are small membrane proteins which are characterized by an FXYD motif, two conserved glycines and a serine residue. Mutation of a conserved glycine residue into an arginine residue in FXYD2 has been linked to cases of renal hypomagnesemia (Delprat, 2006) which probably occurs as a consequence of increased reabsorption of calcium in the loop of Henle (Meij, 2000).

Claudins (CLDN16 and CLDN19) are transmembrane proteins found at tight junctions. Tight junctions form barriers that control the passage of ions and molecules across an epithelial sheet and the movement of proteins and lipids between apical and basolateral domains of epithelial cells. CLDN16 (paracellin 1) is selectively expressed at tight junctions of renal epithelial cells of the thick ascending limb of the Henle loop where it plays a central role in the reabsorption of divalent cations. Genetic defects in claudin 16 were associated with primary hypomagnesemia (Simon, 1999) and defects in claudin 19 were associated with renal hypomagnesemia with ocular involvement (Konrad, 2006).

Ca^{2+}/Mg^{2+}-sensing receptor (**CASR**) is a plasma membrane G protein-coupled receptor that is expressed in the parathyroid gland and the cells lining the kidney tubule. By virtue of its ability to sense small changes in circulating calcium concentration and to couple this information to intracellular signalling pathways, CASR plays an essential role in maintaining mineral ion homeostasis (Nagase, 2002). Defects in this gene were associated both with hypercalcemia and hypocalcemia (Hendy, 2000). CASR activation decreases PKA activity

resulting in a decrease in phosphorylated claudin-16, translocation of claudin-16 to lysosome and a decrease in magnesium reabsorption.

Metallothionein 2A (**MT2A**) may have an important role of cell protection in inflammation reaction (Liang, 2004) and under physiological conditions, the formation of MT disulfide bonds is involved in the regulation of zinc homeostasis. The G-allele of the polymorphism +838 C/G showed increased MCP-1 and decreased zinc, copper and magnesium content in erythrocytes and increased iron in plasma as well as higher incidence of soft carotid plaques (Giacconi, 2007).

APPENDIX V. POLYMORPHISMS ASSOCIATED WITH CONNECTIVE TISSUE DISPLASIAS (CTD)

Despite great variety of rare genetic diseases (such as Ehlers-Danlos disease, Stickler syndrome etc), the common genetic predispositions to undifferentiated CTD are not known. Rare mutations in, say, a number of collagen explain only a few rare monogenic phenotype, but can hardly explain the relatively high population frequency of occurrence CTD. In chapter IV, we mentioned the molecular mechanisms of the Mg-dependent CTDs (see also Torshin, Gromova, 2008). Here, we sum polymorphisms that were associated with CTD-like diseases and which can affect the magnesium requirements of the patients (Torshin, Gromova, 2008a). Such diseases include scoliosis, osteochondrosis and mitral valve prolapse. Data on polymorphism associated with these three groups of diseases summarized in table V-1. These genes belong to very different functional groups. Most genes refer to the structural components of connective tissue and a number of genes are involved in magnesium-dependent mechanisms of CTD (IL6, MMP2, MMP3, and TIMP1).

Table V-1. Polymorphisms associated with, osteochondrosis and mitral valve prolapse. Within each group, genes are located in alphabetical order

Gene ID	Gene name	Polymorphism
Scoliosis		
ESR1	Estrogen receptor, subunit 1	XbaI
IGF1	Insulin-like growth factor	5' C>T
IL6	interleukin-6	G/C pro
MMP3	Stromelysin	5A/6A
VDR	Vitamin D receptor	BsmI
Osteochondrosis of the spine		
ACAN	Aggrecan	A26
COL11A1	Collagen XI A1	C4603T
COL9A2	Collagen IX A2	R103W
IL1B	interleukin-1, subunit B	-511T>C
MMP2	Gelatinase A	-1306C/T
TIMP1	MMP inhibitory protein 1	IVS5 G>T
VDR	Vitamin D receptor	TaqI
Mitral valve prolapse		
COL3A1	Collagen III A1	Ex.31G
MMP3	Stromelysin	5A/6A
PLAU	Plasminogen activated urokinase	C4065T

Scoliosis is a systematic lateral deviation of the spine from the normal straight position. Such deviation could arise due to incorrect posture and does not involve a deficit of connective tissue. In this case, however, we mean rather “adolescent idiopathic scoliosis”, also known as “scoliotic disease”. This is an aggressive disease of the growing spine associated common among children 6-15 years. It is with this disease the genetic associations mentioned in the table were found. Each of the genes implies a specific pathophysiological mechanism by which CTD arises: structure of extracellular matrix such as stromelysin (Aulisa, 2007), hormonal factors IGF1, ESR1, VDR (Xia, 2007), inflammation (IL6).

Generally, the term osteochondrosis ("spinal disc herniation", "degenerative disk disease" and "lumbar disc disease") implies primary degenerative process in the intervertebral disks leading to secondary development of compensatory changes in bone-cartilage system of the spine. One of the reasons of osteochondrosis are microtraumas under physical overload. Pathophysiological mechanisms of emergence of this group of diseases include, again, the structure of connective tissue (ACAN, COL11A1, COL9A2, MMP2, and TIMP1), see (Dong, 2007; Valdes, 2005), inflammation (IL1B) and hormonal factors (VDR). It is important to note that one of the earliest signs of inflammation in the spine osteochondrosis is characteristic of redistribution of the metal ions (such as the accumulation of Mg, Ca, Mn, Fe in granulocytes, the accumulation of excess calcium and reduction in the levels of Mg, Mn and Cu in erythrocytes (Feltelius, 1988).

Damage of the cardiovascular system due to CTD varies but the most common is, apparently, mitral valve prolapse. Idiopathic mitral valve prolapse is found, in most cases, through phonographic changes and could be not only result of uCTD but also the result of the hidden tetany arising due to magnesium deficiency (Bobkowski, 2005). Pathophysiological mechanisms of the CTD-related mitral valve prolapse include changes in the structure of connective tissue (COL3A1, MMP3, TIMP1) (Oceandy, 2007) as well as in hemostasis (PLAU). Although polymorphisms in the TIMP1 gene have not been associated with mitral valve prolapse, they were associated with aneurysms of the abdominal aorta (Ogata, 2005).

APPENDIX VI. MAGNE-B6 FILM-COATED TABLETS

COMPOSITION

Each tablet film-coated contains:

Magnesium lactate-dihydrate .....470 mg
(equivalent with 48 Mg^{2+} = 1,97 mmol)
Pyridoxine hydrochloride (vitamin B6) .....5 mg
Additional components: sucrose, heavy kaolin, talc, magnesium stearate, acacia, carboxy polymethylene 934, titanium dioxide, carnauba wax (powder) etc.

PHARMACOLOGICAL ACTION

Magne-B6 is a combined medication that contains magnesium lactate-dihydrate and pyridoxine hydrochloride (vitamin B6) produced by *Sanofi-Aventis*. Magnesium is required for normal bone structure formation and the proper functioning of more than 300 enzymes, including those involved with ATP-dependent phosphorylation, protein synthesis, and carbohydrate metabolism.

Extracellular magnesium is critical to both the maintenance of nerve and muscle electrical potentials and the transmission of impulses across neuromuscular junctions. Pyridoxine, which is involved in the metabolism of proteins, carbohydrates and lipids, enhances the intestinal absorption of magnesium and its entry into the cell by forming complexes with amino acids which penetrate the cell membrane: these complexes serve as carriers for magnesium.

INDICATIONS

- Established magnesium deficiencies, whether isolated or associated with other deficiencies.

In cases of concomitant calcium deficiency, it is recommended, in the majority of cases, to correct the magnesium deficiency before giving calcium supplementation.

- Therapy of functional signs and symptoms of anxiety attacks with hyperventilation (constitutional tetany also known as idiopathic normocalcemic tetany or spasmophilia).

DOSAGE

Adults:

- Established magnesium deficiency: 6 tablets daily

(12 mmol or 300 mg magnesium per day)

- Spasmophilia: 4 tablets daily (8 mmol or 200 mg magnesium per day)

Children: Not recommended.

Administration

The daily dose should preferably be divided into 2 or 3 intakes in the morning, at midday and in the evening. Swallow the tablets with a large glass of water.

SIDE EFFECTS

Skin reactions have been reported, as well as a few cases of diarrhea and abdominal pain.

Very rare cases of allergic reactions have been observed.

CONTRAINDICATIONS

- Severe kidney failure (creatinine clearance less than 30 ml/min)
- Known allergy to any of the components of the preparation

Special warnings

- In the event of concomitant calcium deficiency, the magnesium deficiency should be corrected before giving supplemental calcium.

SPECIAL PRECAUTIONS

- In the event of moderate kidney failure, caution is required, given the risk related to hypermagnesemia
- Tablets se reserved for adults and children over 6 years
- Information for diabetics: tablets film-coated contain sucrose as an excipient.

Information for the patient

- Magne B6 is a drug: this medication is to be used as directed by the physician
- As with all drugs, an overdose can be dangerous: in this case, contact your doctor immediately
- Do not use this drug after the expiry date printed on the original box (24 months) Pregnancy and lactation

The clinical experience of a sufficient number of pregnancies has not revealed any teratogenic or feto-toxic effects. Consequently, magnesium should be used during pregnancy only if necessary.

Due to the passage of magnesium into maternal milk its use during breast-feeding should be avoided.

Effects on ability to drive and operate machinery

The drug does not affect the ability to drive and operate machinery.

DRUG-DRUG INTERACTIONS

- Concomitant administration of phosphate of calcium salt based preparations is to be avoided because such compounds inhibit the intestinal absorption of magnesium.

- In the event of concomitant treatment with oral tetracyclines, Magne B6 intake should be delayed for at least 3 hours.

- Concomitant administration with levodopa is to be avoided since the activity of levodopa is inhibited when it is not associated with peripheral dopa decarboxylase inhibitors. Any content of pyridoxine should be avoided if levodopa is not associated with dopa decarboxylase inhibitors.

- Concomitant administration with quinidine is not recommended due to the increased plasmatic levels of quinidine and risk of overdose (decrease of renal excretion of quinidine by urine alkalinization).

OVERDOSE

- Oral magnesium overdose does not, in general, induce toxic reactions with the normal renal function. Magnesium poisoning may, however, develop in the event of kidney failure. The toxic effects mainly depend on the serum magnesium levels and the signs are: hypotension, nausea, vomiting, CNS depression, decreased reflexes, ECG abnormalities, onset of respiratory depression, coma, cardiac arrest and respiratory paralysis, anuric syndrome.

Treatment: discontinuation of administration of the drug, and in severe cases, treatment with calcium salts intravenously.

REFERENCES

Aghajanyan IA, Radysh IV, Kutsov GM Physiological features of the females, adaptation and reproductive function. *The manual.* -- Moscow: RUDN, 1996. p98 [publication in Russian]

Akarachkova ES Assessing the effectiveness of MagneB6 in patients with clinical manifestations of stress. *Trudn. Pacient,* 2008, p 53-57. [publication in Russian]

Alberts B, Johnson A, Lewis J, Raff M, Roberts R, Walter P. Molecular Biology of the Cell, 4th edition, *Garland Science,* 2002, ISBN 0815340729.

Aleshin SV Hypertension: retaliatory strike. Moscow, 2004, LLC *"Orto.ru".* [publication in Russian]

Al-Rasheed AK, Blaser SI, Minassian BA, Benson L, Weiss SK. Cyclosporine A neurotoxicity in a patient with idiopathic renal magnesium wasting. *Pediatr. Neurol.* 2002 Apr;26(4):329.

Al-Rasheed AK, Blaser SI, Minassian BA, Benson L, Weiss SK. Cyclosporine A neurotoxicity in a patient with idiopathic renal magnesium wasting. *Pediatr. Neurol.* 2002 Apr;26(4):329.

Amighi J, Sabeti S, Schlager O, Mlekusch W, Exner M, Lalouschek W, Ahmadi R, Minar E, Schillinger M. Low serum magnesium predicts neurological events in patients with advanced atherosclerosis. *Stroke.* 2004;35(1):22-7

Andreev AV. Manual of clinical ultrasound dopplerography in pediatric neurology. St. Petersburg, 1995, 132s.

Anthony M. Nervous system, *J. Metal. Toxicology.* -1995. - P. 199-235.

Armand V., Gabriel S., Hoffmann P., Heinemann U., Ver-gnes M. Epileptiform activity and changes in field potential responses induced by low (Mg2+)0 in a genetic rat model of absence epilepsy. *Brain-Res.* 1998. Aug 24; 803(1-2), P.19-26.

Arnez J.G., Dock-Bregeon A.C., Moras D. Glycyl-tRNA synthetase uses a negatively charged pit for specific recogniti on and activation of glycine. - 1999. *J. Mol. Biol. Mar.* 12; 286 (5), P.1449-1459.

Atakan IH, Kaplan M, Seren G, Aktoz T, Gül H, Inci O. Serum, urinary and stone zinc, iron, magnesium and copper levels in idiopathic calcium oxalate stone patients. *Int. Urol. Nephrol.* 2007;39(2):351-6.

Aufiero E, Stitik TP, Foye PM, Chen B. Pyridoxine hydrochloride treatment of carpal tunnel syndrome: a review. *Nutr. Rev.* - 2004 Mar;62(3):96-104.

Aulisa L, Papaleo P, Pola E, Angelini F, Aulisa AG, Tamburrelli FC, Pola P, Logroscino CA. Association between IL-6 and MMP-3 gene polymorphisms and adolescent idiopathic scoliosis: a case-control study. *Spine.* 2007 Nov 15;32(24):2700-2.

Avsaroglu D, Inal TC, Demir M, Attila G, Acarturk E, Emre Evlice Y, Kayrin L. Biochemical indicators and cardiac function tests in chronic alcohol abusers. *Croat. Med. J.* 2005 Apr;46(2):233-7.

Avtsyn AP, Zhavoronkov AA, Rish MA, Strochkova LS. Microelementoses in human: etiology, classification and organ pathology. Moscow, *Medicina,* 1991, 492pp. [publication in Russian]

Aybak M, Sermet A, Ayyildiz MO, Karakilçik AZ.Effect of oral pyridoxine hydrochloride supplementation on arterial blood pressure in patients with essential hypertension. *Arzneimittelforschung.* 1995 Dec;45(12):1271-3.

Azria E, Tsatsaris V, Goffinet F, Kayem G, Mignon A, Cabrol D. Magnesium sulfate in obstetrics: current data. *J. Gynecol. Obstet. Biol. Reprod.* (Paris). 2004 Oct;33(6 Pt 1):510-7.

Bac P, Pages N, Herrenknecht C, Maurois P, Durlach J. Magnesium deficiency reveals the neurotoxicity of delta-9-tetrahydrocannabinol (THC) low doses in rats. *Magnes Res.* 2003 Mar; 16(1): 21-8.

Bac P, Pages N, Herrenknecht C, Teste JF. Inhibition of mouse-killing behaviour in magnesium-deficient rats: effect of pharmacological doses of magnesium pidolate, magnesium aspartate, magnesium lactate, magnesium gluconate and magnesium chloride. *Magnes Res.* - 1995 Mar;8(1):37-45.

Bakken N.A, Hunt C.D. Dietary boron decreases peak pancreatic in situ insulin release in chicks and plasma insulin concentrations in rats regardless of vitamin D or magnesium status. *J. Nutr.* - 2003 Nov;133(11) P. 3577-83.

Balan VE, Ilyina LM. Premenstrual syndrome. *Lech. Vrach.* 2008, № 3, 55-62.

Banerjee S, Mimouni FB, Mehta R, Llanos A, Bainbridge R, Varada K, Sheffer G. Lower whole blood ionized magnesium concentrations in hypocalcemic infants of gestational diabetic mothers. *Magnes Res.* 2003 Jun; 16(2): 127-30.

Barbagallo M, Dominguez LJ. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. *Arch. Biochem. Biophys.* 2007 Feb 1;458(1):40-7. Epub 2006 Jun 12.

Belfort MA, Clark SL, Sibai B. Cerebral hemodynamics in preeclampsia: cerebral perfusion and the rationale for an alternative to magnesium sulfate. *Obstet. Gynecol. Surv.* 2006 Oct;61(10):655-65.

Belfort MA, Clark SL, Sibai B. Cerebral hemodynamics in preeclampsia: cerebral perfusion and the rationale for an alternative to magnesium sulfate. *Obstet. Gynecol. Surv.* 2006 Oct;61(10):655-65.

Bell IR, Edman JS, Morrow FD, Marby DW, Perrone G, Kayne HL, Greenwald M, Cole JO.Brief communication. Vitamin B1, B2, and B6 augmentation of tricyclic antidepressant treatment in geriatric depression with cognitive dysfunction. *J. Am. Coll. Nutr.* 1992 Apr;11(2):159-63.PMID: 1578091

Benoit-Gonin M., Serin M., Pegaz-Fiornet A. Essais Terapeutigues. Lyon Medical, - 1973, - N230, Vol. 16, - P. 461 - 465.

Bernardini D, Nasulewic A, Mazur A, Maier JA.Magnesium and microvascular endothelial cells: a role in inflammation and angiogenesis. *Front Biosci.* 2005;10:1177-82.

Bloom S. Effects of magnesium deficiency on the pathogenesis of myocardial infarction. *Magnesium.* 1986;5(3-4):154-64.

Blumberg S. Is coral calcium a safe and effective supplement? *J. Am. Diet. Assoc.* 2004 Sep; 104 (9) :1335-6.

Bobkowski W, Nowak A, Durlach J. The importance of magnesium status in the pathophysiology of mitral valve prolapse. *Magnes Res.* 2005 Mar;18(1):35-52.

Boncimino K, McMahon DJ, Addesso V, Bilezikian JP, Shane E. Magnesium deficiency and bone loss after cardiac transplantation. *J. Bone Miner. Res.* - 1999 Feb;14(2):295-303.

Booth JV, Phillips-Bute B, McCants CB, Podgoreanu MV, Smith PK, Mathew JP, Newman MF. Low serum magnesium level predicts major adverse cardiac events after coronary artery bypass graft surgery. *Am. Heart J.* 2003;145(6):1108-1113.

Bourre JM. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. *J. Nutr. Health Aging.* 2006 Sep-Oct;10(5):377-85.

Brewer R.P., Parra A., Borel C.O., Hopkins M.B., Reynolds J.D. Intravenous magnesium sulfate does not increase ventricular CSF ionized magnesium concentration of patients with intracranial hypertension. *Clin. Neuropharmacol.* - 2001 Nov-Dec,24(6):341-5.

Bruno V. Antidegenerativ effecs of Mg2+ - valproate in cultuted cerebellar neurons, *Funct. Neirol.,* - 1995, May-June, 10(3). P. 121-130.

Busserolles J, Gueux E, Rock E, Mazur A, Rayssiguier Y. High fructose feeding of magnesium deficient rats is associated with increased plasma triglyceride concentration and increased oxidative stress. *Magnes Res.* 2003 Mar; 16(1): 7-12.

Calderon AT, Martinez-Sarmiento J, Montes ME, Sobrino JA, Arroyo M, Borque M, Alvarez Fernandez-Represa J.Quantitative study of metals in bile from patients with cholelithiasis. *Rev. Esp. Enferm. Dig.* 2000 Jul;92(7):439-47.

Campbell JD. Lifestyle, minerals and health. *Med. Hypotheses.* 2001 Nov;57(5):521-31.

Canterino J.C., Verma U.L., Visintainer P.F., Figueroa R., Klein S.A., Tejani N.A. Maternal magnesium sulfate and the development of neonatal periventricular leucomalacia and intraventricular hemorrhage. *Obsted-Gynecol*. - 1999 Mar, 93(3), P. 396-402.

Cefaratti C, Romani A. Intravesicular glucose modulates magnesium2+ transport in liver plasma membrane from streptozotocin-treated rats. *Metabolism*. – 2003, - Nov;52(11) - P.1464-1470.

Chakraborti S, Chakraborti T, Mandal M, Mandal A, Das S, Ghosh S.Protective role of magnesium in cardiovascular diseases: a review. *Mol. Cell Biochem.* 2002 Sep;238(1-2):163-79.

Chekman I.S. Gorchakova NA, Nikolai SL Magnesium in medicine. -- 1982 Kishinev, - p 101. [publication in Russian]

Choi JH, Lee J, Park CM. Magnesium therapy improves thromboelastographic findings before liver transplantation: a preliminary study. *Can. J. Anaesth.* 2005 Feb;52(2):156-9.

Choi JW, Pai SH. Serum lipid concentrations change with serum alkaline phosphatase activity during pregnancy. *Ann. Clin. Lab. Sci.* 2000 Oct;30(4):422-8.

Chubanov V, Waldegger S, Mederos y Schnitzler M, Vitzthum H, Sassen MC, et al.Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesemia with secondary hypocalcemia. *Proc. Natl. Acad. Sci. U. S. A.* 2004 Mar 2;101(9):2894-9.

Cohn R, Roth K. Early diagnosis of diseases of metabolism. -- 1986 Moscow: 637 pp. [publication in Russian]

Corica F, Corsonello A, Ientile R, Cucinotta D, Di Benedetto A, Perticone F, Dominguez LJ, Barbagallo M. Serum ionized magnesium levels in relation to metabolic syndrome in type 2 diabetic patients. *J. Am. Coll. Nutr.* 2006 Jun;25(3):210-5.

Coudray C, Feillet-Coudray C, Rambeau M, Tressol JC, Gueux E, Mazur A, Rayssiguier Y. The effect of aging on intestinal absorption and status of calcium, magnesium, zinc, and copper in rats: a stable isotope study. *J. Trace Elem. Med. Biol.* 2006; 20(2): 73-81. Epub 2005 Dec 20.

da Silva F, Willams RJ (eds). The biological chemistry of the elemements/ In inorganic chemistry of life. 2003, Oxford, University Press, 575 p.

De Blasio MJ, Dodic M, Jefferies AJ, Moritz KM, Wintour EM, Owens JA. Maternal exposure to dexamethasone or cortisol in early pregnancy differentially alters insulin secretion and glucose homeostasis in adult male sheep offspring. *Am. J. Physiol. Endocrinol. Metab.* 2007 Mar 13.

Delprat B, Bibert S, Geering K.[FXYD proteins: novel regulators of Na,K-ATPase] *Med. Sci.* (Paris). 2006 Jun-Jul;22(6-7):633-8

Delva P, Lechi A. Intralymphocyte magnesium decrease in patients with primary aldosteronism. Possible links with cardiac remodelling. *Magnes Res*. 2003 Sep; 16(3): 206-9.

Delva Pietro T., Pastori Caterina, Degan Maurizio, Montesi Germana D., Lechi Alessandro. Intralymphocyte free magnesium in a group of subjects with essential hypertension. *Hypertension.* 1996. № 3. – P. 433-439.

Di Ferrante N, Leachman RD, Angelini P, Donnelly PV, Francis G, Almazan A. Lysyl oxidase deficiency in Ehlers-Danlos syndrome type V. *Connect Tissue Res.* 1975;3(1):49-53.

Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. Institute of Medicine, Washington DC, - 2004, 564 p.

Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. Institute of Medicine, Washington DC, - 2004, 564 p.

Dodd JM, Crowther CA, Dare MR, Middleton P. Oral betamimetics for maintenance therapy after threatened preterm labour. *Cochrane Database Syst. Rev.* 2006 Jan 25;(1).

Domnitskaia TM, D'iachenko AV, Kupriianova OO, Domnitskiĭ MV. Clinical value of the use of magnesium orotate in adolescents with syndrome of cardiac connective tissue dysplasia. *Kardiologiia.* 2005;45(3):76-81.

Dong DM, Yao M, Liu B, Sun CY, Jiang YQ, Wang YS. Association between the -1306C/T polymorphism of matrix metalloproteinase-2 gene and lumbar disc disease in Chinese young adults. *Eur. Spine J.* 2007 Nov;16(11):1958-61.

Dontas AS, Zerefos NS, Panagiotakos DB, Valis DA.Mediterranean diet and prevention of coronary heart disease in the elderly. *Clin. Interv. Aging.* 2007;2(1):109-15.

Durlach J, Pages N, Bac P, Bara M, Guiet-Bara A, Agrapart C. Chronopathological forms of magnesium depletion with hypofunction or with hyperfunction of the biological clock. *Review. Magnes Res.* 2002 Dec; 15(3-4): 263-268.

Durlach J, Pages N, Bac P, Bara M, Guiet-Bara A. Magnesium deficit and sudden infant death syndrome (SIDS): SIDS due to magnesium deficiency and SIDS due to various forms of magnesium depletion: possible importance of the chronopathological form. *Magnes Res.* 2002 Dec; 15(3-4): 269-78. Review.

Durlach J, Pages N, Bac P, Bara M, Guiet-Bara A. New data on the importance of gestational Mg deficiency. *Magnes Res.* 2004 Jun;17(2):116-25.

Durlach J. Pages N. Bac P. Bara M. Guiet-Bara A Magnesium deficit and sudden infant death syndrome (SIDS): SIDS due to magnesium deficiency and SIDS due to various forms of magnesium depletion: possible importance of the chronopathological form. *Magnes Res*. 2002 Dec;15(3-4):269-78.

Durlach J. Recommended dietary amounts of magnesium: Mg RDA. *Magnes Res.* 1989 Sep;2(3):195-203.

Durlach J., Pages N., Bac P., Bara M., Guiet-Bara A. Beta-2 mimetics and magnesium: true or false friends? *Magnes Res.* – 2003, - Sep;16(3), - P. 218-233.

Durnev AD, Seredenin SB Mutageny. Screening and prevention pharmacological effects. M., *Medicine,* - 1998. 327 pp. [publication in Russian]

Dyer S.A., Sampson H.W. Magnesium levels in alcohol-treated rodents using different consumption paradigms. *Alcohol.* - 1998 Oct; 16(3), p. 195-199.

Fausto de Silva, Willams P.J., *Biological chemisrty of elements,* Cambrige - 2003, 678 p.

Feldeisen SE, Tucker KL. Nutritional strategies in the prevention and treatment of metabolic syndrome. *Appl. Physiol. Nutr. Metab.* 2007 Feb;32(1):46-60.

Feltelius N, Hällgren R, Lindh U. Redistribution of cellular mineral and trace element stores in HLA-B27 positive relatives of patients with ankylosing spondylitis--a marker of hidden inflammatory disease. *J. Rheumatol.* 1988 Feb;15(2):308-14.

Feltelius N, Hällgren R, Lindh U. Redistribution of cellular mineral and trace element stores in HLA-B27 positive relatives of patients with ankylosing spondylitis--a marker of hidden inflammatory disease. *J. Rheumatol.* 1988 Feb;15(2):308-14.

Filatov E. A neurologic disorder of breathing: hyperventilation syndrome. *Lech. Vrach,* 2007, № 9, 70-72.

Filipenko PS, Malookaia IuS. The role of connective tissue dysplasia in the forming of mitral valve prolapse. *Klin. Med.* (Mosk). 2006;84(12):13-9.

Fitó M, Guxens M, Corella D, Sáez G, Estruch R, de la Torre R, Francés F, Cabezas C et al; for the PREDIMED Study Investigators. Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. *Arch. Intern. Med.* 2007 Jun 11;167(11):1195-203.

Foster H. Cancer mortality and the environment: suggstive evidence from USA. In: Environmental life elements and health, Jian'an T(Ed), 1990,pp21-33.

Friso S, Girelli D, Martinelli N, Olivieri O, Lotto V, Bozzini C, Pizzolo F, Faccini G, Beltrame F, Corrocher R. Low plasma vitamin B-6 concentrations and modulation of coronary artery disease risk. *Am. J. Clin. Nutr.* - 2004 Jun;79(6):992-8.

Gaspar A.Z., Gasser P., Flammer. *J. Ophtalmologica.* – 1995 - N209, - P.11-13.

Giacconi R, Muti E, Malavolta M, Cipriano C, Costarelli L, Bernardini G, Gasparini N, Mariani E, Saba V, Boccoli G, Mocchegiani E.The +838 C/G MT2A polymorphism, metals, and the inflammatory/immune response in carotid artery stenosis in elderly people. *Mol. Med.* 2007 Jul-Aug;13(7-8):388-95.

Gol M, Sisman AR, Guclu S, Altunyurt S, Onvural B, Demir N. Fetal gender affects maternal serum total and placental alkaline phosphatase levels during pregnancy. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2006 Sep-Oct;128(1-2):253-6.

Golf SW, Bender S, Grüttner J. On the significance of magnesium in extreme physical stress. *Cardiovasc. Drugs Ther.* 1998 Sep;12 Suppl 2:197-202.

Golovskoĭ BV, Usol'tseva LV, Khovaeva IaB, Ivanova NV. Clinical presentation of connective tissue dysplasia in adults. *Klin. Med.* (Mosk). 2002;80(12):39-41.

Gonzalez-Revalderia J., Paula M., Pascual T., Rodicio J.L., Alcazar J.M., Miravalles E. Total and ionized serum magnesium in hypertension, obesity and diabetes. Abstr. 12th *Sci. Meet. Inter-Amer. Soc. Hypertens.*, Mexico City, Mex., March 15-18, 1997. Hypertension. - 1997. № 3. – P. 855.

Goodman G. The pharmacological Basis of Therapeutics, *Eight Edition,* - 2002, - vol. 2, - 1236 p.

Gopher VP Geochemical ecology of disease. T.1, 2., Atomovity. Metro: Helios ARV. -- 2000 - p672. [publication in Russian]

Gorbacheva SV, Belenichev IF, Dunaev VV, Bukhtiiarova NV. Pharmacological correction of neuronal damage in sensomotor zone of frontal cortex under conditions of experimental cerebral blood flow pathology. *Eksp. Klin. Farmakol.* 2007 Nov-Dec;70(6):13-6.

Gorodetsky VV, Talibov OB Preparations of magnesium in medical practice. Metro: - 2003 - p41. [publication in Russian]

Goyer RA et al. Role of the chelating agents for prevention, intervention, and treatment of the exposures to toxic metals// *Environ. Health Perspect,* 103(11):1048-1052.

Goytain A, Quamme GA. Functional characterization of human SLC41A1, a Mg2+ transporter with similarity to prokaryotic MgtE Mg2+ transporters. *Physiol. Genomics.* 2005 May 11;21(3):337-42.

Grafe S., Saluz H.P., Grimm B., Hanel F. Mg-chelatase of tobacco: the role of the subunit CHL D in the chelation step of protoporphurin IX, *Proc. Natl. Acad. Sci. U. S. A.* – 1999, - Mar 2; 96(5), - P.1941-1946.

Grether J.K., Hoogstrate J., Walsh-Greene E., Nelson K.B. Magnesium sulfate for tocolysis and risk of spastic cerebral palsy in premature children born to women without preeclampsia. *Am. J. Obstet. Gynecol*. - 2000 183(3):717-25.

Grimes DA, Nanda K. Magnesium sulfate tocolysis: time to quit. *Obstet. Gynecol.* 2006 Oct;108(4):986-9.

Gromova O. A., Serov V. N., Uvarova E. V., Rebrov V. G. Magnesia therapy in obstetrics: a look at the problem from the standpoint of the evidentiary medicine. *Vopr. Gynecol. Akusher. Perinatol.* 2008, V. 7, № 4, p. 42-47.

Gromova O.A., Burtsev E.M., Skalny A.V., Fedotova L.E. Rola magnezu w leczeniu dysfnkcji mozgowej u dzieci. III Zjazdu Towrzystwa Magnezologicznero im. Prof. Dr. Juliana Aleksandrowwicza, Poznan 15-16.10. 1998. P. 92-98 [publication in Polish]

Gromova OA Bukharin EV Halytska SA Grishina TP, Volkov AY, Mokrousov AA Correction of magnesium deficiency in women with premenstrual syndrome / / *Obstetrics and Gynecology.* - 2003, - № 5, - S. 48-52. [publication in Russian]

Gromova OA Clinical Pharmacology of vitamin-mineral complexes for pregnant and nursing// Obstetrics and Gynecology, - 2005, № 10. [publication in Russian]

Gromova OA Vitamins and trace elements in preconception, pregnancy and nursing. Clinical Pharmacology. Training programs UNESCO, the allowance for doctors ed. VM Sidelnikova, Moscow - 2005, 124 pp. [publication in Russian]

Gromova OA, Gogoleva IV. The use of magnesium in the mirror of the evidentiary medicine and basic research: the shortage of magnesium and the concept of stress. Trudn Patient 11, Vol 5, 2007, p3-11. [publication in Russian]

Gromova OA, Grishina T. R., Andreev AV, Krasnykh A., Fedotov L. A., Limanova OA, Semenchikova N. Potentiation of neuroprotective therapy in adolescents with early forms of cerebrovascular pathology. *Pediatric Pharmacology*, 4(3), 2007, 18-25.

Gromova OA, Kataev SI, Mazin SS, Volkov AY Changing homeostasis of microelements in the rat brain with the appointment of drug Magne B6 / / *Neurology and Psychiatry them.* SS Korsakov, 2003. N5, S. 47-49. [publication in Russian]

Gromova OA, Kudrin AV Neurochemistry of macro-and micronutrients. Moscow:, Alev-V, - 2001 - p 300. [publication in Russian]

Gromova OA, Serov VN, Torshin I.Yu. Magnesia therapy in obstetrics and gynecology: magnesium correction and the therapy outcomes. Trudn Patient, Vol 6(8), 2008, 16-25. [publication in Russian]

Gromova OA, Uvarova EV, Grishina T.R., Red-AM, Fedotov L.E., Limanova OA, Semenchikova NN, Potentiation of the neuroprotective therapy in patients with early forms of cerebrovascular pathology// *Reproductive health of children and adolescents* - 2005, № 2. [publication in Russian]

Gromova OA. The role and importance of magnesium in the pathogenesis of diseases of the nervous system / / *Neurology and Psychiatry them*. SS Korsakov - 2002, - № 12, - S.45-49. [publication in Russian]

Guerrero-Romero F, Rodriguez-Moran M. Hypomagnesemia, oxidative stress, inflammation, and metabolic syndrome. *Diabetes Metab. Res. Rev*. 2006 Nov-Dec;22(6):471-6.

Gums JG. Magnesium in cardiovascular and other disorders. *Am. J. Health Syst. Pharm.* - 2004 Aug 1;61(15):1569-76.

Guo H, Lee JD, Uzui H, Yue H, Wang J, Toyoda K, Geshi T, Ueda T.Effects of folic acid and magnesium on the production of homocysteine-induced extracellular matrix metalloproteinase-2 in cultured rat vascular smooth muscle cells. *Circ. J.* 2006 Jan;70(1):141-6.

Gupta BK, Glicklich D, Tellis VA.Magnesium repletion therapy improved lipid metabolism in hypomagnesemic renal transplant recipients: a pilot study. *Transplantation.* - 1999 Jun 15;67(11):1485-7.

Hall WD, Pettinger M, Oberman A, Watts NB, Johnson KC, Paskett ED, Limacher MC, Hays J.Risk factors for kidney stones in older women in the southern United States. *Am. J. Med. Sci*. 2001 Jul;322(1):12-8.

Hans CP, Chaudhary DP, Bansal DD. Effect of magnesium supplementation on oxidative stress in alloxanic diabetic rats. *Magnes Res.* 2003 Mar; 16(1): 13-9.

He K, Liu K, Daviglus ML, Morris SJ, Loria CM, Van Horn L, Jacobs DR Jr, Savage PJ.Magnesium intake and incidence of metabolic syndrome among young adults. *Circulation.* 2006 Apr 4;113(13):1675-82.

He K, Liu K, Daviglus ML, Morris SJ, Loria CM, Van Horn L, Jacobs DR Jr, Savage PJ. Magnesium intake and incidence of metabolic syndrome among young adults. *Circulation.* 2006 Apr 4;113(13):1675-82. Epub 2006 Mar 27.

Heap LC, Pratt OE, Ward RJ, Waller S, Thomson AD, Shaw GK, Peters TJ.Individual susceptibility to Wernicke-Korsakoff syndrome and alcoholism-induced cognitive deficit: impaired thiamine utilization found in alcoholics and alcohol abusers. *Psychiatr. Genet.* - 2002 Dec;12(4):217-24.

Heiser P, Teepker M, Moller JC, Theisen FM, Friedel S, Hebebrand J, Remschmidt H. Neuropathy due to hypovitaminosis following excessive weight loss.// *J. Am. Acad. Child Adolesc. Psychiatry.* - 2004 Aug;43(8):928-9.

Held K., Antonijevic I.A., Kunzel H., Uhr M., Wetter T.C., Golly I.C., Steiger A., Murck H., Oral MG(2+) supplementation reverses age-related neuroendocrine and sleep EEG changes in humans Should we use oral magnesium supplementation to improve sleep in the elderly? *Sleep Med.* - 2003 May;4(3):- P. 263-264.

Henrotte JG, Franck G, Santarromana M, Nakib S, Dauchy F, Boulu RG.Effect of pyridoxine on mice gastric ulcers and brain catecholamines after an immobilization stress. *Ann. Nutr. Metab.* 1992;36(5-6):313-7.PMID: 1492759

Henrotte JG. Type A behavior and magnesium metabolism. *Magnesium.* 1986;5(3-4):201-10.

Hering F, Briellmann T, Lüönd G, Guggenheim H, Seiler H, Rutishauser G. Stone formation in human kidney. *Urol. Res.* 1987;15(2):67-73.

Higashiura K, Shimamoto K. Magnesium and insulin resistance. [Article in Japanese]. *Clin. Calcium.* 2005 Feb;15(2):251-254.

Higdon J. An Evidence-Based Approach to Vitamins and minerals. New York-Stuttgart, 2005.

How HY, Zafaranchi L, Stella CL, Recht K, Maxwell RA, Sibai BM, Spinnato JA. Tocolysis in women with preterm labor between 32 0/7 and 34 6/7 weeks of gestation: a randomized controlled pilot study. *Am. J. Obstet. Gynecol.* 2006 Apr;194(4):976-81.

Hvas AM, Juul S, Bech P, Nexø E.Vitamin B6 level is associated with symptoms of depression. *Psychother. Psychosom.* 2004 Nov-Dec;73(6):340-3.PMID: 15479988

Iannello S, Belfiore F. Hypomagnesemia. A review of pathophysiological, clinical and therapeutical aspects.// *Panminerva Med.* - 2001 Sep; 43(3):177-209.

Iezhitsa IN, Onishchenko NV, Churbakova NV, Parshev VV, Petrov VI, Spasov AA. Effect of magnesium supplementation containing mineral bishofit (MgCl2 x 6H2O) solution and pyridoxine hydrochloride on erythrocyte magnesium depletion and behaviour of rats after three-month alcoholization. *Magnes. Res.* 2002 Dec; 15(3-4): 179-89.

Igondjo-Tchen S, Pages N, Bac P, Godeau G, Durlach J. Marfan syndrome, magnesium status and medical prevention of cardiovascular complications by hemodynamic treatments and antisense gene therapy. *Magnes. Res.* 2003 Mar; 16(1): 59-64.

Itoh Kazue, Kawasaki Terukazu, Nakamura Motoomi.The effects of high oral magnesium supplementation on blood pressure, serum lipids and related variables in apparently healthy Japanese subjects. *Brit. J. Nutr.* - 1997. № 5. – P. 737-750.

Izmerov NF (ed). Professional disease. Vol 2, Moscow, *Medicina,* 1996, pp 393-404. [publication in Russian]

Johnson S. Micronutrient accumulation and depletion in schizophrenia, epilepsy, autism and Parkinson's disease? *Med. Hypotheses.* 2001 May;56(5):641-5.

Kalaitzidis R, Tsimihodimos V, Bairaktari E, Siamopoulos KC, Elisaf M. Disturbances of phosphate metabolism: another feature of metabolic syndrome. *Am. J. Kidney Dis.* 2005 May;45(5):851-8.

Kamilya G, Bharracharyya SK, Mukherji J. Changing trends in the management of eclampsia from a teaching hospital. *J. Indian Med. Assoc.* 2005 Mar;103(3):132, 134-5.

Kantak KM. Magnesium deficiency alters aggressive behavior and catecholamine function. *Behav. Neurosci*. 1988 Apr;102(2):304-11.

Kaplan W, Haymond MW, McKay S, Karaviti LP.Osteopenic effects of MgSO4 in multiple pregnancies. *J. Pediatr. Endocrinol. Metab.* 2006 Oct;19(10):1225-30.

Karpov OI, Zaitsev AA The risk of drug use during pregnancy and lactation, St. Petersburg, - 2003, 376 pp. [publication in Russian]

Keith W.B. Endemic deseases of suspected chemical etiolog: their Detection and Prevention, Science Press, Beijing, 1990, P.16-33.

Kelly PJ, Kistler JP, Shih VE, Mandell R, Atassi N, Barron M, Lee H, Silveira S, Furie KL. Inflammation, homocysteine, and vitamin B6 status after ischemic stroke. *Stroke.* - 2004 Jan;35(1):12-5.

Kerr G.R., Lee E.S., Lam M.K. Relationship between dietary and biochemical measures of nutrition status in Hannes I data." *Am. J. Nutr.,* 1982, 35:294-307.

Kisters K., Spieker C., Tepel M., Zidek W., Rahn K.H. Cellular calcium/magnesium antagonism in primary hypertension.[Abstr.] 31st Annu. Sci. Meet. Eur. Soc. Clin. Invest., Kiel, 19th-22nd March, - 1997. *Eur. J. Clin. Invest.* 1997. - P. 27.

Kiyakbaev GK, Kurbanov RD, Zhalolov B.Z. Potential combination of magnesium lactate and pyridoxine in enhancing the effectiveness and safety of drugs therapy with class 3 antiarrhythmics / / *Cardiology* - 2001. -- 41. N 11. – pp. 62-65. [publication in Russian]

Klein G.L., Langman C.B., Herndon D.N. Persistent hypoparathyroidism following magnesium repletion in burn-injured children. *Pediatr. Nephrol*. - 2000 Apr, 14 (4). - P. 301-304.

Kobusiak Prokopowicz M., Mysiak A. Wplyw dozylnie podawanego roztworu magnezu na zaburzenia rytmu serca u chorych po kardiowersji elektrycznej. *Pol. Merkuriusz. Lek.* - 1999 Aug, 7 (38). - P. 51-53.

Kodentsova VM, Vrzhesinskaya OA. Vitamins in the diet of pregnant / / *Gynecology,* Volume 4, N1, 2002. [publication in Russian]

Konrad M, Schaller A, Seelow D, Pandey AV, Waldegger S, Lesslauer A, Vitzthum H, Suzuki Y et al. Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement. *Am. J. Hum. Genet.* 2006 Nov;79(5):949-57.

Kosheleva NG Hypomagnesemia in obstetrical pathology and its methods of correction. / / Vestn. Ros. Association. *obstetricians and gynecologists.* -- 1999. N 1. – pp. 42-46. [publication in Russian]

Kosheleva NG, Arzhanova O.N., Pluzhnikov TA Premature pregnancy: ethipatholoy, diagnosis, and treatment. St. Petersburg, 2003, pp 70. [publication in Russian]

Kousa A, Havulinna AS, Moltchanova E, Taskinen O, Nikkarinen M, Eriksson J, Karvonen M. Calcium:magnesium ratio in local groundwater and incidence of acute myocardial infarction among males in rural Finland. *Environ. Health Perspect.* 2006 May;114(5):730-4.

Krajcovicova-Kudlackova M, Klvanova J, Dusinska M.Polyunsaturated Fatty Acid Plasma Content in Groups of General Population with lowvitamin B6 or low iron serum levels. *Ann. Nutr. Metab.* - 2004;48(2):118-21.

Kremer JM, Bigaouette J.Nutrient intake of patients with rheumatoid arthritis is deficient in pyridoxine, zinc, copper, and magnesium. *J. Rheumatol.* - 1996 Jun;23(6):990-4.

Kudrin AV, Gromova OA, Microelements in neurology. Training programs UNESCO, M.: *Geotar-Med.* 2006, 274 pp. [publication in Russian]

Kumeda Y, Inaba M. Metabolic syndrome and magnesium. [Article in Japanese]. *Clin. Calcium.* 2005 Nov;15(11):97-104

Kurbanov RD, Dzhusipov AK, Yunusov ZZ. Magnesium chronic deficit in the practice cardiologist, a manual for doctors, Tashkent-Almaty, 2004 (book in Russian).

Kurup RK, Kurup PA.A Hypothalamic digoxin-mediated model for autism. *Int. J. Neurosci.* – 2003, - Nov;113(11), - P. 1537-1559.

Kuznetsova E, Shiliaev R, Gromova O. Dysmicroelementoses in children with nephropathias. *Vopr. Sovr. Pediatr.* N11, 2006, pp 45-50. [publication in Russian]

Lal KJ, Dakshinamurti K.The relationship between low-calcium-induced increase in systolic blood pressure and vitamin B6. *J. Hypertens.* 1995 Mar;13(3):327-32.PMID: 7622854

Launius BK, Brown PA, Cush EM, Mancini MC. Osteoporosis: The dynamic relationship between magnesium and bone mineral density in the heart transplant patient. *Crit. Care Nurs. Q*. - 2004 Jan-Mar;27(1):96-100.

Leatham EW, Holt DW, McKenna WJ. Class III antiarrhythmics in overdose. Presenting features and management principles. *Drug Saf.* 1993 Dec;9(6):450-62.

Lebedev V. A., V. M. Pashkov, P. V. Budanov. Clinical evaluation of magnesium deficiency in women with premenstrual syndrome. *Vopr. Gynecol. Akusher. Perinatol.* 2008, T. 7, № 1, pp 20-25.

Lech T., Garlicka A. Value of magnesium and calcium in serum and hair of children and adolescents with neurologic diseases. *Przegl. Lek.* - 2000 57(7-8):378-381.

Levy GG, Motto DG, Ginsburg D.ADAMTS13 turns 3.Blood. 2005 Jul 1;106(1):11-7.

Li J, Zhang Q, Zhang M, Egger M. Intravenous magnesium for acute myocardial infarction. *Cochrane Database Syst. Rev.* 2007;(2):CD002755.

Li W. Mg2+ antagonism of Ni (2+) - induced in microtubule assembly and cellular thiol homeostasis. *Toxicol. Appl. Pharmaco*l. – 1996 Vol. 136. - № 1. - P.101-111.

Liang Z, Yang Z.Identification and characterization of a novel gene EOLA1 stimulating ECV304 cell proliferation. *Biochem. Biophys. Res. Commun*. 2004 Dec 17;325(3):798-802.

Liese A.D, Roach A.K, Sparks K.C, et al. Whole-grain intake and insulin sensitivity: the Insulin Resistance Atherosclerosis *Study. Am. J. Clin. Nutr.* – 2003, - Nov;78(5) - P.9

Liflyandsky VG, Zakrevsky VV, Andronova MN Medicinal properties of food. -- Moscow: *TERRA,* 1999. -- S.514-525 (book in Russian).

Liu DL, Yan M, Chua YL, Chen C, Lim YL. Effects of molybdenum, silicon and nickel on alpha1-adrenoceptor-induced constriction of rat isolated aorta. *Clin. Exp. Pharmacol. Physiol.* 2002 May-Jun;29(5-6):395-8.

Locksley RM, Killeen N, Lenardo MJ.The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell*. 2001 Feb 23;104(4):487-501.

Luzhnikov EA. [Current principles of detoxification therapy in acute poisoning] *Anesteziol. Reanimatol*. 1998 Nov-Dec;(6):4-6. [publication in Russian]

Lynn E.G., Vazhappilly R., Au-Yeung K.K., Zhu D.Y., Siow Y.L. Magnesium tanshinoate B (MTB) inhibits low density lipoprotein oxidation. *Life Sci.* 2001 Jan 12,68(8):903-12.

MAGIC trial, 2002 [12401244] MAGIC (Magnesium in Coronaries) Trial Investigators. Early administration of intravenous magnesium to high-risk patients with acute myocardial infarction in the Magnesium in Coronaries (MAGIC) Trial: a randomised controlled trial. *Lancet*. 2002;360(9341):1189-1196.

Makiya R, Stigbrand T. Placental alkaline phosphatase as the placental IgG receptor. *Clin. Chem.* 1992 Dec;38(12):2543-5.

Malmierca E, Polo J, Castro JR. Electrocardiographic changes due to pyridoxine deficiency. *Pacing Clin. Electrophysiol.* - 2003 May;26(5):1289-91.

Malouf R, Grimley Evans J.The effect of vitamin B6 on cognition. //*Cochrane Database. Syst. Rev.* 2003;(4):CD004393.

Markov IV, V. Afanasieva, Tsybulkin EK Clinical toxicology children and adolescents. Volume 2. St. Petersburg, *Intermedica,* - 1999, 367 pp. [publication in Russian]

Matsuda Y, Kouno S, Hiroyama Y. Intrauterine infection, magnesium sulfate exposure and cerebral palsy in infants born between 26 and 30 weeks of gestation Eur J. Obstet *Gynecol. Reprod. Biol.* - 2000 Aug,91(2). - P. 159-164.

Mazotta G. Intracellular Mg concetration and electromyographical ishaemic test in juvenile headache, *Cephalalgia*. - 1999; 19(9), - P. 802-809.

Mazur A, Maier JA, Rock E, Gueux E, Nowacki W, Rayssiguier Y. Magnesium and the inflammatory response: potential physiopathological implications. *Arch. Biochem. Biophys.* 2007 Feb 1;458(1):48-56.

McKeown NM, Jacques PF, Zhang XL, Juan W, Sahyoun NR.Dietary magnesium intake is related to metabolic syndrome in older Americans. *Eur. J. Nutr.* 2008 Jun;47(4):210-6.

Mehta R, Petrova A. Intrapartum magnesium sulfate exposure attenuates neutrophil function in preterm neonates. *Biol. Neonate.* 2006;89(2):99-103. Epub 2005 Sep 26.

Meij IC, Koenderink JB, van Bokhoven H, Assink KF, Groenestege WT, de Pont JJ, Bindels RJ, Monnens LA, van den Heuvel LP, Knoers NV.Dominant isolated renal magnesium loss is caused by misrouting of the Na(+),K(+)-ATPase gamma-subunit. *Nat. Genet*. 2000 Nov;26(3):265-6.

Mervaala Eero M.A., Pere Anna-Kaisa, Lindgren Leena, Laakso Juha, Teravainen Terttu-Liisa, Karjala Kirsi, Vapaatalo Heikki, Ahonen Juhani, Karppanen Heikki. Effects of dietary sodium and magnesium on cyclosporin A-induced hypertension and nephrotoxicity in spontaneously hypertensive rats. *Hypertension.* - 1997. № 3. – P. 822-827.

Miller S, Crystal E, Garfinkle M, Lau C, Lashevsky I, Connolly SJ. Effects of magnesium on atrial fibrillation after cardiac surgery: a meta-analysis. *Heart.* 2005;91(5):618-623.

Mitchell LB. Prophylactic therapy to prevent atrial arrhythmia after cardiac surgery. *Curr. Opin. Cardiol.* 2007 Jan;22(1):18-24.

Mittendorf R, Dammann O, Lee KS. Brain lesions in newborns exposed to high-dose magnesium sulfate during preterm labor. *J. Perinatol.* 2006 Jan 1;26(1):57-63.

Mochalov OM, Vlasov AM, Shcherbakov PN, Dankevich NG By the use of magnesium-pyridoxine treatment in craniocerebral injuries, Sat. materials of the scientific conference, Omsk, 21-22 September 1999, - 1999, s.120. [publication in Russian]

Mohsin N, Jha A, Al Maimani Y, Malvathu R, Kallankara S. Hypomagnesemia as a cause of severe cardiac arrythmias in the immediate postoperative period: a renal transplant case report. *Transplant. Proc.* - 2003 Nov;35(7):2652.

Mozgovaya EV, Kosheleva NG. The effectiveness of the use of magnesium products for gestosis prevention. *Ross. Vestn. Akusher. Gynecol.* 5, 2007, 12-15.

Murray MT, Pizzorno JE. Tryptophan load test. In: Pizzorno JE, Murray MT, eds. *Textbook of Natural Medicine.* 2nd ed. New York, NY: Churchhill Livingstone; 1999:225-228.

Nassar AH, Sakhel K, Maarouf H, Naassan GR, Usta IM. Adverse maternal and neonatal outcome of prolonged course of magnesium sulfate tocolysis. *Acta Obstet. Gynecol. Scand.* 2006;85(9):1099-103.

Nechifor M. Magnesium in drug dependences. *Magnes Res.* 2008 Mar;21(1):5-15.

Nielsen FH, Lukaski HC.Update on the relationship between magnesium and exercise. *Magnes. Res*. 2006 Sep;19(3):180-9.

Nikitin A. The harmful environmental factors and human reproductive system. St. Petersburg, ELBI-SPb, 2005, 215 pp. [publication in Russian]

Oceandy D, Yusoff R, Baudoin FM, Neyses L, Ray SG. Promoter polymorphism of the matrix metalloproteinase 3 gene is associated with regurgitation and left ventricular remodelling in mitral valve prolapse patients. *Eur. J. Heart Fail*. 2007 Oct;9(10):1010-7.

Ogata T, Shibamura H, Tromp G, Sinha M, Goddard KA, Sakalihasan N et al. Genetic analysis of polymorphisms in biologically relevant candidate genes in patients with abdominal aortic aneurysms. *J. Vasc. Surg*. 2005 Jun;41(6):1036-42.

Ogunyemi D. Risk factors for acute pulmonary edema in preterm delivery. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2007 Feb 26.

Okajima T, Fukumoto S, Furukawa K, Urano T.Molecular basis for the progeroid variant of Ehlers-Danlos syndrome. Identification and characterization of two mutations in galactosyltransferase I gene. *J. Biol. Chem.* 1999 Oct 8;274(41):28841-4.

Oleszkewicz J. Zaburzenia Koncentracji Nadpobudliwosc to uleczalne choroby cywilizacji, - 1998. Warzawa. - 56 p.

Onalan O, Crystal E, Daoulah A, Lau C, Crystal A, Lashevsky I. Meta-analysis of magnesium therapy for the acute management of rapid atrial fibrillation. *Am. J. Cardiol.* 2007;99(12):1726-32

Pamphlett R, Todd E, Vink R, McQuilty R, Cheema SS. Magnesium supplementation does not delay disease onset or increase survival in a mouse model of familial ALS. *J. Neurol. Sci.* - 2003 Dec 15;216(1):95-8.

Panchenko LF, May IV, Gurevich KG, Clinical chemistry of the trace elements, M., *VUNMTS.* 2004, pp. 363 [publication in Russian]

Patel VB, Why HJ, Richardson PJ, Preedy VR. The effects of alcohol on the heart. *Adverse Drug React. Toxicol. Rev*. 1997 Mar;16(1):15-43.

Pickering LK, Baker CJ, Long SS, McMillan JA, eds. Red Book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics, 2006.

Poenaru S., Manicom R., Rouhani S., Aymard P., Bajenaru O., Rayssiguier Y., Emmanouillidis E., Gueux E., Nkanga N., Kurlach J., Dall'ava J. Stability of brain content of magnesium in experimental hypomagnesemia. *Brain Res.* - 1997. № 2. – P. 329-332.

Pogodaev KI Epileptologiya and pathochemistry of the brain - 1986 - *Metro:* - 237 pp. [publication in Russian]

Poikolainen K, Alho H. Magnesium treatment in alcoholics: a randomized clinical trial. *Subst. Abuse Treat Prev. Policy.* 2008 Jan 25;3:1.

Polderman K.H., Peerdeman S.M., Girbes A.R. Hypophosphatemia and hypomagnesemia induced by cooling in patients with severe head injury. *J. Neurosurg.* – 2001, - May;94(5) – P. 697-705

Prilepskaya VN, Mezhevitinova EA, Nazarova NM The role of magnesium in the development of premenstrual syndrome (PMS), *Methodical,* M.: - 2003, from -23. [publication in Russian]

Quilichini PP, Diabira D, Chiron C, Milh M, Ben-Ari Y, Gozlan H. Effects of antiepileptic drugs on refractory seizures in the intact immature corticohippocampal formation in vitro. *Epilepsia.* - 2003 Nov;44(11), - P.1365-1374.

Rahman MI, Chagoury ME. Selections from current literature: magnesium, myocardial infarction and meta-analysis. *Fam. Pract.* 1994;11(1):96-101.

Raison-Peyron N., Messaad D., Bousquet J., Demoly P. Selective hypersensitivity to pidolate Allergy Volume 57 Issue 1 January - 2002. P. 53

Rajesh R, Girija AS. Pyridoxine-dependent seizures: a review. *Indian Pediatr.* - 2003 Jul;40(7):633-8.

Ranade, V. V., Somberg, J.C. Bioavailability and Pharmacokinetics of Magnesium After Administration of Magnesium Salts to Humans. *American Journal of Therapeutics.* September/October - 2001. 8(5):345-357

Randell EW, Mathews M, Gadag V, Zhang H, Sun G. Relationship between serum magnesium values, lipids and anthropometric risk factors. *Atherosclerosis.* 2006 Dec 7;

Ravn H.B., Vissinger H., Kristensen S.D., Husted S.E. Magnesium inhibits platelet aggregation in vitro. Abstr. 29th Annu. Meet. Eur. Soc. Clin. Invest. and Med.Res. Soc. Gr. Brit., Cambridge, 2-5 Apr., - 1995. *Eur. J. Clin. Invest.* 1995. – P. 39.

Rayssiguier Y, Guezennec CY, Durlach J. New experimental and clinical data on the relationship between magnesium and sport. *Magnes Res.* 1990 Jun;3(2):93-102.

Rayushkin VA Antidepressant effect and therapy with Magne B6. / / Proceedings of the scientific and practical. Conf. 7-8 April - 1998 - M. - pp 383-385. [publication in Russian]

Rebrov, VG, Gromova OA Vitamins and trace elements. M: Alev-V, - 2003 – 648pp. [publication in Russian]

Reddy V, Sivakumar B.Magnesium-dependent vitamin-D-resistant rickets. *Lancet*. 1974 May 18;1(7864):963-5.

Rubenowitz E, Molin I, Axelsson G, Rylander R. Magnesium in drinking water in relation to morbidity and mortality from acute myocardial infarction. *Epidemiology.* 2000;11(4):416-421.

Saha H., Harmoinen A., Pietila K., Morsky P., Pasternack A. Measurement of serum ionised versus total levels of magnesium and calcium in hemodialysis patients. *Clin. Nephrol.* - 1996. № 5. – P. 326-331.

Sameshima H., Ota A., Ikenoue T. Pretreatment with magnesium sulfate protects against hypoxic-ischemic brain in jury but postasphyxial treatment worsens brain damage in seven-day-old rats. *Am. J. Obstet. Gynecol.* - 1999 180(3 pt 1), p. 725-30.

Sanjad SA, Ibrahim A, Al Shorafa S, Al Abbad A, Khauli RB, Shaibani KA, Al Sabban E. Renal tubular dysfunction following kidney transplantation: a prospective study in 31 children. *Transplant Proc.* 2001 Aug;33(5):2830-1.

Schimpf R, Borggrefe M, Wolpert C.Clinical and molecular genetics of the short QT syndrome. *Curr. Opin. Cardiol.* 2008 May;23(3):192-8.

Schlingmann K.P., Konrad M., Seyberth H.W. Genetics of hereditary disorders of magnesium homeostasis. *Pediatr. Nephrol.* – 2003, - Nov. 22, - P.2110-2115.

Schlingmann KP, Weber S, Peters M, Niemann Nejsum L, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E et al.Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat. Genet.* 2002 Jun;31(2):166-70.

Schmidt EV. Vascular diseases of the nervous system, Moscow, Medicine, 1975, 663P.

Schrewe, U.J. Radiation exposure monitoring in civil aircraft. *Nucl. Instr. Meth. Phy. Res. A.* (1999), 422(1):621-625.

Schulze-Bonhage A, Kurthen M, Walger P, Elger CE. Pharmacorefractory status epilepticus due to low vitamin B6 levels during pregnancy. *Epilepsia.* - 2004 Jan;45(1):81-4.

Schumann K., Lebeau A., Gunther T., Vormann J. Mechanismus und Ausma der Sideroseentwicklung im Magnesiummangel. *VitaMinSpur.* - 1996. № 4. – S.186-194.

Seleznyov LM Proper nutrition with hypertension, M.: 2002 - 221 pp.

Senni K, Foucault-Bertaud A, Godeau G. Magnesium and connective tissue. *Magnes Res. Review.* 2003 Mar; 16(1): 70-74.

Shah NC, Pringle SD, Donnan PT, Struthers AD.Spironolactone has antiarrhythmic activity in ischaemic cardiac patients without cardiac failure. *J. Hypertens.* 2007 Nov;25(11):2345-51.

She QB, Mukherjee JJ, Chung T, Kiss Z. Placental alkaline phosphatase, insulin, and adenine nucleotides or adenosine synergistically promote long-term survival of serum-starved mouse embryo and human fetus fibroblasts. *Cell Signal.* 2000 Oct;12(9-10):659-65.

Shechter M, Shechter A.Magnesium and myocardial infarction. *Clin. Calcium.* 2005 Nov;15(11):111-5.

Shechter M.The role of magnesium as antithrombotic therapy. *Wien Med. Wochenschr.* 2000;150(15-16):343-7.

Shepherd J, Jones J, Frampton GK, Tanajewski L, Turner D, Price A. Intravenous magnesium sulphate and sotalol for prevention of atrial fibrillation after coronary artery bypass surgery: a systematic review and economic evaluation. *Health Technol. Assess.* 2008 Jun;12(28):iii-iv, ix-95.

Sheu JR, Hsiao G, Shen MY, Fong TH, Chen YW, Lin CH, Chou DS. Mechanisms involved in the antiplatelet activity of magnesium in human platelets. *Br. J. Haematol.* 2002 Dec;119(4):1033-41.

Shiekhattar R, Aston-Jones G. NMDA-receptor-mediated sensory responses of brain noradrenergic neurons are suppressed by in vivo concentrations of extracellular magnesium. *Synapse.* 1992 Feb;10(2):103-9.

Shiga T, Wajima Z, Inoue T, Ogawa R. Magnesium prophylaxis for arrhythmias after cardiac surgery: a meta-analysis of randomized controlled trials. *Am. J. Med.* 2004 Sep 1;117(5):325-33.

Shilov AM, Rabinovich J. G., M. V. Melnik, St. I. C., L. A. Maksimova, Sokolinskaya I.Yu. The deficit of magnesium and arterial hypertension, *Ros. Med. Zh.* -- 2000. 5. N 2. p. 62-65. [publication in Russian]

Shilov AM, Svyatov I.S., Chubarov MV, Sanodze I.D. Mg-containing drugs for the treatment and prevention of hyper- and dyslipidemies. *Clinical medicine.* - 1998. 76. N 4. p. 35-37. [publication in Russian]

Shkolnikova MA, Chuprova SN, Kalinin LA. Metabolism of magnesium and therapeutic value of its products. -- Moscow: ID *Medpraktika-M.* - 2002. – p. 28. [publication in Russian]

Simon DB, Lu Y, Choate KA, Velazquez H, Al-Sabban E, Praga M, Casari G et al.Paracellin-1, a renal tight junction protein required for paracellular Mg2+ resorption. *Science.* 1999 Jul 2;285(5424):103-6.

Singh RB, Dubnov G, Niaz MA, Ghosh S, Singh R, Rastogi SS, Manor O, Pella D, Berry EM. Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial. *Lancet.* 2002 Nov 9;360(9344):1455-61.

Singh RB, Niaz MA, Moshiri M, Zheng G, Zhu S. Magnesium status and risk of coronary artery disease in rural and urban populations with variable magnesium consumption. *Magnes Res.* 1997;10(3):205-213.

Singh RB, Rastogi SS, Mehta PJ, Cameron EA. Magnesium metabolism in essential hypertension. *Acta Cardiol.* 1989;44(4):313-22.

Skalniy AV, Ordzhonikidze ZG, Gromova OA. Dysmicroelementoses in sports medicine. Moscow, *Fizkul'tura i sport,* 2000, 150pp

Skurikhin IM, VA Tutelyan (eds). Chemical composition of Russian food: Directory /, M., print DELHI, 2002. [publication in Russian]

Skvortsov AA, Mareev VIu, Orlova IaA, Chelmakina SM, Baklanova NA, Belenkov IuN. Effect of long term therapy with spironolactone on parameters of 24-hour heart rhythm variability and ventricular arrhythmias in patients with heart failure receiving optimal therapy. *Kardiologiia.* 2008;48(2):52-64.

Smetnik VP, LB Butareva. Magne B6 correction of psycho-vegetative disorders in women with PMS. *Farmateka,* 2004, № 15, pp. 1-4. [publication in Russian]

Sobczak AJ.The effects of tobacco smoke on the homocysteine level-a risk factor of atherosclerosis. *Addict. Biol.* 2003 Jun;8(2):147-58.

Soldatovic D, Matovic V, Vujanovic D, Guiet-Bara A, Bara M, Durlach J. Metal pollutants and bioelements: retrospective of interactions between magnesium and toxic metals. *Magnes. Res.* 2002 Mar;15(1-2):67-72.

Sosa M, Bregni C. Metabolism of the calcium and bioavailability of the salts of most frequent use. *Boll. Chim. Farm.* 2003 Jan-Feb;142(1):28-33

Spatling L. Society of Magnesium Research Report on the status of the MagNET study, *J. Trace Elements and Electrolytes,* N3, 2004, P.200.

Spirichev VB, Kodentsova VM, Vrzhesinskaya OA Methods of assessing vitamin security of the population, / / handbook, RAMS Nutrition Research Institute of the Russian Federation, Moscow, - 2001, 68 pp. [publication in Russian]

Spirichev VB, Shatnyuk LN, Poznyakovsky VM Enrichment of food with vitamins and minerals. Science and technology. Siberian university publishing house. Novosibirsk. -- 2004, 407 pp. [publication in Russian]

Strizhakov A. N., I. V. Ignatko, N. T. Martirosyan The principles of comprehensive therapy threatened abortion in women with habitual nevynashivaniem. Principles of complex therapy of threatened abortion in women with habitual miscarriage. Methodologic outline for practitioners, Moscow, 2002. Suslikov VL.Geochemical ecology of human disease. In: *Atomovitoses,* V3, Moscow, Gelios ARV, pp-158, 2002. [publication in Russian]

Stühlinger HG, Kiss K, Smetana R. Significance of magnesium in cardiac arrhythmias. *Wien. Med. Wochenschr.* 2000;150(15-16):330-4.

Suter P.M. The effects of potassium, magnesium, calcium and fiber on risk of stroke. *Nutr. Rev.* - 1999 Mar, 57(3), p. 84 –88.

Svyatov I.S. Magnesium in the prevention and treatment of coronary heart disease and its complications. *Diss.* MD - 1999, - p. 214. [publication in Russian]

Swaminathan R. " Nutritional factors and osteoporosis." *Int. J. Clin. Pract.*, 1999, 53:540-548

Takaya J, Higashino H, Kobayashi Y.Intracellular magnesium and insulin resistance. *Magnes Res.* 2004 Jun;17(2):126-36.

Tam M, Gómez S, González-Gross M, Marcos A. Possible roles of magnesium on the immune system. *Eur. J. Clin. Nutr.* 2003 Oct;57(10):1193-7.

TEMA-12. Proceedings of the 12th International Symposium on Trace elements in Man and Animals. Ulster, Ireland, June 19-23, 2005.

Teo KK, Yusuf S, Collins R, Held PH, Peto R. Effects of intravenous magnesium in suspected acute myocardial infarction: overview of randomised trials. *BMJ.* 1991;303(6816):1499-1503.

Tercius AJ, Kluger J, Coleman CI, White CM. Intravenous magnesium sulfate enhances the ability of intravenous ibutilide to successfully convert atrial fibrillation or flutter. *Pacing Clin. Electrophysiol*. 2007;30(11):1331-1335.

Tetruashvili NK. Therapy magnesium in the early stages of pregnancy among patients with habitual miscarriage. *Ross. Vestn. Akusher. Gynecol.* 4, 2007 62-65.

Timio M, Saronio P, Venanzi S, Gentili S, Verdura C, Timio F.Blood pressure in nuns in a secluded order: A 30-year follow-up. *Miner Electrolyte Metab*. 1999 Jan-Apr;25(1-2):73-9.

Timio M, Verdecchia P, Ronconi M, Gentili S, Francucci B, Bichisao E. Blood pressure changes over 20 years in nuns in a secluded order. *J. Hypertens. Suppl.* 1985 Dec;3(3):S387-8.

Tishchenko AL The impact of alcohol on the exchange pyridoxine in patients with psoriasis and the problem of their treatment. *Vestn. dermatol. and venerol.* -- 1997. № 3. – pp 36-38. [publication in Russian]

Tishchenko AL, SM Haddad, Tishchenko LD The influence of solar radiation on pyridoxine levels in the blood in patients with certain dermatoses in Africa. *Vestn. dermatol. and venerol.* -- 1997. № 5. – pp 35-36. [publication in Russian]

Tishchenko LD Vitamins in dermatology. Moscow, Publishing House of UDN, 1987, p 93. [publication in Russian]

Toba Y., Kajita Y., Masuyama R., Takada Y., Suzuki K., Aoe S. Dietary magnesium supplementation affects bone metabolism and dynamic strength of bone in ovariectomized rats. *J. Nutr.* - 2000 Feb, 130 (2). - P. 216-220.

Todorovic T., Vujanovic D. The influence of magnesium on the activity of some enzymes (AST, ALT, ALP) and lead content in some tissues. *Magnes. Res.* 2002 Dec; 15(3-4): 173-7.

Torshin I. Yu, Gromova O.A. Molecular mechanisms of the magnesium action upon cardiovascular system. Kardiologiia, 2009. [publication in Russian] Tovar AR, Torres N, Halhali A, Bourges H.Riboflavin and pyridoxine status in a group of pregnant Mexican women. *Arch. Med. Res.* - 1996 Summer;27(2):195-200.

Torshin I.Yu. Bioinformatics in the post-genomic era: physiology and medicine. *Nova Biomedical Books,* NY, USA (2007), ISBN: 1600217524, pp35-67.

Torshin I.Yu. Sensing the change: from molecular genetics to personalized medicine. *Nova Biomedical Books,* NY, USA (2009, in press).

Torshin IY, Gromova OA, Gusev EI. Molecular mechanisms of the action of magnesium-pyridoxine preparations. *Zh. Neur. Psych. Im. Korsakova,* 2008, (in press). [publication in Russian]

Torshin IY, Gromova OA, Kosheleva NG. Magnesium-dependent placental proteins: a systematic analysis// *Vopr. Akusher Ginecolog,* 2008, (in press) [publication in Russian]

Torshin IY, Gromova OA. Molecular mechanisms of magnesium in the undifferentiated displasias of the connective tissue. *Russ. Med. Zh,* N2, 2008, P 263-269. [publication in Russian]

Torshin IY.Computed energetics of nucleotides in spatial ribozyme structures: an accurate identification of functional regions from structure. *Scientific World Journal.* 2004 Mar 26;4:228-47.

Tovar AR, Torres N, Halhali A, Bourges H.Riboflavin and pyridoxine status in a group of pregnant Mexican women. *Arch. Med. Res.* - 1996 Summer;27(2):195-200.

Trichopoulou A, Bamia C, Norat T, Overvad K, Schmidt EB, Tjønneland A et al. Modified Mediterranean diet and survival after myocardial infarction: the EPIC-Elderly study. *Eur. J. Epidemiol.* 2007;22(12):871-81.

Trichopoulou A, Orfanos P, Norat T, Bueno-de-Mesquita B, Ocké MC, Peeters PH, et al. Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. *BMJ.* 2005 Apr 30;330(7498):991.

Tsuji H, Venditti FJ Jr, Evans JC, Larson MG, Levy D. The associations of levels of serum potassium and magnesium with ventricular premature complexes (the Framingham Heart Study). *Am. J. Cardiol.* 1994;74(3):232-235.

Tsyganenko AJ, Zhukov VI, Myasoedov VV, Zavgorodny IV Clinical chemistry, 2002, Moscow, Triada-H (book in Russian).

Tu Q, Pi M, Quarles LD. Calcyclin mediates serum response element (SRE) activation by an osteoblastic extracellular cation-sensing mechanism. *J. Nutr.* – 2003, - Nov;133 (11): - P. 3625-3629.

Tutelyan VA and VB Spirichev, Sukhanov BP, Kudasheva VA Micronutrients in the diet of healthy and sick. M., *Kolos*, - 2002, 423 pp. [publication in Russian]

Ueshima K, Shibata M, Suzuki T, Endo S, Hiramori K.Extracellular matrix disturbances in acute myocardial infarction: relation between disease severity and matrix metalloproteinase-1, and effects of magnesium pretreatment on reperfusion injury. *Magnes Res.* 2003 Jun;16(2):120-6.

Valdes AM, Hassett G, Hart DJ, Spector TD. Radiographic progression of lumbar spine disc degeneration is influenced by variation at inflammatory genes: a candidate SNP association study in the Chingford cohort. *Spine*. 2005 Nov 1;30(21):2445-51.

van Dijk RA, Rauwerda JA, Steyn M, Twisk JW, Stehouwer CD.Long-term homocysteine-lowering treatment with folic acid plus pyridoxine is associated with decreased blood pressure but not with improved brachial artery endothelium-dependent vasodilation or carotid artery stiffness: a 2-year, randomized, placebo-controlled trial. *Arterioscler. Thromb. Vasc. Biol.* 2001 Dec;21(12):2072-9.PMID: 11742887

Vernadskiy VI. Research problems of biogeochemistry. Moscow, *Nauka,* 1934, pp11-49. [publication in Russian]

Vernadskiy VI. The living substance and the biosphere. Moscow*, Nauka,* 1994, 631pp. [publication in Russian]

Vormann J, Gunther T, Hollriegl V, Schumann K.Pathobiochemical effects of graded magnesium deficiency in rats. *Z. Ernahrungswiss*. 1998;37 Suppl 1:92-7.

Wang Z, Hu SY, Lei DL, Song WX.[Effect of chronic stress on PKA and P-CREB expression in hippocampus of rats and the antagonism of antidepressors]Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2006 Oct;31(5):767-71

Ware J.E., et al. SF-36 Health Survey: Manual and Interpretation Guide / MA: Boston, Nimrod Press. 1993

Wary C., Brillault Salvat C., Bloch G., Leroy Willig A., Roumenov D., Grognet J.M., Leclerc J.H., Carlier P.G. Effect of chronic magnesium supplementation on magnesium distribution in healthy volunteers evaluated by 31P-NMRS and ion selective electrodes. *Br. J. Clin. Pharmacol.* - 1999 Nov, 48 (5). - P. 655-662.

Wedig KE, Kogan J, Schorry EK, Whitsett JA. Skeletal demineralization and fractures caused by fetal magnesium toxicity. *J. Perinatol.* 2006 Jun;26(6):371-4.

Wei EK, Giovannucci E, Selhub J, Fuchs CS, Hankinson SE, Ma J. Plasma vitamin B6 and the risk of colorectal cancer and adenoma in women. *J. Natl. Cancer Inst.* 2005 May 4;97(9):684-92.

Wein 2003 Vegetative disorders. Clinic, diagnosis and treatment. Eds AM Veyna, Moscow, MIA, 2003 [publication in Russian]

Weisinger JR, Bellorín-Font E.Magnesium and phosphorus. *Lancet.* 1998 Aug 1;352(9125):391-6.

Weiss M. Magnesium in cardiology. Praxis (Bern 1994). 1995 May 2;84(18):526-32.

Woods KL, Abrams K. The importance of effect mechanism in the design and interpretation of clinical trials: the role of magnesium in acute myocardial infarction. *Prog. Cardiovasc. Dis.* 2002;44(4):267-274.

Woods KL, Fletcher S, Roffe C, Haider Y. Intravenous magnesium sulphate in suspected acute myocardial infarction: results of the second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2) *Lancet.* 1992 Jun 27;339(8809):1553-8.

Xia CW, Qiu Y, Sun X, Qiu XS, Wang SF, Zhu ZZ, Zhu F. [Vitamin D receptor gene polymorphisms in female adolescent idiopathic scoliosis patients]. Zhonghua Yi Xue Za Zhi. 2007 Jun 5;87(21):1465-9.

Yagodin BS The ring of life. Moscow, *INES,* - 2001 – p. 201. [publication in Russian]

Yang C.Y, Chiu H.F, Tsai S.S, Chang C.C, Chuang H.Y. Magnesium in drinking water and the risk of death from liver cancer. *Magnes. Res.* 2002 Dec; 15(3-4): 223-8.

Yang C.Y, Chiu H.F, Tsai S.S, Wu T.N, Chang C.C.Magnesium and calcium in drinking water and the risk of death from esophageal cancer. *Magnes. Res.* 2002 Dec; 15(3-4): 215-22.

Yang CY, Chang CC, Tsai SS, Chiu HF. Calcium and magnesium in drinking water and risk of death from acute myocardial infarction in Taiwan. *Environ. Res.* 2006 Jul;101(3):407-11.

Yavuz T, Yavuz O, Ozdemir I, Afsar Y. Cord blood lipoprotein profile after magnesium sulphate treatment in pre-eclamptic patients. *Acta Paediatr.* 2006 Oct;95(10):1224-7.

Yershov YA Chemistry nutrients, M., "High School", - 2000. -- 599 pp. [publication in Russian]

Young GL. *Cochrane Database Syst. Rev.* 2002 (1) D000121

Yuan J, Zhou J, Chen BC, Zhang X, Zhou HM, Du DF, Chang S, Chen ZK. Magnesium supplementation prevents chronic cyclosporine nephrotoxicity via adjusting nitric oxide synthase activity. *Transplant. Proc.* 2005 May;37(4):1892-5.

Yue H, Lee JD, Shimizu H, Uzui H, Mitsuke Y, Ueda T. Effects of magnesium on the production of extracellular matrix metalloproteinases in cultured rat vascular smooth muscle cells. *Atherosclerosis.* 2003 Feb;166(2):271-7.

Zaitseva SV, Varfalameeva S.D. The mechanism of regulation two-and trivalent metal ions bind to opioid receptors morphine, M., - 1993. [publication in Russian]

Zehender M, Meinertz T, Faber T, Caspary A, Jeron A, Bremm K, Just H. Antiarrhythmic effects of increasing the daily intake of magnesium and potassium in patients with frequent ventricular arrhythmias. Magnesium in Cardiac Arrhythmias (MAGICA) Investigators. *J. Am. Coll. Cardiol.* 1997 Apr;29(5):1028-34.

Zerwekh JE, Odvina CV, Wuermser LA, Pak CY. Reduction of renal stone risk by potassium-magnesium citrate during 5 weeks of bed rest. *J. Urol.* 2007 Jun;177(6):2179-84.

INDEX

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z