

Yoshiki Nishizawa  
Hirotohi Morii  
Jean Durlach *Editors*

# New Perspectives in Magnesium Research



Nutrition and Health



Springer

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Yoshiki Nishizawa, Hirotoishi Morii, and  
Jean Durlach, Eds.

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# **New Perspectives in Magnesium Research**

**Nutrition and Health**



**Springer**

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# Preface

## Magnesium: New Vistas

Eighteen years after the 5th International Magnesium Symposium was held in Kyoto in 1988, the Japanese Society for Magnesium Research (JSMR) is organizing the 11th International Magnesium Symposium in ISE-Osaka. The vitality of the Japanese Society has been evidenced by the regular publication of its journal (Journal of the Japanese Society of Magnesium Research), which ensures regular updating of current Japanese research on magnesium, and by its participation in the activities of the international Society for the Development of Research on Magnesium (SDRM).

Thanks to the efficient management of Yoshiki Nishizawa, Hirotooshi Morii, and Masaaki Inaba, the publication of this book of proceedings provides new vistas on magnesium research in 2006. It comprises reports presented during the plenary sessions concerning the role of magnesium not only in physiology, chronobiology, nutrition, epidemiology, and internal medicine particularly, cardiology, neurology, rheumatology, gynecology, and nephrology, but also in stomatology, pharmacognosy, and veterinary medicine.

Unlike many books that promise more than their texts deliver, the multiplicity and interest of the discussed topics can but stimulate careful reading of this book, which gives a platform to authors originating from many parts of the world. Published 35 years after the 1st International Magnesium Symposium, it demonstrates that magnesium research stands the test of time and that a wide path remains open for further investigation.

Such as it is, this book provides a valuable tool for interdisciplinary research in the future.

Jean Durlach  
September 2006

# Preface

New Perspectives in Magnesium Research is published as a document of the 11th International Magnesium Symposium, a joint meeting of Japanese Society for Magnesium Research. Professor Jean Durlach has been President of International Society for Magnesium Research since it was established in 1970. There are international meetings every 2 to 3 years in many parts of the world. The last meeting hosted by the Japanese Society for Magnesium Research was in Kyoto in 1988, when the Society President was Professor Yoshinori Itokawa.

Magnesium is the most abundant cation, second only to potassium in the intracellular compartment and to calcium in bone tissues. One of the big differences between calcium and magnesium is the difference between intra- and extracellular levels. Extra- and intracellular ratio is 10,000 for calcium, but 0.33 for magnesium. Such facts have significance for maintenance of life. Intracellular space rich in magnesium is just like seawater, adequate for the origin of life and maintenance of life in sea animals and plants. Bone magnesium constitutes a part of hydroxyapatite and may be important in maintaining bone integrity.

In physiological situations, magnesium is involved in metabolism of fat, amino acids, and sugar. Magnesium plays an important role in PPAR (peroxisome proliferators-activated receptor)-mediated signaling pathways as a key cofactor in the protein phosphorylation.

In bone and calcium metabolism, magnesium seems to play roles as a constituent of bone. Experimentally, magnesium deficiency induces osteoporosis but there have not been definite evidences of correlation between osteoporosis and magnesium. In calcium- and magnesium-deficient animals, calcium restores the bone quality but magnesium only partially. Another aspect of role of magnesium is the regulation of calcium metabolism through calcium-sensing receptor, which requires magnesium for its action. Another point is how magnesium participates in vitamin D action. Vitamin D is another important factor, other than calcium-sensing receptor, that requires magnesium in its action. The correlation between calcium-sensing receptor and vitamin D receptor has been discussed. Thus, magnesium plays some roles in the regulation of calcium and bone metabolism.

Clinically, diabetes mellitus, atherosclerosis, hypertension, cardiovascular diseases, and hyperlipidemia are in some other ways influenced by magnesium. More evidence is needed to have a definite conclusion how to manage clinical problems, problems of public health, and individual nutrition.

Hirotoishi Morii  
September 2006

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# Overview

# 1

## Overview of Magnesium Research: History and Current Trends

Jean Durlach

The lore of magnesium in medicine, starting as far back as the 17th century up to the first quarter of the 20th century, covers a large span of the chemical and pharmacological fields of knowledge.

The modern period, from 1926—when the essential character of magnesium was demonstrated—up to the 1960s, laid the basis for the present development of magnesium research by opening new vistas in the epidemiological and clinical fields where magnesium was acknowledged as being involved.

Several leading ideas deserve to be mentioned: (1) magnesium concerns all areas of medical activities; (2) the main expression of chronic marginal deficit is long-term chronic magnesium deficiency; (3) the specific etiological treatment of the responsible illness is the specific therapeutic measure meant to correct secondary magnesium deficit; (4) atoxic physiological palliative oral magnesium therapy is basically different from potentially noxious pharmacological use of magnesium; (5) the importance of distinguishing between magnesium deficiency and magnesium depletion in case of magnesium deficit; and (6) the interest of studying the chronopathological forms of magnesium deficit with hyper- or hypofunction of the biological clock.

This overview on past, present, and future bears witness to the vitality of magnesium research in health and disease.

Magnesium, the second most important intracellular cation, is found in all tissues and may affect many functions in the body. Its multiple physiological actions have been discovered thanks to the numerous convergent efforts of multinational research.

The aim of this study is to sum up the history of magnesium research and to highlight the current trends with a special stress on the leading ideas developed at the XIth International Magnesium Symposium (ISE, Japan).

This overview, which first considers the early history of the subject during the 18th and 19th centuries and first quarter of the 20th century, is mainly an account of the development of chemical and pharmacological knowledge. The modern history follows, which includes an initial period ending in the 1970s, when physiological, analytical, and epidemiological data established a firm

background for the first clinical studies in the neurological and cardiovascular fields particularly.

The present period is characterized by an exponential development of magnesium research, as will be testified by the subjects of the multiple sessions at the ISE Osaka Symposium.

## History<sup>1-7</sup>

One may consider the recognition by N. Grew, in 1695, of magnesium sulfate as one of the essential constituents of Epsom salts, as marking the entry of magnesium into medicine. N. Grew separated the solid salt in quantity from the bitter-tasting natural water of the Epsom spring. This latter was considered as an internal remedy and purifier of the blood and used by “a great store of citizens” and especially by “persons of quality,” including Marie de Medicis in the 16th century. Other important springs also contained magnesium sulfate and Epsom salts, or *sal anglicum*, synonymous with Sedlitz, or Egra powder, or salt, to designate the first preparation of magnesium sulfate used in medicine, mainly as a purgative. It was considered as a typical saline cathartic.

In 1707, M.B. Valentini of Giessen processed “magnesia alba” from the mother liquors obtained in the manufacture of nitre. This by-product of the preparation of nitre was considered as “a panacea for all bodily ailments,” but then *magnesia alba* and “calcareous earth” were confused. In 1755, J. Black of Edinburgh distinguished between *magnesia* and lime chemically.

In 1808, H. Davy of London isolated magnesium. Conducting his studies on alkali, that is, earth compounds, H. Davy succeeded in producing the amalgams of calcium, barium, strontium, and magnesium. He then isolated the metals by distilling off the mercury. As in the case of the alkali metals, he named these alkali-earth metals after their oxides: *baryta*, *strontia*, *chalk*, and *magnesia*, calling them barium, strontium, calcium, and *magnium*. *Magnium* has long been forgotten, however, and magnesium has been adopted by general usage for the element derived from *magnesia*.

In 1828, the French chemist A.A. Bussy, by reducing anhydrous magnesium chloride with potassium, prepared the metal in a state approximating purity.

In 1833, M. Faraday of London was the first to succeed in producing metallic magnesium by electrolysis of molten magnesium chloride. Electrolytic methods entirely superseded the older ones in the industrial production of magnesium until about 1941, when the carbothermic and ferrosilicon technique employing a thermal process came into use for a small proportion of magnesium production. In industry, magnesium was first used in photography and to make incendiary bombs; now it is in great demand for alloys and structural materials. Because of its lightness and abundance (2.1% of the earth's crust) “it has become the glamour metal of the space age.” Aluminium, the nearest rival for structural purposes, is one and a half times as heavy.<sup>1</sup>

Two Nobel prizes are linked to work concerning the important role of magnesium in organic chemistry. V. Grignard was awarded his in 1912 for the description of organomagnesium compounds. Grignard reagents are those with the composition  $RMgX$  (where  $R$  = an alkyl or aryl group and  $X$  = a halogen). These compounds are primarily important as intermediates in a large scale of synthesis, in biology in the conversion of aldehyde or ketones to alcohols, as well as in organic chemistry in the production of silicones. R. Willstätter was awarded the Nobel prize in 1915 for demonstrating that the structure of chlorophyll consisted of the porphyrin system, the central magnesium atom with its complex linkage and the phytol radical.

These discoveries laid a scientific basis for the use of magnesium in fertilizers and for assessing its importance in phytophysiology.

Around 1900, the essentiality of magnesium was established by studies concerning several algae and inferior fungi.

The same notion concerning animals was demonstrated only after a further quarter of century. However, one can cite as a predecessor J. Gaube Du Gers who, more than a century ago, noticed in mice that a diet deficient in magnesium, that is, composed of bread devoid of magnesium and of distilled water, but also no doubt deficient in other nutrients, caused progressive sterility. Magnesium already appeared to Gaube Du Gers as "the metal of vital activity for what is most precious in life: reproduction and sensation."<sup>1,3,7</sup>

The first modern biological studies<sup>1,2,4,6</sup> concerning the pharmacodynamic properties of parenteral magnesium were carried out by the French.

In 1869, F. Jolyet showed that intravenous injection of magnesium in dogs induced a peripheral paralyzing action similar to that of curare. Unfortunately, these pertinent observations were considered physiological.

Thirty years later, the same misconception, through the assimilation of pharmacological and physiological data, was repeated by the American groups of J. Loeb, who included magnesium among the denominators of his coefficient in which the principal humoral factors controlling neuromuscular excitability were grouped, and of J. Meltzer, who analyzed the sedative pharmacological properties of magnesium. The latter also observed that the effects of intravenous administration of a solution of a calcium salt rapidly reversed the effects of magnesium given parenterally. Correspondingly, parenteral magnesium sulphate was used to treat tetanus and eclamptic, nephritic and tetanic convulsions. In cases of respiratory depression through magnesium therapeutic overload, calcium was administered as an antidote to magnesium intoxication.

Although these observations of the pharmacodynamic effects of high doses of oral or parenteral magnesium may have some interest per se, one should nevertheless not extrapolate from these pharmacodynamic effects to physiological properties. Evidence of the latter can be demonstrated only by the occurrence of symptoms due to magnesium deficiency followed by its specific control by administration of nutritional physiological oral doses of magnesium. One should however, note, the predictive character of hypotheses presented more than a century ago, and subsequently verified, on the etiological

and physiopathological role of magnesium in certain tetanies, convulsions, and cardiovascular or toxic problems. We have cited here only carefully chosen examples, where modern studies have proved past extrapolations to be well founded, and not any others. The latter have, on the contrary, been the source of a number of unwarranted attributions of paternity as to the etiology of magnesium deficit. For this reason few ions have generated as much enthusiasm and as much disdain. Heated zealots, such as the French P. Delbet, have seen in magnesium a sort of panacea, the lack of which plays a major role in the development of cancer, the spread of epidemics, and even in the frequency of suicides. To the skeptics, on the other hand, magnesium remained a trace element of unclear biological physiological and pathological importance.

Between these two extremes it is today possible to find a balance.

## **The Early Modern Period**

Beginning with the seminal experiment of J. Leroy, who proved in 1926 the essential character of the ion in mice, this era ended in the 1960s. The physiological properties of magnesium were mainly revealed in the 1930s by the remarkable studies in the rat of the American groups of E.V. MacCollum and D.M. Greenberg, who showed the multiple effects of a lack of magnesium intake on development, reproduction, the neuromuscular apparatus, and humoral balance. The specific reversibility of such defects by oral loading of magnesium provided the experimental basis for the diagnostic test for magnesium deficiency by oral loading of nutritional physiological doses of magnesium.

These physiological data constituted the background for the first clinical observations establishing the importance of abnormalities in magnesium metabolism in the period between the 1930s and 1960s. These observations concerned acute syndromes, for example, in veterinary medicine according to the work of the Dutchman B. Sjollema in 1932 on grass tetany (in cows or ponies) and the tetany of milk-fed calves and human pathology, where Americans E.B. Flink and W.E.C. Wacker in particular led the way with reports of acute neurological manifestations of primary or secondary magnesium deficit due to alcoholism or endocrinometabolic disorders.

Epidemiological data introduced two major considerations. First, in the 1960s, the American M. Seelig, as well as J. Durlach in France, emphasized the fact that dietary magnesium in many regions appeared to be insufficient to meet daily needs. Indeed, it may be said that magnesium intake in developed countries is marginal. Previously, the Japanese J.A. Kobayashi (1957) and the American H.A. Schroeder (1958) stressed an inverse relation between total drinking water hardness and cardiovascular risk.

In 1955, the Australian A. Walsh reported the first application of atomic absorption spectrophotometry to the determination of magnesium. Henceforth magnesium could be measured with ease and accuracy.

## Current Trends<sup>2-8</sup>

The first international symposium on magnesium deficit in human pathology (1971) may be considered as evidence of the present importance of magnesium research in medicine: 350 participants from 52 countries contributed to the publication of two heavy volumes of proceedings corresponding to a synthesis of more than 25,000 references and which still offer a wide range of valuable knowledge.

The first International Symposium on Magnesium fostered the creation of the Society for the Development of Research on Magnesium (SDRM), an international coordinating structure.

The Society for the Development of Research on Magnesium promoted the publication of magnesium books: volume of proceedings<sup>2,3,5,11-13</sup> and monographs.<sup>6</sup>

The Society for the Development of Research on Magnesium organized the publication of diverse magnesium journals. First of all, *Magnesium Research*, the official organ of the international Society for the Development of Research on Magnesium (18 volumes), but also national journals such as the *Journal of the Japanese Society for Magnesium Research* (Japan), the *Buletin informatic al societatii romane de cercetare a magneziului* (Romania), and the *Journal of Elementology* (Poland).

Several leading ideas expressed in the course of the modern period of magnesium research deserve special mention:

- In the organism, all the systems and all the functions are involved. Therefore, magnesium research is not only relevant in certain fields but in all areas of medical activity.
- The main expression of primary magnesium deficit is closely linked with the consequences of long-term chronic marginal deficiency. Experimentally, it has been widely studied after the seminal report of O. Heroux in 1972. Clinical forms of chronic magnesium deficit are better and better identified, particularly in the neuromuscular system: for example, normocalcaemic latent tetany with or without mitral valve prolapse, and in the cardiovascular system, where the importance of magnesium deficit is recognized among cardiovascular risk factors.
- Specific therapeutic measures that seek to correct secondary magnesium deficit are justified only if the etiological treatment of the responsible illness is either impossible or ineffective. Such measures are then adjuvant treatments only justified by the ultimate importance of the secondary magnesium deficits in the physiopathology of the original illness.

Pharmacological effects of magnesium are observed irrespective of the magnesium status. They are established either in vitro, in situ, or in vivo when a parenteral or an oral intake is high enough to exceed any homeostatic capacity that may prevent magnesium overload. These basic differences between pharmacological and physiological magnesium actions lead us to discriminate

between the two types of magnesium load tests. Clinical efficiency of parenteral magnesium administration should not be used as a diagnostic tool attesting to magnesium deficiency. Conversely, physiological oral doses of magnesium are totally devoid of the pharmacodynamic effects of parenteral magnesium and without clinical effects when magnesium status is normal. Correction of symptoms by this oral magnesium load constitutes the best proof that they were due to magnesium deficiency.

But the main consequence of the differentiation between the pharmacological properties of magnesium is to allow us to distinguish between two different types of therapy with magnesium: atoxic physiological oral therapy and pharmacological magnesium therapy that may induce toxicity. Today the main form of magnesium therapy is oral magnesium physiological nutritional supplementation of magnesium deficiency. The palliative oral doses meant to balance magnesium deficiency are obviously devoid of any toxicity because their purpose is to restore to normal the insufficiency of the magnesium intake. In order to use the pharmacological properties of magnesium, no matter what the magnesium status is, it is necessary to go beyond the mechanisms of magnesium homeostasis to induce a therapeutic magnesium overload. Large doses of magnesium given orally are advisable when there are chronic indications and the parenteral route suitable for acute applications. Both types of pharmacological magnesium treatment are capable of inducing toxicity. It is a real scientific fraud and an ethical misconduct to fail to differentiate between the absent toxic consequences of a physiological magnesium supplementation and the effects of high pharmacological magnesium doses that are potentially dangerous.

In the case of magnesium deficit it is important to differentiate *magnesium deficiency*, where the disorder corresponds to a negative balance, and which merely requires an increased intake of magnesium as treatment, from *magnesium depletion*, where the disorder is related to a dysregulation of the control mechanisms of magnesium metabolism, and which requires appropriate correction. Modern research is concerned with the analysis of the neuro-endocrino-metabolic factors that control or disturb magnesium metabolism associated with genetic factors either linked with major leucocyte complex or studied as an etiopathogenic factor of various congenital hypomagnesemia.<sup>6,18</sup> The nervous forms of magnesium deficit may be considered as typical examples.

The nervous form of primary chronic magnesium deficiency represents the best documented experimental and clinical aspect of chronic magnesium deficit. This neurotic, neuromuscular, and autonomic nervous form induced by magnesium deficiency merely requires nutritional oral physiological magnesium supplementation.<sup>4,10,13</sup>

In the case of neurodegenerative diseases linked with various types of magnesium depletion, simple nutritional magnesium supplementation is inefficient. Magnesium depletion requires more or less specific correction of its causal dysregulation. Amyotrophic Lateral Sclerosis/Parkinsonism/Dementia complex (ALSPDC) may be considered as the type of a neurodegenerative



disease linked with magnesium depletion due to the sum of magnesium- (and calcium-) deficient intake plus slow neurotoxins (either inorganic, i.e., Al, or organic, particularly cycad neurotoxins). Cycad seeds can be directly eaten or indirectly through traditional feasting on flying foxes (with possible biomagnification of neurotoxins).<sup>6,12–15</sup>

Both in clinical forms and in animal experiments, the dysregulation mechanism of magnesium depletion associates a reduced magnesium intake with various types of stress. Among these are the biological clock dysrhythmias with two opposite groups: (1) with a biological clock hyperfunction and (2) with a biological clock hypofunction. These symmetrical physiopathologies lead to opposed therapies: phototherapy in cases of a biological clock hyperfunction and scototherapy in cases of a biological clock hypofunction.<sup>11,16–18</sup>

## Conclusion

Upon the initiative of SDRM and of Hirotoishi Morii, President of the Japanese Society for the Development of Research on Magnesium, the Eleventh International Magnesium Symposium will be held in ISE Osaka (October 24–28, 2006) under Yoshiki Nishizawa and Mieko Kimura. Once again, after the remarkable meeting organized 18 years ago in Kyoto (1988) by Yoshinori Itokawa, the Japanese branch of SDRM is organizing a meeting testifying to the exponential development of research on magnesium covering not only basic research (cellular and subcellular channels particularly), nutrition, epidemiology, and metabolic diseases, but also multifaceted clinical forms: cardiovascular, neuromuscular, psychiatric, rheumatologic, nephrologic, and even in dental and sport medicine.

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## 2

# Magnesium and Calcium in Drinking Water

Hirotooshi Morii, Ken-ichi Matsumoto, Ginji Endo, Mieko Kimura, and Yoshitomo Takaishi

While magnesium is one of the elements that supports life, many studies have been performed regarding physiological functions as well as correlation with diseases. Seelig and Rosanoff reviewed interesting data describing correlations between magnesium deficiency and disease.<sup>1</sup> Magnesium deficiency causes arrhythmia, overactivity to stress hormones (adrenalin), overproduction of cholesterol, blood clotting in blood vessels, constriction of blood vessels, high sodium–potassium ratio, insulin resistance, coronary atherosclerosis, and vulnerability to oxidative stress. Thus, Seelig and Rosanoff showed that magnesium content in hearts from cadavers of those who died of heart disease were much less than controls. Cadaver hearts from people who had lived in areas with hard drinking water had higher amounts of magnesium than cadaver hearts from soft-water areas. In 1957, Kobayashi indicated that the hardness of drinking water is related to the incidence of apoplexy.<sup>2</sup>

## Materials and Methods

Water samples were collected from various sources in the world: hotel aqueduct, river, spring, and other sources. Countries included the United States, France, Belgium, Turkey, Greece, Chili, Egypt, China, Korea, Mongol, Indonesia, and Japan. Mineral content was measured at Sakai Institute of Public Health, Sakai, Japan, and Takeda Research Institute Life Science and Preventive Medicine, Kyoto, Japan.

## Results

Calcium and magnesium as well as calcium/magnesium in drinking water showed considerably different levels in various parts of the world. Generally speaking, both calcium and magnesium levels are higher in Europe compared to other areas. Mineral content in Pamukkale in Turkey was the highest among sampled water. Contrexville in France had high levels of calcium and

magnesium. In Asian countries, some areas showed relatively higher levels of calcium and magnesium. Sang Sa Village has been known as a longevity village in Korea and has its own spring from which drinking water is obtained. Zhoukoudian area, located 50 kilometers southwest of Beijing, where Peking man (*Sinanthropus pekinensis*) was discovered, has wells nearby still being utilized by neighborhood populations; water from one had calcium and magnesium levels that were remarkably high. Marie Eugene Francois Thomas Dubois (1858–1940) discovered fossils of *Pithecanthropus* near Solo River in 1890. Drinking water was collected in this region and compared with that collected near Jakarta, Indonesia. Calcium and magnesium content in the former was higher compared with the latter. In Japan, calcium and magnesium content are not so high compared with other areas in the world, especially in comparison with those in Europe.

## Discussion

### *Cardiovascular Disease and Mineral Content*

Regarding the effect of both magnesium and calcium on blood pressure and incidence of cardiovascular disease, there have been many studies that are contradictory.<sup>1</sup> However, Seelig and Rosanoff showed that cardiovascular and overall rates were found to be lower in hard-water areas than in soft-water areas. Deaths rates from coronary heart disease are approximately 300 per 1,000,000 people in Lincoln, Nebraska, where water hardness level was 147 ppm, a little more than 600 in Washington, DC, where water hardness level was 96 ppm, and more than 800 in Savannah, Georgia, where water hardness level was 41 ppm.

Recent statistics in the United States (Table 2.1)<sup>3</sup> indicate that areas with high incidence of total death (as well as cardiovascular deaths) are located in the southeastern part of the country, including approximately 10 states. Among the top 10 areas with high mortality rates for both total deaths and cardiovascular deaths, six areas are included for both causes of death: Mississippi, the District of Columbia, Kentucky, Alabama, Tennessee, and Oklahoma (Table 2.1). In the state of Tennessee, the water hardness is not so high in the present study.

Water hardness in France seems to be high compared with other countries outside Europe. The idea of a French paradox has been proposed for the low incidence of cardiovascular deaths in France compared with some neighboring countries in Europe.<sup>4</sup> One of the contributing factors was ascribed to the high consumption of polyphenol supplied from red wine, but the water hardness could be another factor. Marque collected information about all deaths of 14,311 individuals in 69 parishes of southwest France from 1990–1996. A significant relationship was observed between calcium and cardiovascular mortality with relative risk (RR) = 0.90 for noncerebrovascular causes and

**TABLE 2.1.** Number of total and heart disease deaths in the United States in 2002.<sup>3</sup>

Rank	Total deaths		Heart disease deaths	
1		Mississippi	Mississippi	
2	1037		326.9	
3		District of Columbia	Oklahoma	
4	1027.4		307.1	
5		Louisiana	District of Columbia	293.5
6	1001.1		Kentucky	
7		Kentucky	288.9	
8	1000.6		West Virginia	
9		Alabama	288.0	
10	999.9		Alabama	
11		West Virginia	286.3	
12	991.7		Tennessee	
13		Tennessee	283.7	
14	985.5		Arkansas	
15		Oklahoma	279.5	
16	976.2		New York	
17		Arkansas	277.6	
18	966.9		Missouri	
19		Georgia	270.6	
20	953.4		Louisiana	
21		South Carolina	269.6	
22	949.5		Michigan	
23		Nevada	265.7	
24	919.1		Georgia	
25		Missouri	263.0	
26	917.1		Ohio	
27		Ohio	258.0	
28	907.8		Texas	
29		North Carolina	252.7	
30	907.5		Pennsylvania	250.6
31		Indiana	Indiana	248.0
32	919.1		Illinois	246.4
33		Texas	Nevada	246
34	878.4		South Carolina	244.7
35		Michigan	New Jersey	244.4
36	875.8		Rhode Island	240.3
37		Wyoming	Maryland	238.7
38	868.0		Delaware	236.7
39		Pennsylvania	North Carolina	235.6
40	863.5		Virginia	226.6
41		Maryland	California	225.9
42	863.5		Florida	222.3
43		Illinois	Kansas	221.0
44	855.8		New Hampshire	220.1
45		Virginia	Iowa	
46	855.6		219.1	
47		Maine	Connecticut	216.9
48	848.3		Wisconsin	216.4
49		Montana	Nebraska	213.5
50	848.3		Wyoming	211.3
51		Kansas	South Dakota	209.9
	845.1		Maine	209.0

TABLE 2.1. *Continued*

Rank	Total deaths	Heart disease deaths	
	Delaware	Vermont	208.2
840.9		Arizona	204.2
	Oregon	Massachusetts	202.3
832.6		Idaho	200.9
	New Mexico	North Dakota	200.8
817.7		New Mexico	194.1
	Nebraska	Washington	193.1
814.6		Oregon	191.9
	Rhode Island	Montana	190.6
813.3		Hawaii	187.7
	New Jersey	Utah	186.0
811.2		Colorado	180.3
	Arizona	Alaska	167.0
800.5		Minnesota	163.9
	Wisconsin		
799.3			
	Massachusetts		
796.1			
	Colorado		
795.7			
	Alaska		
794.1			
	Florida		
787.8			
	Utah		
785.8			
	Idaho		
785.7			
	Washington		
784.4			
	New York		
783.3			
	New Hampshire		
781.1			
	Vermont		
774.2			
	Iowa		
773.3			
	South Dakota		
771.7			
	Conneticut		
760.3			
	California		
758.1			
	North Dakota		
748.3			
	Minnesota		
743.8			
	Hawaii		
659.6			

RR = 0.86 for cerebrovascular deaths when calcium is higher than 94 mg/L. There was a protective effect of magnesium between 4 and 11 mg/L with RR = 0.92 for noncerebrovascular and RR = 0.77 for cerebrovascular mortality at concentration lower than 4 mg/L.<sup>5</sup>

Cardiovascular deaths in Japan are much less compared with those in western countries: 85.8 in males and 48.5 in females per 100,000 people in 2000, although the rank is the second next to neoplastic diseases.<sup>6</sup> Hardness of water is less in most of the areas in Japan compared with countries in Europe and North America (Table 2.2). But there are still differences in mineral content among areas in Japan. One of areas in Kyushu Island showed the higher level of calcium and magnesium compared to other areas (Table 2.2). This area belongs to Miyazaki Prefecture, where the cardiovascular death rate was shown to be lower than average. There are so many factors other than minerals that effect the incidence of cardiovascular death.

### ***Mineral Intake of Prehistoric Man***

Mineral content in the well of Zhoukoudian area, where the Peking man fossils were discovered, showed very high levels compared with those in Beijing City water and in the Yong Ding Hu river (Table 2.2). In Indonesia, calcium and magnesium content in natural water were not so high. However, in nearby Solo River, near where Java man was discovered 1859 by Dubois, mineral content was higher than in another area near Jakarta (Table 2.2).

Mineral intake will be influenced by mineral content in drinking water, contributing to the requirement of minerals. Total amount of minerals ingested from drinking water not only supplement the dietary allowances, but also modulates mineral metabolism by affecting various factors depending on the concentration of minerals. Meunier compared serum parathyroid hormone and biochemical markers of bone remodeling between females ingesting high- (596 mg/L) and low- (10 mg/L) calcium drinking water. One hundred and eighty healthy postmenopausal women with mean age of  $70.1 \pm 4.0$  years and with daily average intake of calcium less than 700 mg were studied, and 152 completed the 6-month trial. There was a significant 14.1% decrease of PTH, osteocalcin (−8.6%), bone alkaline phosphatase (−11.5%), serum (−16.3%), and urine (−13.0%) type-1 collagen C-telopeptide in those who ingested high-calcium drinking water compared with control group. The additive effect of vitamin D supplement at a dose of 400 IU was not significant.<sup>7</sup> Regarding the effect of magnesium, Marie demonstrated that in weanling male mice, magnesium supplementation in drinking water increased serum and urinary magnesium concentrations and bone magnesium content and that both calcification rate and the extent of tetracycline double-labeled osteoid surface increased progressively in magnesium-treated mice.<sup>8</sup>

These data indicate that calcium and magnesium content in drinking water, as well as in diet, influence calcium and bone metabolism, thus helping to adjust individuals to the environment depending on the quantity of minerals

**TABLE 2.2.** Calcium, magnesium, and calcium/magnesium in drinking water in some parts of the world.

Continent	Country	Area	Measured minerals (mg/L)		
			Calcium	Magnesium	Ca/Mg
Europe	Turkey	Pamukkale	427	108	3.95
	Greece	Athens city	54.9	9.9	5.55
		Kos Island	131.1	18.5	7.09
		ITI mountain	64.4	24.2	2.70
	Belgium	Mountain natural	5.3	1.90	2.79
	France	Lourdes Fountain	77.9	5.20	14.98
		Contexeville	486	84	6.14
	Norway	North pole sea	309.5	1200	0.26
The Americas	United States	Tennessee River	28.9	6.71	4.31
		Nashville city	30.1	6.70	4.49
	Chili	Santiago city	53.4	8.7	6.14
Oceania	Australia	Sydney city	11.6	4.9	2.37
		Sydney suburb	nd	nd	—
Asia	Korea	Kwang-ju city	38.6	6.0	6.43
		Sang Sa village	53.5	7.0	7.64
	China	Zhoukoudian well	97.1	19.8	4.90
		Town near Beijing	62.1	17.4	3.57
		Beijing city	60.5	20.4	2.97
		Yong Ding River near Beijing	28.9	14.5	1.99
	Indonesia	Mountain near Jakarta	7.0	2.2	3.18
		Wonogiri Mountain near Solo River	27.3	7.1	3.85
	Mongol	Well in the plain	40.9	7.20	5.68
		Ulaanbaartar City	15.1	2.25	6.71
		Tur River	nd	0.01	—
	Japan	Kyoto City w (Biwa Lake)	13.7	2.64	5.19
		Niigata Mountain area	2.1	0.97	2.16
		R river in Osaka	10.0	1.64	6.10
		Kirishima Mountain	42.0	9.70	4.33

Abbreviation: nd, not determined.

ingested. Prehistoric man may have needed strong physical activity to have better access to foods and protection from enemies. Guillemant studied the effect of exercise on calcium and bone metabolism in 12 well-trained elite male triathletes with and without ingesting high-calcium mineral water. When exercise was performed without calcium load, both serum concentrations and total amount of crosslinking telopeptide of type-1 collagen (CTX) began to increase progressively 30 min after the start of the exercise and were still significantly elevated, by 45% to 50%, 2 h after the end of the exercise. Ingestion of high-calcium mineral water completely suppressed the CTX response.<sup>9</sup> Thus, high-mineral drinking water and foods may have had beneficial effects in prehistoric men.



Kaifu showed the difference in mandibular structure between inhabitants of the Jomon period (ca. 10,000 BCE–300 BCE hunter/gatherer/fishers) and those in Yayoi period (ca. 300 BCE–300 CE agriculturists). The underdevelopment of major masticatory muscle attachment in the former populations may have been caused by diminished chewing stress.<sup>10</sup>

## Summary and Conclusion

Magnesium and calcium in drinking water were measured in various sources in the world. There were considerable differences in the mineral content in drinking water. In Europe, both minerals showed higher levels compared to other areas in the world. There have been some studies indicating the importance of magnesium content in drinking water. But there are other important other constituents in drinking water, as well as other risk and protective factors for cardiovascular diseases that should be taken into account to explain the cause of cardiovascular death.<sup>11</sup> The lifestyle of prehistoric man is another aspect of interest from the standpoint of the role of minerals in cardiovascular disease.

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# **Magnesium Homeostasis**

# 3

## Cellular $\text{Mg}^{2+}$ Transport and Homeostasis: An Overview

Martin Kolisek, Rudolf J. Schweyen, and Monika Schweigel

Magnesium plays a vital role as a cofactor for many enzymes, as a binding partner of nucleotides, and in stabilizing nucleic acids and membranes. It acts as a modulator of ion channels, and it affects many other cellular processes such as neuromuscular excitability, secretion of hormones, and it antagonizes the actions of  $\text{Ca}^{2+}$ , to name a few effects.<sup>1-4</sup>  $\text{Mg}^{2+}$  deficiency was found to be associated with hypertension, ischemic heart disease, inflammation, eclampsia, diabetes, cystic fibrosis, and in the establishment of human immunodeficiency virus 1 (HIV-1) reservoirs.<sup>5-10</sup> Several disease phenotypes have been shown to be due to inherited disorders of  $\text{Mg}^{2+}$  homeostasis.<sup>11-15</sup> Therefore, the regulation of extracellular and intracellular magnesium levels by transmembrane and transepithelial transport processes is critical for numerous cellular and organ functions.

Free cytosolic  $[\text{Mg}^{2+}]$  is maintained between 0.3 to 1.2 mM, with the higher levels in excitable cells. Yet the extracellular  $\text{Mg}^{2+}$  concentration can vary considerably and the inside negative membrane potential of most cells constitutes a strong driving force for influx of the ion. This suggests a low membrane permeability to  $\text{Mg}^{2+}$  in the resting state and the presence of transport proteins allowing regulated uptake and extrusion of  $\text{Mg}^{2+}$ . Although studies of  $\text{Mg}^{2+}$  fluxes in mammalian cells and in microorganisms have indicated the presence of functionally active plasma membrane  $\text{Mg}^{2+}$  transport mechanisms, most pathways lack molecular identification and/or elucidation of the mechanisms regulating transporter expression and activity.

### Mechanisms of $\text{Mg}^{2+}$ Influx

The inside negative membrane potential is thought to serve as a driving force for cellular uptake of  $\text{Mg}^{2+}$ , either mediated by ion channels or by carriers. Whether there exist such transport pathways has been unclear until recently.  $\text{Mg}^{2+}$  uptake in some gastrointestinal and renal epithelial cells,<sup>16,17</sup> in cardiac myocytes,<sup>18</sup> and human platelets<sup>19</sup> have been observed to be influenced by the transmembrane voltage and/or by ion channel blockers. These findings are

consistent with channel-mediated  $\text{Mg}^{2+}$  influx in these cells, but the applied methods, such as fluorescence spectroscopic measurements of  $[\text{Mg}^{2+}]_i$  by the aid of Magfura-2 and/or  $^{28}\text{Mg}$  uptake measurements, are not the methods of choice unequivocally to establish the presence of channels. In some cellular models,  $\text{Mg}^{2+}$  uptake has also been shown to involve  $\text{Mg}^{2+}/\text{Cl}^-$  or  $\text{Mg}^{2+}/\text{HCO}_3^-$  cotransport.<sup>20–22</sup>

Progress towards the identification and function of plasma membrane  $\text{Mg}^{2+}$  channels comes from genetic studies both in microorganisms and in mammalia. Maguire and coworkers have characterized various proteins to be involved in bacterial  $\text{Mg}^{2+}$  transport.<sup>23</sup> Among them, the CorA protein has been recognized as the major, constitutively expressed  $\text{Mg}^{2+}$  transport protein in eubacteria as well as in archaea. Distant homologues of the CorA protein have been shown to occur in the plasma membrane of lower eukaryotes and plants and in mitochondria of yeast, plants, and mammalia, where they constitute the major  $\text{Mg}^{2+}$  uptake channels (cf. Chapter 5 of this book). Mammalian cells lack proteins of the CorA superfamily in their plasma membrane. However, recent genetic studies identified two novel ion channels of the melastatin-related, transient receptor potential (TRPM) ion channel subfamily, TRPM6 and TRPM7, as being essential for  $\text{Mg}^{2+}$  uptake and homeostasis.<sup>24–28</sup> Additional candidate genes for  $\text{Mg}^{2+}$  transport proteins were identified by Goytain and Quamme<sup>29–32</sup> based on a screen for genes showing  $\text{Mg}^{2+}$ -regulated expression in renal epithelial cells. Two of these putative  $\text{Mg}^{2+}$  transporters belong to the solute carrier transporter superfamily (SLC) and are termed SLC41 member a1 (SLC41a1) and SLC41 member a2 (SLC41a2). Another putative transporter, named ACDP2, belongs to the family of ancient conserved domain proteins (ACDP). MagT1, finally, represents a member of a novel protein family without similarity to any other transporter protein family known to date. Table 3.1 gives an overview of identified and putative  $\text{Mg}^{2+}$  channels in mammalian cells.

### ***TRPM6 and TRPM7 $\text{Mg}^{2+}$ Channel Proteins of the Plasma Membrane***

Patch-clamp studies described TRPM7 as a constitutively active ion channel with a specific permeation profile of ( $\text{Zn}^{2+} \approx \text{Ni}^{2+} \gg \text{Ba}^{2+} > \text{Co}^{2+} > \text{Mg}^{2+} \geq \text{Mn}^{2+} \geq \text{Sr}^{2+} \geq \text{Cd}^{2+} \geq \text{Ca}^{2+}$ ). Native TRPM7-mediated currents were designated as magnesium-nucleotide-regulated metal ion currents (MagNum). Under standard physiological conditions, it is a primarily  $\text{Mg}^{2+}$ -conducting ion channel that is regulated by  $[\text{Mg}^{2+}]_i$  and/or intracellular magnesium-nucleotide complexes. At potentials where divalent ions do not experience sufficient driving force to enter the cell and therefore no longer impede monovalent outward fluxes, TRPM7 channel mediates  $\text{Na}^+$  and  $\text{K}^+$  efflux.<sup>24,27,33</sup>

TRPM7 and TRPM6 share similar molecular characteristics, such as six transmembrane (TM) domains participating on channel formation and a

**TABLE 3.1.** Overview of known or putative mammalian Mg<sup>2+</sup> channels and transporters.

Gene	Protein superfamily	Protein family	TM	Cellular localization	Similarity	Channel	Chanzyme	Influx / efflux
Trpm7	TRP	TRPM (8) <sup>a</sup>	6	PM (EC)		Yes	Yes	I
Trpm6	TRP	TRPM (8) <sup>a</sup>	6	PM (EC)		Yes	Yes	I
Slc41a1	SLC	SLC41 (3) <sup>a</sup>	10	PM (EC)	MgtE (p)	??	No	I / E?
Slc41a2	SLC	SLC41 (3) <sup>a</sup>	10	PM ???	MgtE (p)	??	No	I / E?
MagT1		MagT (2) <sup>a</sup>	5	PM ???		??	No	I / E?
Acdp2		ACDP (4) <sup>a</sup>	4	PM ???	CorC (p)	??	No	I / E?
hMrs2	CorA-Mrs2-Alr1	MRS2 (2) <sup>a</sup>	2	IMM (EC)	CorA (p)	Yes	No	I

Abbreviations: ?, unknown; ??, speculative experimental evidence; E, predominantly efflux; EC, experimentally confirmed; I, predominantly influx; IMM, inner mitochondrial membrane; p, prokaryotic; PM, cytoplasmic membrane; PU, putative; TM, transmembrane domain.

<sup>a</sup>Numbers of family members are given in parentheses.

C-terminal functional kinase domain. TRPM6 and TRPM7 proteins (together with TRPM2 protein) in fact represent unique natural chimeras between a channel-forming polypeptide and an enzyme. To emphasize uniqueness of this kind of proteins they were named *chanzymes*.<sup>34</sup> Schmitz and colleagues<sup>27,35</sup> showed that the TRPM7 kinase domain is not essentially required for TRPM7 activation, but functional coupling between kinase and channel exist such that structural alterations of the kinase modify the sensitivity of channel activation to Mg<sup>2+</sup>. Direct phosphorylation of the channel by its kinase was proposed being unlikely.

Both TRPM6 and TRPM7 have overlapping expression profiles that was confirmed by real-time polymerase chain reaction (RT-PCR) and Northern blot analyses.<sup>36,37</sup> TRPM7 is able to form a functional homooligomeric complex as well as heterooligomers together with TRPM6. To date, it has not been shown that TRPM6 is able to form homooligomeric complexes. Chubanov and colleagues<sup>36</sup> elegantly demonstrated that TRPM6 without TRPM7 is unable to target the membrane, thus it is retained intracellularly.

The naturally occurring TRPM6 missense mutation S141L was identified as associated with hypomagnesemia with secondary hypocalcemia (HSH).<sup>25,26</sup> This mutation prevents interaction of TRPM6 with TRPM7 and thus TRPM6/TRPM7 oligomerization. Hermosura and coworkers<sup>14</sup> identified a TRPM7 variant containing a missense mutation (T14821) in DNA of a group of individuals suffering from guamanian lathral sclerosis (ALS-G) and parkinsonism dementia (PD-G). For more details, see Chapter 4 in this book.

## Novel Candidate $\text{Mg}^{2+}$ Transport Proteins in Mammalia

### *MagT1*

Human as well as mouse *MagT1* genes are X-chromosome-linked and encode proteins with a relative molecular mass of 38 kDa with five putative TM domains.<sup>29</sup> *MagT1* expression was found to be high in kidney, colon, liver, and heart, while weak in the intestine, brain, spleen, and lung.  $\text{Mg}^{2+}$  starvation enhances *MagT1* expression in epithelium of the renal distal tubule in mice when compared to animals fed with normal diet. When expressed in oocytes of *Xenopus laevis*, *MagT1* creates currents that are induced by elevated  $[\text{Mg}^{2+}]_{\text{ext}}$ , but not by elevated concentrations of other divalent cations. Goytain and Quamme<sup>29</sup> excluded direct coupling of  $\text{Mg}^{2+}$ -evoked currents to  $\text{H}^+$  movement but claimed their sensitivity to external pH with maximum currents at pH 7.4. *MagT1*-induced currents were inhibited by  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Mn}^{2+}$ , as well as some other cations at high external concentrations. *MagT1*-mediated  $\text{Mg}^{2+}$  uptake was linear for at least 20 min and did not display any time-dependent decay during repetitive stimulation with voltage steps. From these data, the authors concluded that *MagT1* is a voltage-dependent magnesium transporter with channel-like properties. The authors state that many characteristics of *MagT1* expressed in oocytes parallel physiological observations with kidney distal convoluted tubule cells.<sup>29</sup> It remains to be shown in mammalian cells in which membrane the *MagT1* protein is localized and what its contribution to  $\text{Mg}^{2+}$  in the respective compartment might be.

### *ACDP2*

The ACDP (ancient conserved domain protein) gene family encodes large proteins with four predicted TM domains. Mouse and human genomes contain four genes (*Acdp1*, *Acdp2*, *Acdp3*, and *Acdp4*) with deduced proteins of 951, 874, 713, and 771 amino acids, respectively. ACDP sequence motifs are highly conserved in various taxonomic entities.<sup>38</sup> Among these homologues is the bacterial CorC protein that had previously been implicated in  $\text{Mg}^{2+}$  and  $\text{Co}^{2+}$  transport<sup>39</sup> and the yeast MAM3 gene product involved in vacuolar  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Zn}^{2+}$  homeostasis.<sup>40</sup> Expression of these ACDP proteins in mouse varied from tissue to tissue, but was generally strong in kidney. Low  $\text{Mg}^{2+}$  diet led to a increase of ACDP2 expression relative to normal diet in mouse kidney. A similar observation was made when MDCT epithelial cells were cultivated in low- $\text{Mg}^{2+}$  medium.<sup>30,38</sup> Upon expression of mouse ACDP2 in oocytes of *Xenopus laevis*, Goytain and Quamme<sup>30</sup> attributed saturable transport of  $\text{Mg}^{2+}$  and several other cations ( $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Fe}^{2+}$ ).  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ni}^{2+}$  did not induce currents, but  $\text{Zn}^{2+}$  inhibited  $\text{Mg}^{2+}$  permeation significantly. These and further results suggested that ACDP2 may constitute a regulated transporter of  $\text{Mg}^{2+}$  and other divalent cations.<sup>30</sup>

## SLC41A1

Wabbaken and colleagues<sup>41</sup> identified the human gene *Slc41A1* as encoding a protein with a predicted mass of 56 kDa. Based on computer prediction, SLC41A1 contains 10 TM domains and is localized in the cytoplasmic membrane (Kolisek et al., unpublished data). It has significant sequence homology to the bacterial MgtE, which had previously been shown to be involved in  $Mg^{2+}$  transport.<sup>42</sup> In human and mouse *Slc41a1*, transcripts are detected in most tissues (notably in heart, muscle, testis, thyroid, kidney) and low  $Mg^{2+}$  diet in mice led to significant increase of *Slc41a1* gene expression in the kidney, colon, and heart.<sup>31,41</sup> The TEV clamp of *Xenopus* oocytes heterologously expressing SLC41A1 revealed  $Mg^{2+}$ -evoked currents with a large amplitude of  $1.0 \mu M$ . Uptake was linear for at least 20 min and did not display a time-dependent decay during repetitive stimulation with voltage steps. Steady-state  $Mg^{2+}$  currents were saturable. Substitution of external NaCl with choline  $Cl^-$  had no effect on SLC41A1 related  $Mg^{2+}$  currents. Similarly, substitution of external NaCl and  $MgCl_2$  with appropriate amounts of  $Na^+$  gluconate and  $MgSO_4$  did not alter SLC41A1-mediated  $Mg^{2+}$  conductance. It was concluded that transport neither depends on extracellular  $Na^+$  or  $Cl^-$ , nor is it influenced by these electrolytes.<sup>31</sup> A number of other divalent cations was reported to be conducted by SLC41A1, namely  $Sr^{2+}$ ,  $Fe^{2+}$ ,  $Ba^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ , and  $Co^{2+}$ , but neither  $Ni^{2+}$  nor  $Ca^{2+}$  or  $Gd^{3+}$ .  $Gd^{3+}$  and  $La^{3+}$  were identified as antagonists of  $Mg^{2+}$ -induced SLC41A1 currents. SLC41A1 thus may constitute another transporter of  $Mg^{2+}$  and other divalent cations.

Taken together, the identification of SLC41, ACDP2, and MagT1 proteins is a major step forward in the characterization of mammalian  $Mg^{2+}$  transport proteins. While their role as electrogenic ion transporters rests on a single series of experiments in a heterologous system only, their function in cation homeostasis is supported by their mode of expression in mammalia and, as far as SLC41 and ACDP2 are concerned, by studies on the roles of their homologues in ion homeostasis in microorganisms. Future ion transport studies only will reveal the capacities of these proteins in cation transport, their location in plasma membrane or an intracellular membrane, and the contribution of these proteins to  $Mg^{2+}$  homeostasis of various mammalian cell types and organs.

## Mechanisms of $Mg^{2+}$ Extrusion

Extrusion of  $Mg^{2+}$  must occur against a steep electrochemical gradient. Because no evidence exists that  $Mg^{2+}$  is extruded from the cell via an outwardly oriented  $Mg^{2+}$ -adenosine triphosphatase (ATPase), other driving forces, such as the  $Na^+$  (mammalian) or  $H^+$  (microorganisms) gradient, must be used to transport the ion by secondary active transport mechanisms. In accordance with



this, a  $\text{Na}^+/\text{Mg}^{2+}$  exchanger, whose presence and characteristics have been characterized mainly by functional studies, seems to be an important efflux system in many mammalian cells. The  $\text{Na}^+/\text{Mg}^{2+}$  exchanger is also a candidate mechanism for the extrusion of  $\text{Mg}^{2+}$  across the basolateral membrane of the  $\text{Mg}^{2+}$  absorbing and reabsorbing intestinal and renal epithelia.<sup>43,44</sup> Although some authors suggest that  $\text{Mg}^{2+}$  extrusion also occurs through the  $\text{Na}^+/\text{Ca}^{2+}$  antiporter,<sup>45</sup> through a  $\text{Mg}^{2+}/\text{Ca}^{2+}$  exchanger,<sup>46</sup> or through anion-dependent mechanisms,<sup>47</sup> we will concentrate here on the proposed  $\text{Na}^+/\text{Mg}^{2+}$  antiport which is best characterized  $\text{Mg}^{2+}$  transporter of nonepithelial cells and tissues.

### ***$\text{Na}^+/\text{Mg}^{2+}$ Exchange***

$\text{Na}^+/\text{Mg}^{2+}$  exchange was first described by Günther and Vormann in chicken and turkey red blood cells,<sup>48</sup> and they and others studied the  $\text{Na}^+$ -dependent  $\text{Mg}^{2+}$  transport in numerous vertebrate erythrocytes.<sup>49–51</sup> Over the years, the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger has been shown to operate in the majority of examined cells pointing to an ubiquitous expression.<sup>52–57</sup>

With physiological transmembrane  $\text{Na}^+$  ( $[\text{Na}^+]_i < [\text{Na}^+]_e$ ) and  $\text{Mg}^{2+}$  ( $[\text{Mg}^{2+}]_i \leq [\text{Mg}^{2+}]_e$ ) gradients the  $\text{Na}^+/\text{Mg}^{2+}$  antiport mediates efflux of intracellular  $\text{Mg}^{2+}$  in exchange for extracellular  $\text{Na}^+$  ions. The driving force for this exchange is the inwardly directed concentration gradient of  $\text{Na}^+$ , making the transporter indirectly dependent on the  $\text{Na}^+/\text{K}^+$  pump and thereby on adenosine triphosphate (ATP).<sup>19,58</sup> The stoichiometry of the exchange seems to vary between cell types and possibly according to the functional state, but in most cases an electroneutral ratio of 2  $\text{Na}^+$  to 1  $\text{Mg}^{2+}$  has been reported.<sup>48</sup> In some cell systems, the  $\text{Mg}^{2+}$  efflux via  $\text{Na}^+/\text{Mg}^{2+}$  antiport can be switched to  $\text{Mg}^{2+}$  influx by reversing the  $\text{Na}^+$  gradient.<sup>59</sup> Until now, the physiological meaning of a reverse  $\text{Na}^+/\text{Mg}^{2+}$  is not clear.

A characteristic feature of  $\text{Mg}^{2+}$  extrusion via the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger is its strict dependence on extracellular  $\text{Na}^+$ .<sup>52,56</sup> Substitution of extracellular  $\text{Na}^+$  by  $\text{K}^+$ ,  $\text{Li}^+$ , or choline<sup>+</sup> can not support  $\text{Mg}^{2+}$  efflux.<sup>46,60</sup> As expected for a carrier system,  $\text{Mg}^{2+}$  efflux is saturable and its activation by  $[\text{Na}^+]_e$  follows a Michael–Menten relationship with  $K_m$  values between 11 to 30 mM.<sup>61,62</sup>

It is long known that a measurable  $\text{Mg}^{2+}$  efflux took place only when  $[\text{Mg}^{2+}]_i$  was increased and that it stopped when the normal  $\text{Mg}^{2+}$  content is achieved,<sup>60</sup> showing that the rate of  $\text{Na}^+/\text{Mg}^{2+}$  antiport is dependent on the  $[\text{Mg}^{2+}]_i$ .<sup>49</sup> Günther<sup>63</sup> suggested that the transporter performs  $\text{Mg}^{2+}/\text{Mg}^{2+}$  exchange in cells with normal  $[\text{Mg}^{2+}]_i$  and that binding of two  $\text{Mg}^{2+}$  to an intracellular modifier site is required for the allosteric transformation of the  $\text{Mg}^{2+}/\text{Mg}^{2+}$   $\text{Na}^+/\text{Mg}^{2+}$  exchanger. In most cell systems unstimulated  $\text{Mg}^{2+}$  influx is not high enough to activate the exchanger.<sup>49,60</sup> However, in some cell types, a basal  $\text{Na}^+/\text{Mg}^{2+}$  exchanger activity has been shown to exist without manipulation of intra- or extracellular ion concentrations.<sup>64,65</sup> The  $K_m$  for  $[\text{Mg}^{2+}]_i$  at half maximal rate has been calculated to be in the range between 1.3 to 4 mM.<sup>48,49,66</sup>

Differences in cell type,  $[\text{Mg}^{2+}]_i$  (loaded or unloaded cells, loading concentration, use of metabolic inhibitors, intracellular buffering capacity for  $\text{Mg}^{2+}$ ) and in the method used to determine  $\text{Mg}^{2+}$  efflux (flux studies with  $^{28}\text{Mg}$ , determination of intra- or extracellular  $[\text{Mg}^{2+}]$  and  $[\text{Na}^+]$  by AAS or by fluorescence probes) are partly responsible for the observed differences in the transport capacity. Therefore, a comparison of the available data is difficult. Under nearly  $V_{\max}$  conditions (after preloading with 6 mM  $\text{Mg}^{2+}$ ), we found a  $\text{Na}^+/\text{Mg}^{2+}$  exchanger mediated  $\text{Mg}^{2+}$  efflux of  $9.4 \pm 4.7 \text{ mM/l cell/15 min}$  in ruminal epithelial cells.<sup>56</sup> This rate of  $\text{Mg}^{2+}$  loss is even greater than the rate reported under the same or very similar conditions for most of the other cell systems.<sup>49,55,65</sup> We interpret the higher efflux rate in the epithelial cell system as to reflect the capability to maintain a normal  $[\text{Mg}^{2+}]_i$  of 0.6 to 1.2 mM in spite of disturbances induced by marked  $\text{Mg}^{2+}$  influx ( $37.5\text{--}42 \mu\text{M/min}$ ), which is a characteristic of this  $\text{Mg}^{2+}$ -absorbing cell.<sup>17,22</sup>

### Regulation

In perfused heart and liver, a transient  $\text{Mg}^{2+}$  efflux can be induced by  $\beta$ -adrenergic substances or by application of db-cyclic adenosine monophosphate (cAMP) and different cell types respond to cAMP stimulation with a 50% to 70 % increase of  $\text{Mg}^{2+}$  efflux.<sup>67–69</sup> Stimulation with cAMP was ineffective in  $\text{Na}^+$ -free NMDG medium, indicating that elevation of the intracellular cAMP concentration specifically triggers the  $\text{Na}^+$ -dependent  $\text{Mg}^{2+}$  efflux.<sup>56,70</sup> An increased affinity of the  $\text{Na}^+/\text{Mg}^{2+}$  transporter for intracellular  $\text{Mg}^{2+}$  induced by phosphorylation of the transport protein has been postulated as the underlying mechanism for cAMP activated  $\text{Mg}^{2+}$  extrusion.<sup>53</sup>

Besides acting on the antiport directly, cAMP and other agents could also induce the release of  $\text{Mg}^{2+}$  from intracellular stores, for example, from mitochondria, and activation of the exchanger via the increase in cytosolic free  $\text{Mg}^{2+}$  content.<sup>71</sup> Alternatively to the cAMP activation,  $\text{Na}^+$ -dependent  $\text{Mg}^{2+}$  extrusion is activated by changes in intracellular  $\text{Ca}^{2+}$  content.<sup>52</sup>

Most likely, the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger is a pathway that different hormones or mediators activate to induce  $\text{Mg}^{2+}$  extrusion. Angiotensin II,<sup>72</sup>  $\text{PGE}_2$ ,<sup>56</sup> catecholamines,<sup>52</sup> and  $\text{INF}\alpha$ <sup>70</sup> are some examples. Recently, it has been shown that the  $[\text{Mg}^{2+}]_i$  decrease resulting from  $\text{Na}^+/\text{Mg}^{2+}$  exchanger activation influences directly or indirectly cellular transport mechanisms and physiological functions. He and colleagues<sup>72</sup> demonstrated  $\text{Na}^+/\text{Mg}^{2+}$  exchanger-mediated  $\text{Mg}^{2+}$  efflux from smooth vascular muscle cells after acute angiotensin II stimulation. The induced reduction of  $[\text{Mg}^{2+}]_i$  led than to vasoconstriction. TRPM7, which facilitates  $\text{Mg}^{2+}$  influx, may also be activated by decreased  $[\text{Mg}^{2+}]_i$ . A TRPM7-mediated increase of the intracellular  $\text{Mg}^{2+}$  level is postulated to play a role in smooth vascular muscle cell growth.<sup>72</sup> In ruminal epithelial cells (REC), a cAMP-induced reduction of  $[\text{Mg}^{2+}]_i$  has been demonstrated to activate a nonselective cation conductance expressed in the apical membrane of these cells and, thereby to play an important role in the regulation of ruminal  $\text{Na}^+$

transport.<sup>44</sup> By use of the Ussing chamber technique, the same authors showed a stimulating effect of cAMP on the unidirectional  $^{28}\text{Mg}^{2+}$  flux across the isolated ruminal epithelium, which is in line with the stimulation of the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger seen in ruminal epithelial cells.<sup>56</sup> For years a role of the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger in the transepithelial  $\text{Mg}^{2+}$  transport performed by gastrointestinal and renal epithelia has been postulated,<sup>43</sup> but is shown here for the first time. Moreover, the suggestion of a regulation of this transport by cAMP is of general importance, and there is a need to investigate what hormones or other signals are involved.

### ***Towards Identification of the $\text{Na}^+/\text{Mg}^{2+}$ Exchanger***

Despite the extensive evidence for a functional  $\text{Na}^+/\text{Mg}^{2+}$  exchanger in the cell membrane of nearly all mammalian cells, encoding gene(s), structural properties, and the modalities of its operation and regulation remain for the most part unknown. Among other reasons this can be explained by the fact that there is no highly selective blocker available. A reversible inhibition of the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger was observed after application of amiloride, quinidine, and imipramine, compounds known to inhibit other transport systems ( $\text{Na}^+$  channels,  $\text{Na}^+$ -dependent carriers,  $\text{K}^+$  channels) as well.<sup>59,70</sup> Imipramine binds to the  $\text{Na}^+$  site of the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger and competition between these two compounds slows  $\text{Mg}^{2+}$  efflux. Using imipramine concentrations between 10 and  $500\mu\text{M}$ , most investigators achieved a 33% to 85% inhibition of  $\text{Mg}^{2+}$  efflux.<sup>52,60,65</sup> However, the imipramine inhibition was always incomplete and an even greater reduction of  $\text{Mg}^{2+}$  extrusion could be obtained by omitting extracellular  $\text{Na}^+$ .<sup>56,59</sup> To overcome this problem, monoclonal antibodies (mabs) raised against the porcine erythrocyte  $\text{Na}^+/\text{Mg}^{2+}$  exchanger were prepared in our laboratory.<sup>73</sup> Because of the unknown protein structure of the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger, the screening for effective antibodies has been done by a specific functional test using the capability of the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger of porcine red cells to transport  $\text{Mn}^{2+}$  instead of  $\text{Na}^+$ .<sup>49</sup> Supernatants of antibody-producing hybridoma cell clones were checked for their ability to inhibit  $^{54}\text{Mn}^{2+}$  influx as a measure of the  $\text{Na}^+/\text{Mg}^{2+}$  exchange activity. Compared with control conditions, effective antibody-containing supernatants decreased the rate of  $\text{Mn}^{2+}$  uptake in porcine red cells by  $68\% \pm 2\%$  and the inhibition effect varies from 60% to 74% between single clones. Hybridoma supernatants or purified mabs were subsequently used to inhibit  $\text{Mg}^{2+}$  transport and to detect the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger in REC.<sup>56</sup> Transport studies were performed with unloaded REC under conditions promoting influx (high- $\text{K}^+$ , low- $\text{Na}^+$  medium) or efflux (high- $\text{Na}^+$ , low- $\text{K}^+$  medium) of  $\text{Mg}^{2+}$  via the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger, respectively. Addition of the mab effectively inhibited  $\text{Mg}^{2+}$  transport as reflected by a 50% reduction or 23% increase of the REC  $[\text{Mg}^{2+}]_i$ . In addition, the antibody specifically labeled a protein with an apparent molecular mass of 70 kDa in immunoblots of proteins from REC as well as from porcine red blood cell membranes. Also, in flow cytometric measurements the anti- $\text{Na}^+/\text{Mg}^{2+}$  exchanger mab was

specifically bound to a significant proportion of REC. With these new tools, we should be able to purify and to characterize the protein by molecularbiological techniques. The considerable variation of functional properties, such as maximum transport capacity, reversibility, capability of Mn<sup>2+</sup> transport, and stoichiometry of Mg<sup>2+</sup> transport via Na<sup>+</sup>/Mg<sup>2+</sup> exchanger between cell types point, to the existence of various isoforms of the transport protein. Its molecular characterization is of greatest interest because disturbance or dysregulation of the Na<sup>+</sup>/Mg<sup>2+</sup> exchanger has been assumed to participate in the pathogenesis of diseases, such as primary hypertension<sup>6</sup> and cystic fibrosis.<sup>9</sup>

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## 4

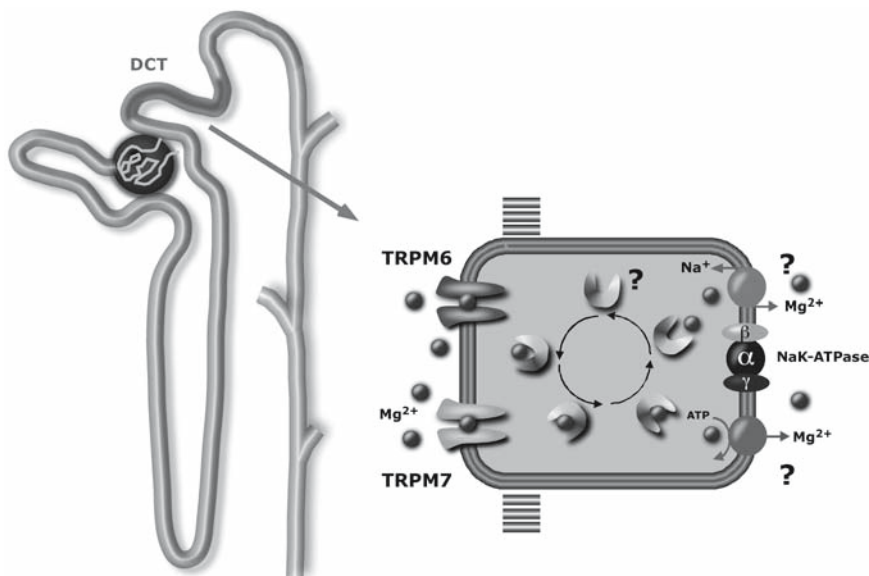
# TRPM6 and TRPM7 Chanzymes Essential for Magnesium Homeostasis

Wouter M. Tiel Groenestege, Joost G. J. Hoenderop,  
and René J. M. Bindels

$\text{Mg}^{2+}$  is the second most abundant intracellular cation and plays an essential role as cofactor in many enzymatic reactions. Regulation of the total body  $\text{Mg}^{2+}$  balance principally resides within the kidney that tightly matches the intestinal absorption of  $\text{Mg}^{2+}$ . The identification of epithelial  $\text{Mg}^{2+}$  transporters in the kidney has been greatly facilitated by studying hereditary disorders with primary hypomagnesemia. Identification of the gene defect in hypomagnesemia with secondary hypocalcemia has recently elucidated the TRPM6 protein, a member of the transient receptor potential melastatin (TRPM) family. TRPM6 shows the highest homology with TRPM7, which has been identified as a  $\text{Mg}^{2+}$ -permeable ion channel primarily required for cellular  $\text{Mg}^{2+}$  homeostasis. TRPM6 and TRPM7 are distinct from all other ion channels because they are composed of a channel linked to a protein kinase domain and therefore referred to as chanzymes. These chanzymes are essential for  $\text{Mg}^{2+}$  homeostasis, which is critical for human health and cell viability. This chapter describes the characteristics of epithelial  $\text{Mg}^{2+}$  transport in general and highlights the distinctive features and the physiological relevance of these new chanzymes in (patho)physiological situations.

## $\text{Mg}^{2+}$ (Re)absorption in Kidney and Intestine

The kidney is the principal organ responsible for the regulation of the body  $\text{Mg}^{2+}$  balance. About 80% of the total plasma  $\text{Mg}^{2+}$  is filtered in the glomeruli,<sup>1,2</sup> of which the majority is subsequently reabsorbed along the nephron.<sup>3</sup> Approximately 10% to 20% of  $\text{Mg}^{2+}$  is reabsorbed by the proximal tubule. However, the bulk amount of  $\text{Mg}^{2+}$  (50%–70%) is reabsorbed by the thick ascending limb of Henle (TAL), which mediates  $\text{Mg}^{2+}$  reabsorption via paracellular transport. The distal convoluted tubule (DCT) reabsorbs 5% to 10% of the filtered  $\text{Mg}^{2+}$  and the reabsorption rate in this segment defines the final urinary  $\text{Mg}^{2+}$  concentration, because virtually no reabsorption takes places beyond this segment.<sup>3</sup>  $\text{Mg}^{2+}$  transport in DCT is transcellular in nature and influenced by



**FIGURE 4.1.** Transcellular  $\text{Mg}^{2+}$  reabsorption. Active  $\text{Mg}^{2+}$  reabsorption is carried out as a three-step process in the distal convoluted tubule (DCT). Following entry of  $\text{Mg}^{2+}$  in DCT through the epithelial  $\text{Mg}^{2+}$  channel, TRPM6 (and TRPM7),  $\text{Mg}^{2+}$  diffuses to the basolateral membrane. As extrusion mechanisms are postulated a basolateral  $\text{Na}^+/\text{Mg}^{2+}$  exchanger and/or ATP-dependent  $\text{Mg}^{2+}$  pump. The  $\text{Na}^+/\text{K}^+$ -ATPase complex including the  $\gamma$ -subunit controls this transepithelial  $\text{Mg}^{2+}$  transport. In this way, there is net  $\text{Mg}^{2+}$  reabsorption from the luminal space to the extracellular compartment.

dietary  $\text{Mg}^{2+}$  restriction and various hormones.<sup>4,5</sup> However, the molecular details and regulation of this pathway remain largely unknown.<sup>3,4,6,7</sup>

Hypothetically, the process of transcellular  $\text{Mg}^{2+}$  transport is envisaged by the following sequential steps (Figure 4.1). Driven by a favorable transmembrane potential,  $\text{Mg}^{2+}$  enters the epithelial cell through the apical epithelial  $\text{Mg}^{2+}$  channel TRPM6. Next,  $\text{Mg}^{2+}$  will diffuse through the cytosol to be extruded actively against an electrochemical negative gradient across the basolateral membrane.<sup>8</sup> The molecular identity of these latter basolateral  $\text{Mg}^{2+}$  transporters is not known. Most physiological studies favor a  $\text{Na}^+$ -dependent exchange mechanism.<sup>9</sup> Other candidate mechanisms include an ATP-dependent  $\text{Mg}^{2+}$  pump. The  $\text{Mg}^{2+}$  entry appears to be the rate-limiting step and the site of regulation.

## Genes Involved in Primary Inherited Hypomagnesemia

In the last decade, several genes encoding proteins that are directly involved in renal  $\text{Mg}^{2+}$  handling have been identified following a positional cloning strategy in families with primary hereditary hypomagnesemia. First, the gene

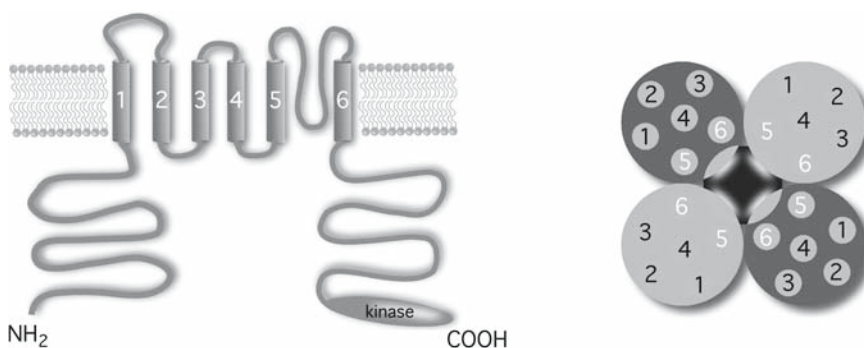
*PCLN-1* (or *CLDN16*) encoding the protein paracellin-1 (or claudin-16) was found to be mutated in patients who have hypomagnesemia, hypercalciuria, and nephrocalcinosis (HHN; MIM 248250).<sup>10</sup> The tight junction protein paracellin-1 is specifically expressed in TAL and is important for paracellular  $Mg^{2+}$  and  $Ca^{2+}$  reabsorption in this segment.<sup>10</sup> Second, the gene *FXYD2*, encoding the  $\gamma$ -subunit of the  $Na^+, K^+$ -adenosine triphosphatase (ATPase) pump, is mutated in patients with autosomal dominant renal hypomagnesemia associated with hypocalciuria (IDH; MIM 154020). The  $\gamma$ -subunit of the  $Na^+, K^+$ -ATPase pump is predominantly expressed in the kidney and shows the highest expression levels in DCT and medullary TAL.<sup>11</sup> Although the  $\gamma$ -subunit of the  $Na^+, K^+$ -ATPase pump is critical for active transcellular  $Mg^{2+}$  reabsorption in DCT, the molecular mechanism for renal  $Mg^{2+}$  loss in this autosomal dominant type of primary hypomagnesemia remains to be elucidated.<sup>11</sup> The key molecules that represent the basolateral  $Mg^{2+}$  extrusion mechanism in the process of transcellular  $Mg^{2+}$  transport are still elusive. Until recently the molecular identity of the protein that facilitates the apical influx of  $Mg^{2+}$  in active  $Mg^{2+}$  transport was unknown. A promising candidate was found by the elucidation of the genetic basis of hypomagnesemia with secondary hypocalcemia (HSH; MIM 602014). HSH is an autosomal recessive disease of which the gene locus was mapped on chromosome 9q22.<sup>12</sup> Affected individuals show neurological symptoms of hypomagnesemic hypocalcemia, including seizures and muscle spasms during infancy.<sup>13–15</sup> Physiological studies indicate that the pathophysiology of HSH is primarily caused by a primary defect in intestinal  $Mg^{2+}$  transport.<sup>16,17</sup> In most patients, renal  $Mg^{2+}$  conservation has been reported to be normal. However, for some patients, inappropriately high fractional  $Mg^{2+}$  excretion rates with respect to their low serum  $Mg^{2+}$  levels were reported.<sup>14,18</sup> This renal  $Mg^{2+}$  leak suggests an impaired renal  $Mg^{2+}$  reabsorption. HSH can be treated by high dietary  $Mg^{2+}$  intake because passive  $Mg^{2+}$  absorption is not affected.<sup>19</sup> When untreated, the disease may be fatal or may lead to severe neurologic damage. The observed hypocalcemia is a secondary effect possibly caused by parathyroid failure resulting from  $Mg^{2+}$  deficiency.<sup>20</sup> Using a positional candidate gene-cloning approach, mutations in TRPM6 were found to be the cause of autosomal recessive HSH.<sup>13,14,21</sup> The TRPM6 protein shows 52% homology with TRPM7, which has been identified as a  $Mg^{2+}$ -permeable ion channel particularly required for cellular  $Mg^{2+}$  homeostasis.<sup>8,22,23</sup> In summary, a genetic screen in patients with primary hypomagnesemia revealed the identification of the transient receptor potential (TRP) cation channel TRPM6 as potential gatekeeper in the maintenance of the  $Mg^{2+}$  balance.

## TRPM6 and TRPM7

The TRP superfamily is a newly discovered family of cation-permeable ion channels.<sup>24</sup> There are at least three previously recognized subfamilies of proteins; TRPC (conical), TRPV (vanilloid), and TRPM (metastatin), that are

expressed throughout the animal kingdom (<http://clapham.tch.harvard.edu/trps/>). Recently, the polycystins were also included in the TRP superfamily abbreviated as TRPP (polycystin).<sup>25</sup> Each of the proteins is a cation channel composed of six transmembrane-spanning domains and a conserved pore-forming region that assemble in a tetrameric configuration (Figure 4.2).<sup>24,26,27</sup> TRPM6 is one of the eight members of the identified TRPM cation channel subfamily and is composed of 2022 amino acids encoded by a large gene that contains 39 exons.<sup>13,14,24</sup>

TRPM6 and its closest homologue TRPM7 are unique bifunctional proteins combining  $Mg^{2+}$ -permeable cation channel properties with protein kinase activity.<sup>24,28</sup> However, the precise function of this kinase domain remains to be established. To date, TRPM7 regulation has received most of the attention. TRPM7 is ubiquitously expressed and implicated in cellular  $Mg^{2+}$  homeostasis. In contrast to TRPM7, TRPM6 has a more restricted expression pattern and is predominantly present in (re)absorbing epithelia.<sup>8,13,14</sup> In mice, TRPM6 is predominantly present in kidney, lung, cecum, and colon, that therefore likely constitute the main sites of active  $Mg^{2+}$  (re)absorption.<sup>29</sup> TRPM6 and TRPM7 are not functionally redundant in humans because the concurrent co-expression of TRPM7 cannot rescue the severe phenotype of HSH patients.<sup>30</sup> In kidney, TRPM6 is localized along the apical membrane of DCT, known as the main site of active transcellular  $Mg^{2+}$  reabsorption along the nephron.<sup>8</sup> In line with the expected function of being the gatekeeper of  $Mg^{2+}$  influx, TRPM6 was predominantly localized along the apical membrane of these immunopositive tubules. Immunohistochemical studies of TRPM6 and the  $Na^+$ ,  $Cl^-$  cotrans-



**FIGURE 4.2.** Structural organization of TRPM6 and TRPM7. TRPM6 and TRPM7 belong to the largest TRP channels, consisting of around 2000 amino acids including very large cytosolic amino- and carboxyl-termini including an atypical protein kinase domain. The six transmembrane unit is one of four identical or homologous subunits presumed to surround the central pore. The gate and selectivity filter are formed by the four 2 transmembrane domains (TM5 pore loop TM6) facing the center of the channel. Cations are selected for permeation by the extracellular-facing pore loop, held in place by the TM5 and TM6  $\alpha$ -helices.

porter (NCC), which were used as specific markers for DCT, indicated a complete colocalization of these transport proteins in kidney.<sup>8</sup> Until now, specific  $Mg^{2+}$ -binding proteins have not been identified, but it is interesting to mention that the  $Ca^{2+}$ -binding proteins parvalbumin and calbindins also bind  $Mg^{2+}$ .<sup>31</sup> Importantly, TRPM6 colocalized with parvalbumin in the first part of the DCT and with calbindin- $D_{28K}$  in the last part of the DCT.<sup>8</sup> In the small intestine, absorptive epithelial cells stained positively for TRPM6 detected by in situ hybridization and immunohistochemistry.<sup>8,14</sup> Voets and colleagues demonstrated that TRPM6 was localized along the brush-border membrane of these intestinal cells.<sup>8</sup>

Interestingly, Schmitz and coworkers<sup>23</sup> demonstrated that  $Mg^{2+}$  supplementation of cells that lack TRPM7 expression rescued growth arrest and cell lethality that was caused by TRPM7 inactivation. Although TRPM7 is permeable for  $Ca^{2+}$ , as well as trace divalents such as  $Zn^{2+}$ ,  $Ni^{2+}$ ,  $Ba^{2+}$ , and  $Co^{2+}$ , supplementation with these cations was ineffective, indicating the specific effect of  $Mg^{2+}$  on these cellular processes. Thus, the ubiquitously expressed TRPM7 is important for cellular viability and cellular  $Mg^{2+}$  homeostasis rather than the extracellular  $Mg^{2+}$  homeostasis. TRPM7 has also been implicated to play a role in anoxic neuronal death<sup>22,32,33</sup> and regulation of cell adhesion as a result of a combined effect of kinase-dependent and -independent pathways on actomyosin contractility.<sup>34</sup> This suggests that TRPM7 is also important for various cell biological processes, including cytokinesis, adhesion, and migration, because these processes are regulated by actomyosin contractility.

## Regulation of TRPM6 and TRPM7

Although in HSH the defect was originally established at the level of the intestine, there is also evidence for impaired renal  $Mg^{2+}$  reabsorption.<sup>14,18,35</sup> It was demonstrated that individuals with HSH, with respect to their low serum  $Mg^{2+}$  levels, show inappropriately high fractional  $Mg^{2+}$  excretion rates. This indicates an additional role of impaired renal  $Mg^{2+}$  reabsorption in HSH.<sup>18,35</sup> This is confirmed by Walder and colleagues, who characterized a considerable renal leak of  $Mg^{2+}$  in HSH patients.<sup>13</sup> The renal expression of TRPM6, in addition to the renal  $Mg^{2+}$  leak in patients with HSH, emphasizes the important role of TRPM6 in renal  $Mg^{2+}$  reabsorption. The gatekeeper function of TRPM6 in transepithelial  $Mg^{2+}$  transport is further supported by studies investigating the regulation of TRPM6 by dietary  $Mg^{2+}$  and hormones. Dietary  $Mg^{2+}$  restriction in mice resulted in hypomagnesemia and renal  $Mg^{2+}$  conservation, and significantly upregulated renal TRPM6 mRNA levels. The role of TRPM7 in cellular and not total body  $Mg^{2+}$  homeostasis is supported by the fact that dietary  $Mg^{2+}$  did not alter TRPM7 mRNA expression in mouse kidney and colon. Furthermore, it was shown that the hormone  $17\beta$ -estradiol, but not  $1,25$ -dihydroxyvitamin  $D_3$  and parathyroid hormone, regulates renal TRPM6 mRNA levels.<sup>29</sup> Renal TRPM7 mRNA abundance remained unaltered under

these conditions. This study demonstrated that the renal TRPM6 mRNA level in ovariectomized rats is significantly reduced, whereas  $17\beta$ -estradiol treatment normalized TRPM6 mRNA levels.<sup>29</sup> Future studies should point out if these hormones regulate the expression of TRPM6 in the intestine.

Chronic metabolic acidosis results in renal  $Mg^{2+}$  wasting, whereas chronic metabolic alkalosis is known to exert the reverse effect. It was hypothesized that these adaptations are mediated at least in part by the renal  $Mg^{2+}$  transport protein TRPM6. Chronic metabolic acidosis decreased renal TRPM6 expression, increased  $Mg^{2+}$  excretion, and decreased serum  $Mg^{2+}$  concentration, whereas chronic metabolic alkalosis resulted in the exact opposite effects.<sup>36</sup> Thus, these data suggest that regulation of TRPM6 contributes importantly to the effects of acid–base status on renal  $Mg^{2+}$  handling. In addition, TRPM6 is regulated in NCC-knockout mice and upon NCC inhibition.<sup>37</sup> Thiazide diuretics enhance renal  $Na^+$  excretion by blocking NCC, and mutations in NCC result in Gitelman syndrome. The mechanism underlying the accompanying hypomagnesemia remains debated. In NCC-knockout mice, an animal model of Gitelman syndrome and during chronic HCTZ administration, the hypomagnesemia is accompanied by downregulation of TRPM6. Thus, TRPM6 downregulation may represent a general mechanism involved in the pathogenesis of hypomagnesemia accompanying NCC inhibition or inactivation.

## Functional Characteristics of TRPM6 and TRPM7

To functionally characterize TRPM6, the protein was heterogeneously expressed in human embryonic kidney 293 (HEK293) cells. TRPM6-transfected HEK293 cells perfused with an extracellular solution that contained 1 mM  $Mg^{2+}$  or  $Ca^{2+}$  exhibited characteristic outwardly rectifying currents upon establishment of the whole-cell configuration as demonstrated for TRPM7.<sup>8,22,28</sup> It is intriguing that at physiological membrane potentials of the DCT cell ( $-80$  mV), small but significant inward currents were observed in TRPM6-expressing HEK293 cells with all tested divalent cations as the sole charge carrier.<sup>8</sup> However, mutations in TRPM6 are linked directly to HSH, emphasizing that this channel is an essential component of the epithelial  $Mg^{2+}$  uptake machinery. Interestingly, HEK293 cells transfected with the TRPM6 mutants identified in HSH patients (TRPM6<sup>Ser590X</sup> and TRPM6<sup>Arg736fsX737</sup>) displayed currents with similar amplitude and activation kinetics as nontransfected HEK293 cells, indicating that these mutant proteins are nonfunctional, in line with the postulated function of TRPM6 being  $Mg^{2+}$  influx step in epithelial  $Mg^{2+}$  transport.<sup>8</sup> It is possible that the TRPM6-mediated  $Mg^{2+}$  inward current is more pronounced in native DCT and intestinal cells as a result of specific cofactors, such as intracellular  $Mg^{2+}$  buffers, that are missing in overexpression systems, for example, HEK293 cells. The unique permeation rank order determined from the inward current amplitude at  $-80$  mV was comparable to TRPM7 ( $Ba^{2+} \geq Ni^{2+} > Mg^{2+} > Ca^{2+}$ ).<sup>8,28</sup> Micropuncture studies have demonstrated that the

luminal concentration of  $\text{Mg}^{2+}$  in DCT ranges from 0.2 to 0.7 mM.<sup>3</sup> Because the  $\text{Ca}^{2+}$  concentration is in the millimolar range, the apical  $\text{Mg}^{2+}$  influx pathway should exhibit a higher affinity for  $\text{Mg}^{2+}$  than for  $\text{Ca}^{2+}$ . It is interesting that dose-response curves for the  $\text{Na}^+$  current block at  $-80$  mV indicated four times higher  $K_D$  values for  $\text{Ca}^{2+}$  compared with  $\text{Mg}^{2+}$ .<sup>8</sup> These data suggest that the pore of TRPM6 has a higher affinity for  $\text{Mg}^{2+}$  than for  $\text{Ca}^{2+}$ . In this way, TRPM6 comprises a unique channel because all known  $\text{Ca}^{2+}$ -permeable channels, including members of the TRP superfamily, generally display a 10 to 1000 times lower affinity for  $\text{Mg}^{2+}$  than for  $\text{Ca}^{2+}$ .

Voets and colleagues demonstrated a coherent relationship between the applied extracellular  $\text{Mg}^{2+}$  concentration and the measured intracellular  $\text{Mg}^{2+}$  level in TRPM6-expressing cells by using the  $\text{Mg}^{2+}$ -sensitive radiometric fluorescent dye Magfura-2.<sup>8</sup> To study the effect of intracellular  $\text{Mg}^{2+}$  on TRPM6 activity, the  $\text{Mg}^{2+}$  concentration was altered directly in a spatially uniform manner using flash photolysis of the photolabile  $\text{Mg}^{2+}$  chelator DM-nitrophen.<sup>8</sup> Elevation of the intracellular  $\text{Mg}^{2+}$  concentration reduced the TRPM6-induced current, indicating that the channel is regulated by intracellular  $\text{Mg}^{2+}$  or  $\text{Mg}^{2+}$ -ATP. Previous studies reported that TRPM7 channel activity is strongly suppressed by  $\text{Mg}^{2+}$ -ATP concentrations in the millimolar range.<sup>22,38</sup> Kozak and Cahalan demonstrated that intracellular  $\text{Mg}^{2+}$  rather than ATP inhibits TRPM7 channel activity.<sup>39</sup> Runnels and coworkers demonstrated that in addition to  $\text{Mg}^{2+}$  inhibition, TRPM7 is also inactivated by hydrolysis of the phosphoinositide lipid  $\text{PIP}_2$ .<sup>40</sup> It is interesting that TRPM7 was discovered on the basis of binding to phospholipase C (PLC), which cleaves  $\text{PIP}_2$ .<sup>28</sup> This raises the question whether there is a link between the two apparently dissimilar modes of channel inhibition: an increase in  $\text{Mg}^{2+}$  and hydrolysis of  $\text{PIP}_2$ . It was postulated that  $\text{Mg}^{2+}$  could possibly activate PLC that associates with TRPM7.<sup>41</sup> This possibility is strengthened by a study in B lymphocytes that demonstrates the presence of phosphoinositide-specific PLC, which is activated as the  $\text{Mg}^{2+}$  concentration is raised from 30 to 1000  $\mu\text{M}$ .<sup>42</sup> Although, a  $\text{Mg}^{2+}$ -activated PLC has not been described in other cell types, it may be present in various cell types including other lymphoid-derived cell lines, for example, Jurkat T cells and rat basophilic leukemia cells. The latter mentioned cell lines contain an endogenous TRPM7-like current, referred to as a  $\text{Mg}^{2+}$ -inhibited cation channel (MIC), which makes them attractive candidates for the presence a  $\text{Mg}^{2+}$ -activated PLC.<sup>43,44</sup>

Recently, it was suggested that at physiological pH (7.4),  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  bind to TRPM7 and inhibit the monovalent cationic currents, whereas at high  $\text{H}^+$  concentrations, the affinity of TRPM7 for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  is decreased, thereby allowing monovalent cations to pass through TRPM7. In addition, these investigators demonstrated that the endogenous TRPM7-like current, which is known as  $\text{Mg}^{2+}$ -inhibitable cation current (MIC) or  $\text{Mg}^{2+}$  nucleotide-regulated metal ion current (MagNuM) in rat basophilic leukemia (RBL) cells was also significantly potentiated by acidic pH, suggesting that MIC/MagNuM is



encoded by TRPM7. In conclusion, the pH sensitivity represents a novel feature of TRPM7 and implies that TRPM7 may play a role under acidic pathological conditions.<sup>45</sup>

It was postulated that TRPM6 requires assembly with TRPM7 to form functional channel complexes in the plasma membrane and that disruption of multimer formation by a mutated TRPM6 variant, TRPM6<sup>S141L</sup>, results in HSH.<sup>46</sup> In this study, TRPM6<sup>S141L</sup> was not directed to the cell surface by TRPM7 and failed to interact with the co-expressed TRPM7. Remarkably, in contrast to TRPM7, Gudermann and coworkers found that TRPM6 expression in *Xenopus* oocytes and HEK293 cells did not entail significant ion currents.<sup>46</sup> In addition, Schmitz and colleagues also supported the idea that TRPM6 and TRPM7 associate and that trafficking of TRPM6 to the cell surface is strongly dependent on TRPM7 co-expression.<sup>30</sup> In variance, Voets and colleagues measured significantly larger currents in TRPM6-transfected HEK293 cells compared to mock-transfected cells.<sup>8</sup> An explanation for this discrepancy might be the existence of specific TRPM6 splice variants with different functional properties. Chubanov and colleagues demonstrated that 5' rapid amplification of cDNA ends revealed three short alternative 5' exons, called 1A, 1B, and 1C, that were found to be individually spliced onto exon 2, suggesting that the TRPM6 gene harbors a promoter with alternative transcription start sites.<sup>46</sup> These transcripts have been named, accordingly, TRPM6a, TRPM6b, and TRPM6c, and additional functional measurements are needed to explain possible biophysical differences.

## **$\alpha$ -Kinase Domain**

Unlike other members of the TRP channel family, TRPM6 and TRPM7 contain long carboxy-terminal domains including an active threonine/serine kinase, which belongs to the atypical family of eukaryotic  $\alpha$ -kinases.<sup>28</sup> Genomic studies identified six  $\alpha$ -kinases in mammals, including the ones fused to TRPM6 and TRPM7. The  $\alpha$ -kinases share no sequence homology with the conventional kinases.<sup>47</sup> A key question concerns the nature of the mechanisms underlying the activation and regulation of TRPM6 and TRPM7. In particular, what is the function of the atypical protein  $\alpha$ -kinase domain located in the carboxyl terminus? To characterize the TRPM7 kinase activity in vitro, the catalytic domain was expressed in bacteria.<sup>48</sup> This kinase is able to undergo autophosphorylation and to phosphorylate substrates such as myelin basic protein and histone H3 on serine and threonine residues. The kinase is specific for ATP and  $Mg^{2+}$  or  $Mn^{2+}$  is required for optimal activity. Schmitz and coworkers demonstrated that, using a phosphothreonine-specific antibody, TRPM6 can phosphorylate TRPM7, but not vice versa.<sup>30</sup> Recently, annexin 1 and myosin II A have been identified as endogenous substrates of the TRPM7 kinase.<sup>34,49</sup> Although, the biological role of annexin 1 phosphorylation via



TRPM7 is currently unknown, both proteins have been linked to processes of cell survival and growth.<sup>33,50</sup> Future studies should elucidate whether the kinase domain of TRPM6 and TRPM7 has specific cellular phosphorylation targets that modulate ion channel activity and, therefore, the  $Mg^{2+}$  balance. It has been suggested that TRPM7 could potentially serve both as a  $Mg^{2+}$  uptake mechanism and a  $Mg^{2+}$  sensor when the ion channel domain could modulate the phosphotransferase activity by increasing intracellular  $Mg^{2+}$  or directly via conformational changes induced by gating of the channel.<sup>51</sup> This modification could result in the phosphorylation of yet unidentified substrates providing real time information on channel activity or cellular  $Mg^{2+}$  status. While there is general consensus that TRPM7 is inhibited by free intracellular  $Mg^{2+}$ , the functional role of intracellular levels of  $Mg^{2+}$  ATP and the kinase domain in regulating TRPM7 channel activity remain controversial. Several groups suggested that the kinase domain is essential for channel activity,<sup>28,52</sup> whereas others indicated that it is not involved.<sup>23,53</sup> Furthermore, the phosphotransferase activity of the TRPM7 kinase domain affected channel activity by regulating the sensitivity of the channel to inhibition by  $Mg^{2+}$  and  $Mg^{2+}$ ·ATP,<sup>23,54,55</sup> but this finding was not generally confirmed.<sup>39,52</sup> Further experiments are needed to elucidate these inconsistent results and firmly establish the functional role of the kinase domain and intracellular levels of  $Mg^{2+}$ ·ATP in regulating TRPM6 and TRPM7 channel activity.

## Prospectives

This chapter focused on the identification, function, and regulation of the epithelial  $Mg^{2+}$  channels TRPM6 and TRPM7. The discovery of these channels was a significant advance in our knowledge about cellular  $Mg^{2+}$  transport and hormonal regulation of the  $Mg^{2+}$  balance. Although, several studies have investigated the regulation of TRPM6 and TRPM7, many questions remain to be investigated. Future research is needed to unravel the nature of the mechanisms underlying the activation and regulation of TRPM6 and TRPM7 and to elucidate the reported controversy in the functioning and necessity of the kinase domain in regulating TRPM7 channel activity. In addition, the phosphorylation targets of these channels that can modulate channel activity and, therefore, the  $Mg^{2+}$  balance remain to be identified. Taken together, the discovery of TRPM6 and TRPM7 provides a unique opportunity to investigate the molecular mechanisms determining the specificity, regulation, and activation of these channels in detail with respect to their distinct roles in  $Mg^{2+}$  homeostasis.

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# 5

## CorA-Mrs2-Alr1 Superfamily of $\text{Mg}^{2+}$ Channel Proteins

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CorA proteins are ubiquitously expressed in eubacteria and archaea. They constitute the first ever described proteins for cellular  $\text{Mg}^{2+}$  uptake. Nearly all eukaryotic genomes encode distant relatives of CorA, named Alr1p and Mrs2p. Members of each of these subfamilies have been shown to form oligomeric membrane complexes mediating electrogenic uptake of  $\text{Mg}^{2+}$ . The recently published crystal structure of a CorA-type protein and electrophysiological data of a Mrs2-type protein consistently show that this superfamily of proteins forms  $\text{Mg}^{2+}$  permeable channels.

Uptake of  $\text{Mg}^{2+}$  into bacteria, mitochondria, or eukaryotic cells has been described as a diffusive process, driven by the inside negative membrane potential.<sup>1–6</sup> If these processes were unlimited, free ionized  $\text{Mg}^{2+}$  ( $[\text{Mg}^{2+}]$ ) would reach unphysiologically high values, particularly in compartments with high inside negative membrane potentials like bacterial cells or eukaryotic mitochondria. Yet concentrations of  $[\text{Mg}^{2+}]$  in cells or mitochondria were found to be small, ranging according to most determinations from 0.3 to 1.2. Accordingly, cells and organelles can be assumed to have developed mechanisms to control intracellular and intraorganellar  $[\text{Mg}^{2+}]$ . This may either be mediated by proteins involved in  $\text{Mg}^{2+}$  uptake processes or via activation of  $\text{Mg}^{2+}$  extrusion systems, which may involve  $\text{Na}^+/\text{Mg}^{2+}$  or  $\text{H}^+/\text{Mg}^{2+}$  exchange or anion/ $\text{Mg}^{2+}$  cotransport.<sup>1</sup> The latter processes occur at the expense of other, actively generated ion gradients ( $\text{Na}^+$ ,  $\text{H}^+$ ; cf. Chapter 3, this book). Therefore, it will be energetically favorable to limit influx to the needs of cells or organelles via regulation of  $\text{Mg}^{2+}$  influx systems.

Early literature essentially agreed on the presence of very slow turnover of  $\text{Mg}^{2+}$  across cell or mitochondrial membranes under quiescent conditions. Yet more recent reports provided a number of experimental observations suggesting that large fluxes of  $\text{Mg}^{2+}$  can cross these membranes following a variety of stimuli.<sup>5–7</sup>

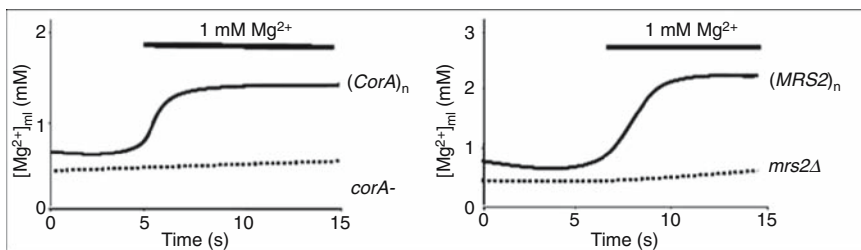
## Prokaryotic $\text{Mg}^{2+}$ Transport Proteins

Genetic screens for bacteria resistant to toxic levels of cobalt led to the identification of *corA*, *mgtA*, and *mgtBC* as the first known loci encoding  $\text{Mg}^{2+}$  transport proteins. A screen for suppressors of CorA-deficient bacteria resulted in the identification of an additional transporter named MgtE.<sup>3,8,9</sup>

CorA proteins are constitutively expressed proteins of the plasma membrane without any similarity to other proteins families. They constitute the major  $\text{Mg}^{2+}$  transporters in *Eubacteria* and *Archaea*.  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  also serve as CorA transport substrates, but with lower affinities and velocities than  $\text{Mg}^{2+}$ . CorA of the bacterium *Salmonella enterica* serovar *Typhimurium* has been reported to transport  $\text{Mg}^{2+}$  with an apparent  $K_m$  of 15 to 20  $\mu\text{M}$ , allowing them to grow in media with very low  $[\text{Mg}^{2+}]$ .<sup>3</sup>

While earlier studies on CorA mediated cation transport involved radioactively labeled isotopes, Froschauer and colleagues<sup>6</sup> introduced measurements of free intracellular  $[\text{Mg}^{2+}]$  by use of the  $\text{Mg}^{2+}$  sensitive dye Magfura-2. Bacterial cells were found to exhibit a rapid change of internal free  $[\text{Mg}^{2+}]$  upon increase of extracellular  $[\text{Mg}^{2+}]$ , consistent with a high capacity of  $\text{Mg}^{2+}$  uptake (Figure 5.1). Driving force of this  $\text{Mg}^{2+}$  uptake is the high inside-negative membrane potential of bacterial cells. Efflux from bacterial cells was comparatively small, and only visible upon dissipation of the membrane potential.<sup>6</sup> CorA-mediated efflux, as invoked previously,<sup>10</sup> is at variance with the findings that this protein forms a channel (see below) mediating  $\text{Mg}^{2+}$  influx driven by the inside negative membrane potential.

MgtA and its homologue MgtB are polytopic membrane proteins belonging to the large group of P-type ATPases in pro- and eukaryotes. MgtA is present in widely divergent bacteria, but it is not as common as CorA. Expression of MgtA and MgtB is under the control of a two-component signal-transduction



**FIGURE 5.1.** Influx of  $\text{Mg}^{2+}$  into bacterial cells (left) and isolated yeast mitochondria (right). Bacterial cells or isolated yeast mitochondria were loaded with the  $\text{Mg}^{2+}$ -sensitive fluorescent dye Magfura-2 and intramitochondrial  $[\text{Mg}^{2+}]$  was determined by ratiometric measurements<sup>5,6</sup> at resting conditions (nominally  $\text{Mg}^{2+}$ -free buffer) and upon addition of  $\text{Mg}^{2+}$  to the buffer to final concentrations of 1 mM. (Left)  $(\text{CorA})_n$  and  $\text{corA}^-$ : *S. typhimurium*  $\text{corA}^-$ ,  $\text{mgtA}^-$ , and  $\text{mgtB}^-$  cells transformed with multicopy plasmid expressing CorA or lacking this gene, respectively. (Right)  $(\text{Mrs2})_n$  and  $\text{mrs2}\Delta$ : mitochondria isolated from cells expressing *MRS2* from a high copy number vector or lacking the *MRS2* gene, respectively.

system regulated by  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ . Their expression is thus restricted to conditions when bacteria are starved for  $\text{Mg}^{2+}$ .<sup>3</sup>

Triple mutants lacking MgtA, MgtB, and CorA have a  $\text{Mg}^{2+}$ -dependent growth phenotype. Compared to CorA-expressing cells, mutant mgtA, mgtB, and corA cells had about twofold reduced steady state  $[\text{Mg}^{2+}]$  and exhibited about tenfold reduced rates of  $\text{Mg}^{2+}$  uptake when provided with 1 mM external  $\text{Mg}^{2+}$  (Figure 5.1).<sup>6</sup>

Bacterial MgtE proteins have been poorly studied, but the recent finding of putative homologues in mammalian cells (SLC41) has raised renewed interest in this protein family (cf. Chapter 3, this book). This polytopic membrane protein has been shown to be present in various *Eubacteria* as well as *Archaea*, but not as ubiquitously as CorA. MgtE has been found to transport  $\text{Mg}^{2+}$  and  $\text{Co}^{2+}$ , but (unlike CorA) not  $\text{Ni}^{2+}$ . It remains to be shown if  $\text{Mg}^{2+}$  transport is its physiological role in bacteria.<sup>11</sup>

## Structure and Function of CorA Proteins

CorA proteins constitute a gene family of their own.<sup>11,12</sup> Comparisons of proteins from distantly related prokaryotes reveal very little primary sequence conservation. As a common denominator all CorA proteins have two predicted transmembrane domains, separated by a short loop-oriented to the outside of the membrane, and a YGMN/F motif at the end of the N-terminal of one of these two transmembrane (TM) domains, frequently followed by a MPEL motif in the loop sequence. Mutational studies identified this motif as critical for CorA function in *S. typhimurium* and recognized further residues in each of the two conserved TM domains as important for ion transport and/or for ion selectivity.<sup>13,14</sup> These studies and the finding of a CorA homotetrameric complex were consistent with the formation of  $\text{Mg}^{2+}$ - (as well as  $\text{Ni}^{2+}$ - and  $\text{Co}^{2+}$ -) permeable channels.<sup>15</sup>

Expressing different tagged isomers of partially deleted CorA proteins in *S. typhimurium*, suggested the presence of the large N-terminal protein sequence in the periplasm, and the short C-terminus in the cytoplasm. A third TM segment, N-terminal to the two predicted TM domains near the C-terminus, was postulated according to Smith and colleagues.<sup>16</sup> Yet, this additional TM segment frequently contained charged residues and thus remained questionable. Other CorA-related proteins have been reported to have N-in, C-in orientation, much like the eukaryotic homologues Mrs2p and Alr1p (see below).

A crystal structure of the CorA protein from the bacterium *Thermotoga maritima* has recently been published.<sup>17</sup> It forms a channel, composed of five CorA monomers each of which spans the membrane twice. These TM domains form two concentric rings in the membrane. The inner ring is made up of the five N-terminal domains and forms the pore. The five C-terminal TM domains envelope this inner ring. The short loop connecting the TM domains is oriented towards the outside and presumably contains an ion selective filter. The



short C-terminal as well as the long N-terminal sequence are oriented towards the cytoplasm.

The structure published appears to represent the closed form of the channel. While it is not informative with respect to predict ion conductance values, it reveals details about amino acid residues in the pore, their putative interaction with  $Mg^{2+}$  and their role in gating of the pore. The five large N-terminal domains fold into a funnel, opening from the membrane pore towards the cytoplasm. Clusters of negatively charged or hydroxyl-bearing amino acids inside this funnel may constitute an electrostatic sink for incoming ions. A magnesium binding site has been observed in the N-terminal sequences, involving two neighboring monomers. Metal binding at this site might locally alter helix conformation with possible long-range effects to the membrane pore affecting its conductance.<sup>17</sup>

## Alr1-Type Proteins

MacDiarmid and Gardner<sup>18</sup> screened for genes of which overexpression rendered cells of the yeast *Saccharomyces cerevisiae* tolerant to growth on toxic levels of  $Al^{3+}$ . Two genes, *ALR1* and *ALR2*, were shown to encode closely related proteins with some similarity to the bacterial CorA proteins, particularly two adjacent TM domains near the proteins C-terminus, and a GMN motif in the short loop connecting them. Studies on the cellular topology of Alr1p revealed its location in the plasma membrane.<sup>19</sup> Based on splitubiquitin assays both N- and C-termini were shown to be in the cytoplasm and, accordingly, the TM-connecting loop on the outer side of the plasma membrane.<sup>20</sup>

Disruption of the *ALR1* gene resulted in a dependence of yeast cells for  $[Mg^{2+}]$  less than 20 mM in growth media and led to about twofold reduced levels in intracellular  $[Mg^{2+}]$  and thus suggested a major role of the Alr1 protein in cellular  $Mg^{2+}$  homeostasis. Patch-clamp studies confirmed that Alr1p mediates  $Mg^{2+}$  transport into yeast cells.<sup>9,19,21</sup> Interestingly, turnover of Alr1p via endocytosis and vacuolar degradation were shown to become active when  $[Mg^{2+}]$  in growth media was less than 0.1 mM.<sup>19</sup> Besides transcriptional regulation of *ALR1* expression,  $Mg^{2+}$ -dependent degradation of Alr1p appears to regulate the  $Mg^{2+}$  transport capacity of yeast cells.

Disruption of *ALR2* did not affect viability of yeast cells, but overexpression of Alr2p partially suppressed the growth phenotype of *alr1Δ* mutant cells. This gene is poorly expressed in yeast and, additionally, it appears to have mutations rendering it more inefficient in  $Mg^{2+}$  transport than Alr1p.<sup>18,20</sup>

Alr1-related proteins are ubiquitous in fungi and occasionally found in other lower eukaryotes. They appear to be absent in metazoa and plants.<sup>12</sup> Compared to CorA and to Mrs2 proteins, Alr1-related proteins frequently have extended N-terminal sequences, exceeding those of CorA or Mrs2 proteins by up to 300 residues. These extensions show little sequence conservation within the Alr1-type proteins, except when phylogenetically related fungi are com-



pared, and partial deletions had little effect on  $\text{Mg}^{2+}$  transport activity of Alr1p (Graschopf, unpublished observations).

## Mrs2

A genetic screen for proteins affecting splicing of group II introns in *Saccharomyces cerevisiae* mitochondria led to the identification of Mrs2p, an integral protein of the mitochondrial inner membrane. While Mrs2p had little similarity to CorA proteins, the presence of two adjacent TM domains and the GMN motif suggested it might be a distant relative of this bacterial  $\text{Mg}^{2+}$  transporter. Homologues of the yeast Mrs2 protein were found to be encoded by most eukaryotic genomes. The single functional *MRS2* gene in the human genome codes for a mitochondrial protein. The genome of the plant *Arabidopsis thaliana*, in contrast, contains 15 genes encoding CorA-related proteins. Most of them are Mrs2-like and appear to occur in mitochondrial as well as in plasma membranes.<sup>12</sup>

Evidence for the contribution of Mrs2p in mitochondrial  $\text{Mg}^{2+}$  uptake came from the findings that (1) knockout of the *MRS2* gene (*mrs2Δ* mutant) resulted in functional defects of mitochondria associated with RNA splice defects and reduced  $[\text{Mg}^{2+}]$  and (2) that expression of a bacterial CorA protein in yeast could partially compensate for these mutant effects.<sup>22</sup> Moreover, RNA splicing was restored in isolated *mrs2Δ* mutant mitochondria when provided with high  $[\text{Mg}^{2+}]$  in the buffer, consistent with low intramitochondrial  $[\text{Mg}^{2+}]$  inhibiting RNA processing.<sup>23</sup>

The use of the fluorescent,  $\text{Mg}^{2+}$ -sensitive dye Magfura-2 in isolated mitochondria revealed the presence of a high-capacity influx system into wild-type yeast as well as mammalian mitochondria, resulting in the increase of free  $[\text{Mg}^{2+}]$  from 0.6 mM to about 2 mM within seconds [Fig. 1(B)]. Flux rates were dependent on the mitochondrial membrane potential  $\Delta\psi$  and on the expression level of Mrs2p. The rapid, high-capacity influx was absent in *mrs2Δ* knockout yeast cells as well as in knockdown cultured mammalian cells. A slow  $\text{Mg}^{2+}$  uptake remained to be seen in these mutant mitochondria, which led to steady state  $[\text{Mg}^{2+}]$  of about half of those seen in wild-type cells over extended periods of time. The strict correlation between  $\text{Mg}^{2+}$ -influx rates and expression levels of Mrs2p, and the restoration of this influx by expression of related proteins (human or plant Mrs2p, bacterial CorA) in *mrs2Δ* mutant mitochondria led to the conclusion that this protein constitutes the  $\text{Mg}^{2+}$  transporter.<sup>5,22,24</sup> In addition, a plant Mrs2-type protein was shown to complement a bacterial CorA mutant.<sup>26</sup>

Previous studies on mammalian heart mitochondria also involved the Magfura-2 potentiometric method and determined similar steady state  $[\text{Mg}^{2+}]$  values and revealed a slow  $\text{Mg}^{2+}$  uptake dependent on the extramitochondrial  $[\text{Mg}^{2+}]$ .<sup>2</sup> Stimulation of  $\Delta\psi$  by nigericin addition led to an increased  $\text{Mg}^{2+}$  uptake. Based on their results, Jung and colleagues<sup>2</sup> suggested that mitochondria take up  $\text{Mg}^{2+}$  by electrophoretic flux through membrane leak pathways, rather than via a specific  $\text{Mg}^{2+}$  transporter.

In contrast, Kolisek and colleagues<sup>5</sup> and Piskacek<sup>27</sup> and coworkers observed high-capacity  $Mg^{2+}$  influx into yeast and human mitochondria, mediated by the Mrs2 protein and driven by  $\Delta\psi$ . Both yeast and human Mrs proteins were found to form homooligomeric complexes in mitochondrial membranes.<sup>5,27</sup> Accordingly, they concluded that the Mrs2 protein might form a high-conductance ion channel. Patch-clamp studies involving liposomes fused with yeast mitochondrial membranes now have fully confirmed this notion.<sup>28</sup>  $Mg^{2+}$  conductance of this mitochondrial channel has been found to be surprisingly high (155 pS), exceeding those of most cation channels in plasma membranes. Features of this channel varied with the mutational state of Mrs2p, leaving no doubt that currents measured were mediated by the Mrs2p homooligomer.<sup>28</sup>

The requirement for a high-conductance  $Mg^{2+}$  channel may be specific for bacterial cells and mitochondria with their rapid shifts of adenosine diphosphate/adenosine triphosphate (ADP/ATP) ratios, for example, when switching from anaerobiosis to aerobiosis or upon ischemic reperfusion. The affinity of  $ATP^{4-}$  ( $K_{Mg-ATP} 1 \times 10^{-4}$ ) is close to tenfold greater than that of  $ADP^{3-}$ , and mitochondrial adenine nucleotide concentrations are in the same molar range as free  $[Mg^{2+}]$ . A rapid conversion of ADP to ATP thus is expected to require influx of  $Mg^{2+}$  into mitochondria, and this is supported by experimental data.<sup>29</sup>

## Common Features of CorA, Mrs2, and Alr1 Channel Proteins

Sequence conservation among the many members of the superfamily of proteins is restricted to the very C-terminal part with its two adjacent TM domains of which the N-terminal one ends with a GMN signature motif. All studies place the short loop connecting these two TM domains towards the outer side of the membrane. The long N-terminal and short C-terminal parts are highly variable in length and show very little sequence similarity. The crystal structure of the *T. maritima* CorA protein<sup>17</sup> is oriented towards the cytoplasm (N-in, C-in orientation), and this orientation has also been reported for yeast Alr1p and Mrs2p.<sup>20,22</sup> At variance with these data is the report of an N-out, C-in orientation of the CorA protein of *S. typhimurium*<sup>16</sup> and further experiments will be required to determine if membrane topologies of bacterial CorA proteins can differ.

Mutations in the signature sequence of this superfamily of proteins, GMN, almost invariably abolish transport function.<sup>5,14</sup> It appears to be critical for the positioning of the external loop and to form part of the entrance to the pore. Most proteins exhibit a surplus of negatively charged residues in the external loop, notably a conserved glutamic acid residue at position +6 of those proteins shown to transport  $Mg^{2+}$ . Mutational studies revealed a key role of this residue for  $Mg^{2+}$  transport by yeast Mrs2 and Alr1 channels.<sup>20,28,30</sup> Forming the mouth of the channels, these charged residues may electrostatically attract  $Mg^{2+}$  and may constitute a part of the selectivity filter. Various mutations in the TM domains of *S. typhimurium* have been shown to affect  $Mg^{2+}$  transport function and ion selectivity. A series of mutations in the soluble part of yeast

Mrs2p N-terminal to the TM domains are known to result increased  $\text{Mg}^{2+}$  flux, consistent with a role of these sequences in homeostasis control. A full interpretation of their effects will only be possible when three-dimensional (3D) structures are modeled.

Patch clamping provided physiological evidence for the presence of  $\text{Mg}^{2+}$  permeable channels<sup>28</sup> and will be the method of choice for further characterization of these channel proteins and mutations therein. Conductance of yeast Mrs2p was found to be exceptionally high (155 ps). It may reflect that the physiology of mitochondria (and probably also in bacterial cells) requires rapid  $\text{Mg}^{2+}$  influx to meet fast changes in components with highly different  $\text{Mg}^{2+}$  affinities, for example, shifts of ADP to ATP.

Control of intracellular or mitochondrial  $\text{Mg}^{2+}$  homeostasis is likely to be mediated by the channel proteins themselves.<sup>5</sup> Steady-state  $[\text{Mg}^{2+}]$  in mitochondria is kept rather independent of the level of Mrs2p expression, but mutations in this protein can result in a considerable upshift of free  $[\text{Mg}^{2+}]$  and in  $\text{Mg}^{2+}$  conductance.<sup>28,30</sup> The finding of a putative  $\text{Mg}^{2+}$  binding site in the *T. maritima* structure is consistent with the notion that this cation directly may interfere with the channel to adjust internal  $[\text{Mg}^{2+}]$  to physiological needs of cells and organelles.

## Perspectives

The crystal structure of the *T. maritima* CorA protein forms a basis to model respective folding structures of Alr1 and Mrs2 proteins, although sequence conservation is very low. In combination with already available or future data on effects of mutations in CorA, Alr1, and/or Mrs2, a more detailed picture of structure/function relationships of this interesting protein superfamily can be expected in the near future.

A major focus of future work will be on sequences defining ion selectivity and on  $\text{Mg}^{2+}$  homeostasis control via opening/closing of the channel. Mutations in CorA, Alr1, or Mrs2 proteins with effects on  $\text{Mg}^{2+}$  homeostasis will help to evaluate the contribution of these proteins themselves and of other factors in  $\text{Mg}^{2+}$  homeostasis control. Yet a full understanding of  $\text{Mg}^{2+}$  homeostasis will require the identification of other genes and proteins contributing to the maintenance of physiological  $[\text{Mg}^{2+}]$  and for  $\text{Mg}^{2+}$  extrusion from cells or organelles (presumably  $\text{H}^+/\text{Mg}^{2+}$  or  $\text{Na}^+/\text{Mg}^{2+}$  exchangers).

Finally, mutations in genes encoding the  $\text{Mg}^{2+}$  transport systems will experimentally allow obtaining unphysiologically high or low  $[\text{Mg}^{2+}]$  in cells and organelles and thus will help to recognize and evaluate possible pathological effects of  $\text{Mg}^{2+}$  homeostasis disorders. Mutant CorA bacterial cells arrest growth while the absence of Mrs2p in yeast causes mitochondrial dysfunction, but no growth arrest. In contrast, mammalian cells die when expression of Mrs2p in their mitochondria is severely reduced. Low steady-state  $[\text{Mg}^{2+}]$  obtained with yeast Mrs2 and bacterial CorA mutants indicated that RNA

processing is a  $Mg^{2+}$ -sensitive function, possibly because  $Mg^{2+}$  has a key function in RNA structure formation.<sup>23,31,32</sup> Yet rapid influx of  $Mg^{2+}$  in response to sudden increase of  $Mg^{2+}$  binding components (e.g., ATP) may be equally or even more important for cellular function and a lack of this function may explain lethality observed in mammalian mMrs2 knockdown cells.

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## 6

# Practical Interest of Circulating Total and Ionized Magnesium Concentration Evaluation in Experimental and Clinical Magnesium Disorders

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Magnesium (Mg) is essentially an intracellular ion, which makes it difficult to evaluate Mg status. Both circulating total (MgT) and ionized  $\text{Mg}^{2+}$  (MgI) are used in clinical practice but their respective interest is still debated. In the present review, we list various studies comparing MgT and MgI in either Mg imbalances or Mg status dysregulations. In simple Mg imbalances (either therapeutic Mg overload or Mg deficiency), the evaluation of MgT appears a better marker than MgI because it seems that a subtle physiological homeostatic compensatory reaction modifies the proportion of MgI, the most biologically active fraction of blood Mg, in order to reduce the effects of Mg imbalance. In contrast, in Mg dysregulations (either Mg pathological overload or Mg depletion) both fractions may vary independently, depending mainly on the renal status and on the equilibrium between MgI and Mg complexed with proteins and anions. The choice of the more appropriate marker is discussed.

Magnesium is an essential cofactor in numerous cellular functions.<sup>1</sup> In addition to a balance between intestinal absorption and renal excretion, the bone is involved in magnesium homeostasis.<sup>2</sup> Exchanges between the circulating Mg forms also occur.<sup>1,3</sup>

Although MgI is considered as the bioactive fraction and generally presented as the best marker in Mg imbalances, most laboratories still measure only MgT. In healthy humans, MgT concentration is maintained within narrow limits ( $0.88 \pm 0.05 \text{ mmol/L}^{-1}$ ).<sup>1</sup> MgT consists of three different circulating Mg forms: (1) ionized (65%); (2) complexed to anions (15%) phosphate, bicarbonate, lactate, citrate; and (3) bound to proteins, mainly albumin (20%).

MgI concentration, measured by using Mg-selective electrodes, is about  $0.54 \pm 0.05 \text{ mmol/L}^{-1}$ .<sup>1,3</sup>

The less severe disorders are Mg imbalances. Hypermagnesaemia is unusual, observed after inadequate parenteral Mg treatment and requires only a posologic adaptation. Mg deficiency, resulting from low Mg dietary intake, is more common and may be corrected by dietary supplementation.<sup>3,4</sup>

More worrying, Mg status may be dysregulated.<sup>3,5</sup> Dysregulated hypermagnesemia appear mainly in renal failure. Dysregulated hypomagnesemia (Mg depletion) appears when both Mg deficiency and any type of stress, including clinical or iatrogenic disorders, coexist.

In the present review, the practical interest of both markers were compared in various clinical or experimental situations of Mg disorders.

## Magnesium Imbalances

In magnesium imbalances, MgT varies more importantly than the finely regulated MgI form. Magnesium balance and the ratio  $R$  (MgI/MgT) in serum or plasma are inversely correlated: in Mg excess,  $R$  decreases; in Mg deficiency,  $R$  increases.<sup>3</sup> This might reflect a buffer function of the bound serum fraction, allowing the concentration of the functionally active MgI fraction to stay constant as long as possible to reduce the effects of Mg imbalances.<sup>6</sup> This is the case in magnesium excess<sup>1,7,8</sup> or in experimental and clinical situations of dietary-induced Mg deficiency,<sup>1-3,6,9-13</sup> leading most authors to the conclusion that this mechanism erodes MgI diagnostic usefulness and that MgT is the best clinical marker.<sup>12</sup>

The mechanisms implicated in this adaptative phenomenon are poorly documented but include various factors involved in Mg homeostasis, such as neuroendocrine factors or compensatory inter- or intracell or tissue exchanges. For example, (1) the parathyroid hormone–calcitonin couple and perhaps some digestive polypeptide hormones mainly controlling exchanges between the extra cellular compartment and hard tissues; (2) catecholamines and insulin, especially presiding over exchanges between extracellular components and the soft tissues; (3) taurine and L-glutamyl taurine, which may oppose the harmful intracellular effects of hypersecretion of epinephrine; and (4) a central regulation of magnesemia accounting for the biological links between photo-periods and Mg status.<sup>1,3,14</sup>

## Magnesium Dysregulations

Magnesium dysregulations may occur during failure of the Mg homeostasis controlling mechanisms or after intervention of Mg status disturbing factors. Generally, the compensatory exchanges between blood fractions are insufficient to face the major disturbances induced by pathological or iatrogenic agents.

### *Dysregulated Hypermagnesemia*

Chronic renal failure is known to increase both MgI and MgT, their levels raising progressively with deterioration in renal function.<sup>15-19</sup> Although some



studies reported similar  $R$  in patients and controls,<sup>20–22</sup> most studies showed a mild tendency towards a reduced  $R$  in patients because of a relative decrease in MgI compared to MgT.<sup>16–20,23,24</sup> It has been postulated that, before dialysis, the decreased MgI would result from Mg complexation to anions (inorganic phosphate) or from Mg binding to anionic toxins that accumulate in the setting of uremia<sup>16,18,23</sup> but, even after dialysis that removes these anions, MgI complexation persisted.<sup>22</sup> It may be assumed that in the postdialysis period, the tendency to metabolic alkalosis leads to a higher affinity of albumin for MgI, and consequently to a decrease in MgI, as observed in vitro.<sup>25</sup> These complexations might reflect a trend for the homeostatic adaptation proposed above, even in this critical situation, in order to preserve as long as possible MgI levels.

### ***Dysregulated Hypomagnesaemia***

Magnesium depletion cannot be corrected by a simple oral physiological Mg supplementation, but requires the more specific correction of its causal dysregulation.<sup>1,3</sup> It is often linked with renal loss: when the renal Mg handling is impaired, hypomagnesaemia ensues because, unlike calcium, equilibration with cellular stores (mainly the bones) does not occur for several weeks.<sup>26</sup>

Studies on various diseased human subjects indicate that plasma MgI often exhibit slight significant alterations from normality, despite no change in MgT.<sup>27</sup> Consequently, in most of the following studies taken as examples, circulating MgI is regarded as the best marker for an accurate estimate of Mg status, but this result is sometimes controversial.

### **Postoperative, Injured, and Critically Ill Patients**

This heterogeneous population often suffers hypomagnesaemia, but the fractions concerned vary according to the clinical feature and do not reflect the Mg body stores.<sup>28</sup> Even though some studies in adult intensive care unit (ICU) patients showed a decrease in both MgT and MgI,<sup>29,30</sup> most studies revealed a decrease in MgI (associated with a worse prognosis) with normal MgT concentration.<sup>31–33</sup> The same pattern was also observed after the administration of chelating agents such as citrate with blood transfusions.<sup>34</sup> Consequently, these patients would not be recognized as Mg-deficient based on routine MgT.

Conversely, in the following examples taken among other ICU patients, MgI is unnecessary to detect low Mg levels.<sup>34</sup> For example, after cardiopulmonary bypass and in agreement with our hypothesis, the protein-bound fraction of Mg decreased consecutively to the dilutional hypoalbuminemia following surgery, without affecting the MgI levels.<sup>35,36</sup> Finally, in abdominal surgery, without massive transfusion and no cardiopulmonary bypass, MgI and MgT both decreased. The MgT decrease correlated with the degree of hypoalbuminemia. The MgI decrease may be linked to the intraoperative administration of fluids.<sup>34</sup>



## Cardiovascular Diseases and Risk Factors

Magnesium deficits have been shown to be associated with fatal cardiovascular diseases, such as cardiac arrhythmias and coronary heart diseases, as well as with risk factors for these diseases, such as essential hypertension, hypercholesterolemia, and diabetes mellitus.<sup>37</sup> In all these patient groups, MgT concentration was similar to that of controls, whereas patients with arrhythmia and diabetes mellitus presented a decrease in MgI. In those patients, MgT measurement obscured the diagnosis of an abnormality in Mg metabolism.<sup>37</sup>

Low MgT level has been reported in 25% to 38% of patients with poorly controlled type 1 and type 2 diabetes.<sup>38,39</sup> It is associated with late complications (cardiovascular disease, retinopathy) and might contribute to insulin resistance.<sup>40</sup> Magnesium depletion is related to enhanced urinary loss, associated with the diabetic state.<sup>41</sup> Most studies have reported decreases in MgT, whereas only sparse information is available regarding MgI.<sup>42</sup> Well-controlled type 1 or 2 diabetes patients have MgT generally comparable to controls,<sup>43,44</sup> whereas MgI are significantly lower<sup>44–46</sup> except in one study.<sup>47</sup>

Hypomagnesemia appears during gestation, mainly in diabetics; similar decreases in both MgT and MgI levels were reported in control and diet-controlled diabetic gestational women, whereas the intracellular free Mg decreased only in diabetics, indicative of a true Mg depletion.<sup>48</sup> Infants of either insulin-dependent diabetic mothers<sup>49,50</sup> or gestational-diabetic mothers<sup>51</sup> showed that Mg deficiency plays a role in neonatal hypocalcemia. Hypocalcemia was more accurately correlated through MgI that decreased significantly than using MgT, which remained similar to controls.<sup>49–51</sup>

Finally, in chronic renal failure, one of the major complication of diabetes, both MgI and MgT levels were lower in diabetic than in nondiabetic patients (with a mild tendency towards a reduced *R* in diabetics because of a relative decrease in MgI).<sup>52</sup>

## Kidney Diseases

### Primary Renal Tubular Disorders

They represent unusual and heterogeneous disorders marked by renal hypokalemic alkalosis and various alterations of calcium and Mg homeostasis.<sup>53</sup> According to the authors, MgI determination is of little value in the diagnostic workup of most of these primary disorders because MgI levels generally correlates with either normal or decreased MgT. In contrast, low MgI with normal MgT levels might perhaps disclose latent hypomagnesaemia in nephrocalcinosis of unknown cause.<sup>53</sup>

### Renal Transplants Patients

Hypomagnesemia is a well-documented metabolic derangement of renal transplantation, mainly occurring after surgery, in close correlation with acute tubular necrosis and exposure to high levels of immunosuppressive

drugs.<sup>54–56</sup> However, it frequently persists even in the long term, probably as a consequence of metabolic acidosis, hypercalcemia, hypophosphatemia, increased urinary excretion fraction, diuretics, and cyclosporin A (CyA).<sup>56–58</sup> In feline renal transplants, MgT concentrations were within the normal range, whereas MgI were below normal in the postoperative period.<sup>59</sup> In human transplant patients, the significantly lower MgI was confirmed but only a minority (7%) had really pathologically low values.<sup>60</sup> In other studies taking into account the urinary Mg excretion and the possible role of confounding factors (CyA, acid–base balance, other divalent cations), MgT values were decreased consecutively to a slight hypoalbuminemia or an altered plasma Mg binding (with 26% of patients having pathological levels) and MgI concentrations were also lower (with only 10% of patients having pathologically low values), indicating that the prevalence of a true ionized hypomagnesemia is low, especially in the absence of diuretics.<sup>56,61</sup> In conclusion, the diagnostic of hypomagnesaemia in renal transplant cannot lie only on the determination of plasma MgT or MgI and requires a more complete workup, including evaluation of urinary excretion and search for clinical signs of Mg deficit.

### **Acid–Base Imbalances**

MgI concentrations decrease with increasing pH, indicating the stronger binding of MgI with protein, mainly albumin, in an alkaline environment.<sup>62</sup> In addition, evidence from micropuncture studies indicates that acid–base changes alter active Mg absorption within the distal convoluted tubule (DCT), leading to alterations in urinary Mg excretion: alkalosis stimulates Mg<sup>2+</sup> uptake, whereas acidosis diminishes entry rates.<sup>63</sup>

#### ***Alkalosis***

Respiratory alkalosis induced by 30-min hyperventilation, in healthy subjects, induced no change in MgT and a significant decrease in MgI levels.<sup>64</sup>

Similarly, chemical alkalosis induced by addition of bicarbonate in vitro to neonatal serum decreased MgI.<sup>65</sup>

#### ***Acidosis***

Despite major advances in the care of diabetes, ketoacidosis (DKA) remains a leading cause of morbidity in children and adolescents with type 1 diabetes. In 62% of children with DKA, MgT was significantly lower than in diabetic and nondiabetic control groups but returned to normal value with correction of DKA,<sup>66</sup> whereas MgI was not measured. But in dogs with DKA, MgI was significantly higher than in diabetic and nondiabetic control groups, in agreement with the homeostatic adaptative mechanism.<sup>67</sup>

### **Iatrogenic Dysregulations**

Many therapeutics drugs (diuretics, chemotherapeutics, immunosuppressive agents, some antibiotics) cause hypomagnesaemia due to increased urinary

loss. Thiazide diuretics or tacrolimus chronic administrations result in a similar defect in active  $\text{Mg}^{2+}$  reabsorption, that is, downregulation of the epithelial  $\text{Mg}^{2+}$ -channel transient receptor potential melastatin 6 (TRPM6) localized along the apical membrane of DCT to which active  $\text{Mg}^{2+}$  reabsorption is restricted.<sup>68,69</sup>

### ***Immunosuppressants***

Many studies showed that in renal transplant patients on CyA with allografts functioning stably for more than 6 months, both MgT and MgI were significantly lower than in control or renal transplant patients not on cyclosporin.<sup>61,70–74</sup> The MgI decrease was more pronounced and inversely correlated with urinary Mg and directly correlated with platelet Mg.<sup>71</sup> The MgT decrease was inversely correlated with blood CyA concentration.<sup>74</sup>

### ***Chemotherapeutics***

Patients with transient severe hypomagnesemia due to cisplatin or interleukin-2 therapies had MgT profoundly decreased, whereas R rapidly increased to values up to 93% to 128% of the total, indicative of the previously evoked adaptative mechanism.<sup>3</sup> In that case, MgI measurement failed to detect a severe hypomagnesemia.<sup>75</sup>

### ***Antibiotics: Aminoglycosids***

Renal Mg wasting is a rare complication of treatment with aminoglycosides, which is mostly associated with acute renal failure.<sup>76,77</sup> However, recent observations in animals<sup>78–81</sup> and humans<sup>26</sup> indicate the frequent occurrence of renal magnesium wasting even in the absence of renal failure. For example, in cystic fibrosis patients both MgT and MgI significantly decreased in a rather similar way.<sup>26</sup>

### ***Antiviral Agent: Foscarnet***

As a pyrophosphate analogue, foscarnet is a potent chelator of ionized  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and acutely reduces their corresponding plasma ionized concentrations, whereas the decrease MgT is less severe<sup>82</sup> or absent.<sup>76</sup>

## **Conclusion**

In Mg imbalances or dysregulated hypermagnesemia (renal failure), MgT measurement should be preferred in clinical practice because, in addition to its clinical value, it is available and cheap. In most of the pathological situations leading to Mg depletion, generally by  $\text{Mg}^{2+}$  renal loss, MgI often exhibits slight significant decreases despite no change in MgT. Consequently, circulating MgI is sometimes regarded as the best marker for an accurate estimate of Mg status or considered as having low added value, because the variations are

always discrete and do not reflect the intracellular stores.<sup>5,83</sup> It reflects sometimes only changes in either blood pH or anion concentrations. More frequently, this ultrafiltrable Mg fraction decreases after pathological or iatrogenic renal Mg wasting that cannot be completely reversed by homeostatic corrections involving bone or blood exchanges. However, this review pointed out that in some severe situations, the exchanges between the different blood fractions still occur, thus complicating the MgI measurement accuracy. In addition, technical and chronobiological factors must be considered. Some evidences have shown that MgI may be quite variable and thus not a reliable measure<sup>84</sup>: the MgI assay could be improved with respect to the sensitivity, selectivity, and nonspecific interferences.<sup>83</sup> Finally, because of a circadian rhythm for both markers, the collection of specimens at appropriate times is important.<sup>83</sup>

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# Nutrition

# 7

## Overview of Magnesium Nutrition

Mieko Kimura

Magnesium plays a very important role in wide-range biochemical metabolism, especially fundamental cellular reactions. It is explained that magnesium is essential ion for cofactors of over 300 enzyme systems, for example, as a part of the magnesium–adenosine triphosphate (Mg-ATP) complex or as an enzyme activator in experimental and clinical observations.

In 1932, McCollum and colleagues<sup>1</sup> observed the first symptomatic results from magnesium deprivation in rats and dogs. In a small human population of patients with various diseases, the first clinical depletion was reported by Hirschfelder and Hauary in 1934,<sup>2</sup> and in 1942 Hauary and Cantarow found magnesium deficiency in normal and pathological patients assessed for magnesium serum concentration.<sup>3</sup> Flink and coworkers discovered the clinical depletion of magnesium under prolonged parenteral fluid administration and chronic alcoholism in 1954.<sup>4</sup> Since then, many experimental and clinical studies for magnesium deficiency appeared in cases in unhealthy subjects as clinical disorders with magnesium depletion.

### Chemistry

The atomic number of magnesium is 12; its atomic weight is 24.305. Magnesium is an alkaline earth metal and has an s orbital that can be filled with two electrons, that is, ionized magnesium ( $\text{Mg}^{2+}$ ) and has potential to bind six ligands, which is characteristic for the octahedral structure of magnesium complexes. In nature, magnesium is existent magnesium carbonate ( $\text{MgCO}_3$ ), calcium magnesium carbonate [ $\text{CaMg}(\text{CO}_3)_2$ ], calcium magnesium silicate [ $\text{CaMg}(\text{SiO}_3)_2$ ], calcium magnesium hydroxide [ $\text{Ca}_2\text{Mg}_5(\text{OH})_2$ ], magnesium sulfate ( $\text{MgSO}_4$ ) and magnesium chloride ( $\text{MgCl}_2$ ) etc for magnesium complexes.

### Body Composition

Magnesium is the second most common cation found in the body, and the total body content in healthy adults is about 25 g. It is distributed with 50% to 60% of the total found in the bone and 40% to 50% of the total in the soft tissues,

**TABLE 7.1.** Distribution and concentrations of magnesium in a healthy adult. (Total Body: 833–1170 Mmol<sup>a</sup> or 20–28 g).<sup>2</sup>

Distribution Percentage	Concentration
Bone	0.5% of bone ash
Muscle	3.5–5 mmol/kg wet weight
Other cells	3.5–5 mmol/kg wet weight
Extracellular	
Erythrocytes	1.65–2.73 <sup>d</sup> mmol/L <sup>b</sup>
Serum	0.65–0.88 <sup>d</sup> mmol/L <sup>c</sup>
55% free Mg <sup>2+</sup> , 13% complexed	Ultrafilterable: 0.48–0.66 mmol/L <sup>d</sup>
(citrate, phosphate, etc)	Ion electrode 0.53–0.66 mmol/L <sup>c</sup>
32% bound primarily to albumin	0.1–0.3 mmol/L
Mononuclear blood cells <sup>f</sup>	2.91 ± 0.6 fmol/cell <sup>c</sup>
	2.79 ± 0.6 fmol/cell <sup>b</sup>
	3.00 ± 0.04 fmol/cell <sup>i</sup>
Cerebrospinal fluid	1.25 mmol/L
55% free Mg <sup>2+</sup>	
45% complexed	
Sweat	0.3 mmol/L (in hot environment)
Secretions (saliva, gastric, bile)	0.3–0.7 mmol/L

1 mmol = 2 mEq = 24 mg. <sup>†</sup>Magnesium falls slowly with aging. <sup>‡</sup>Similar at various ages. <sup>§</sup>Data from Hosseini, E.: Trace Elem. Med., 5:47–51, 1988. <sup>¶</sup>Data from Altura: Magnesium Trace Elem., 9:311, 1990. <sup>\*\*</sup>Monocytes and lymphocytes in venous blood. <sup>††</sup>Data from Elin, Hoseini: Clin. Chem., 31:377–380, 1985. 1 fmol = 24.3 fg. <sup>\*\*</sup>Data from Reinhart, et al.: Clin. Clim. Acta, 167:187–195, 1987. <sup>§§</sup>Data from Yang, et al.: J. Am. Coll. Nutr., 9:328, 1990.

as shown in Table 7.1. Although there are limited data for human bone, magnesium content is approximately 200 mmol/kg of bone ash.<sup>5</sup> Magnesium is the most cation mineral in cells, second to potassium. Most intracellular magnesium is in the bound form and in frog muscle cells magnesium is bound to adeonsine triphosphate (ATP) at 5.8 mmol/L, to phosphocreatine at 1.7 mmol/L, to myosin at 0.3 mmol/L, and to free Mg<sup>2+</sup> at 0.6 mmol/L.<sup>6</sup> In the skeleton, one third of intracellular magnesium is exchangeable and may be reserved to maintain normality in the intracellular concentration of magnesium.<sup>5</sup> The extracellular magnesium in humans is only 1% of total body magnesium, that is, 0.7 to 0.9 mmol/L in serum, with 55% being in the free ionized form, 15% in complexes to anions, and 30% bound to proteins (mostly albumin).<sup>5</sup> Magnesium is distributed in all of the parts within cells. That content is 5 to 20 mmol/L and is associated with metabolic activity of cells, for example, 75% in erythrocytes, fundamentally combined with anions with 80% to 90% being bound to ATP.<sup>7</sup> Intracellular free magnesium is an important metabolic regular factor, but only 1% to 5% of total intracellular magnesium—0.3 to 0.6 mmol/L.<sup>8</sup>

Biochemical and Physiological Roles

The chemical character of ionized magnesium (Mg<sup>2+</sup>) binds to anions, and it becomes stable, such as in enzymes, or neutral, such as for substrate use. Magnesium has many biochemically important roles, mainly required as a

cofactor for over 300 vital enzymatic reaction steps.<sup>9</sup> For example, there are the synthesis of protein synthesis, fatty acids, activation of amino acids, phosphorylation of glucose and its derivatives in the glycolytic pathway, oxidative decarboxylation of citrate, and transketolase reactions.<sup>10</sup> For aerobic and anaerobic energy production, for glycolysis that is as a part of the magnesium-adenosine triphosphate (Mg-ATP) complex, and as an enzyme activator, magnesium is demanded.<sup>9</sup> In the glycolytic cycle to convert glucose to pyruvate, seven key enzymes are requested:  $Mg^{2+}$  alone or associated with ATP or adenosine monophosphate (AMP). The role of magnesium as Mg-ATP, magnesium adenosine diphosphate (Mg-ADP), or Mg-GTP, etc., is evident in that intracellular magnesium plays an essential role for cofactor of enzymes in energy storage, transfer, use, and ion transport.<sup>11</sup> Magnesium is also required for a substrate in all enzymes employing ATP or ADP functions, such as pyruvate kinase and hexokinase from glycolysis, adenylate, and guanylate cyclase, and ATP-dependent pumps [sodium-potassium adenosine triphosphatase (Na-K ATPase), calcium-adenosine triphosphatase (Ca-ATPase)].<sup>12</sup>

In other biological functions, magnesium is important, too.  $Mg^{2+}$  has led that regulated changes in its level are compatible with its functions as a physiological modulator affecting cardiac physiology, with coupling of neurotransmitters and enzymes, to receptors, with activation proteins and with modulation of various types of ion channels.<sup>13</sup>

## Absorption, Metabolism, and Excretion

Magnesium homeostasis is preserved by control of intestinal absorption and losses through the urine as other minerals. The excretion process from urine is the most powerful regulator for magnesium control mechanism.

Intestinal magnesium absorption is barely relied on the opposite amount of magnesium ingested in adults or children. Although magnesium is absorbed from the entire intestinal tract, the maximal magnesium absorption is found in the distal jejunum and ileum.<sup>14</sup> In rat studies, magnesium is absorbed in the proximal small intestine and in the lower bowel.<sup>15</sup> An *in vitro* study using isolated segments of intestine, the capability for magnesium absorption was shown in following order: colon = ileum > jejunum > duodenum. But in rats with low magnesium intakes, quantitative colonic absorption may be very important.

It is reported that magnesium absorption is throughout the small intestine in humans, that is, in the experiment using stable  $^{26}Mg$ ,  $^{26}Mg$  is found in the plasma at 1 h after an oral dose and its absorption is 80% within 6 h.<sup>16</sup> In balance studies of healthy men under controlled diet, 380 mg/day magnesium was absorbed on average in net absorption of 40% to 60%, with true absorption 51% to 60% under various foodstuffs. Under higher magnesium levels, daily intakes of 550 to 570 mg or with dietary bran and oxalate, net absorption is estimated at 15% to 36%.<sup>17</sup>

Magnesium goes through the intestinal epithelium following one of three types of mechanisms: passive diffusion; solvent drag, for example, water movement; and active transport. Intestinal absorption of nutrients is based generally

in the active transport system. The higher rate magnesium absorption under low dietary magnesium intakes is done in both an unsaturable passive and saturable active system.<sup>18</sup> In the experiments for humans given an oral  $^{26}\text{Mg}$  and magnesium retention 5 days later,<sup>19</sup> for perfused human jejunum and ileum,<sup>20</sup> for perfused rat small intestine,<sup>21</sup> and for isolated colonic segments of rat intestine,<sup>22</sup> active transport is distinct according to a linear relationship between the rate of nutrient transport and the amount present in the intestine.

The mechanism for intestinal magnesium absorption is not yet made clear. The regulation of magnesium absorption by vitamin D status is adopted based the chemical similarity of magnesium to calcium. Intestinal magnesium absorption is enhanced a little by vitamin D and its metabolites: 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D.<sup>23,24</sup> Magnesium absorption in vitamin D-deficient rats was reduced and by vitamin D repletion reversed,<sup>25</sup> and also promoted magnesium absorption by elevated parathyroid hormone (PTH) levels.

Magnesium absorption is prevented by high calcium concentrations in rats. This interactive effect may be indirect, depending on the reduction of tight junction permeability with calcium and reducing of the paracellular magnesium movement. Magnesium absorption in humans has been not influenced by increased calcium intake (for example, augmenting from 300 mg to 2000 mg),<sup>26</sup> rather, calcium and magnesium have different sites for maximal absorption, so that any effects may be a segment specialty.<sup>27</sup> It is sometimes reported that magnesium absorption was inhibited by phosphate. That is, free phosphate may shape insoluble salt complexes with magnesium or phytate inhibit magnesium absorption.<sup>28</sup> Fiber-rich foods induce lower magnesium absorption, possibly because there is an independent effect of fiber or an effect from the phytate content in food.<sup>29</sup>

The kidney plays an important role as the principal organ in magnesium homeostasis, which is controlled in renal excretion.<sup>30</sup> Renal excretion of magnesium is reduced in a low-magnesium diet, but output of other minerals is not affected by low magnesium intake and the effect will rapidly adapt.<sup>31</sup>

Renal magnesium re-absorption is carried out in the thick leading loop of Henle and the distal convoluted tubule. Renal magnesium handling in humans is done in a filtration-re-absorption process to be no tubular secretion of magnesium. The filtrated magnesium is re-absorbed about 65% in the loop of Henle by active transport, namely a decrease of magnesium re-absorption is independent of sodium chloride transport in either hypermagnesemia or hypercalcemia.<sup>32</sup> The re-absorption of magnesium (20%–30%) in the proximal convoluted tubule is passive and mainly paracellular, associated with the changes in salt and water re-absorption, and with the rate of fluid flow. So the tubule in humans has a positive charged rather than a serosal cell side movement; the electrochemical gradient is down. This paracellular pathway has especially high permeability for magnesium and some calcium. High magnesium on the peritubular site of cell is induced on increasing excretion, so that it inhibits reabsorption of magnesium. High calcium in plasma also inhibits magnesium reabsorption. Two means of magnesium reabsorption in paracellular are recognized, because magnesium can alter reabsorption and calcium alters reabsorption of calcium, magnesium, and water.

The reabsorption of magnesium in the loop of Henle is an active transport; namely, a decrease in magnesium reabsorption is independent of sodium chloride transport in either hypermagnesium or hypercalcemia.<sup>33</sup> Magnesium reabsorption in the proximal convoluted tubule is less important than in the loop of Henle, but it is very efficiently routed. That is, magnesium that is divided in the proximal convoluted tubule has a reabsorption rate of 80%. This result suggests that for magnesium absorption, both reabsorption routes are important, but there are remarkable differences in the transport mechanism in these sites. The specific pathway controlled by the receptor is sensitive to magnesium and calcium may not be in the peritubular side of the loop of Henle.<sup>33</sup> The genetic disease for the autosomal recessive renal hypomagnesium with hypercalciuria may induce the specific magnesium reabsorption. This disease is caused by a mutation in the gene encoding the protein paracellin-1.<sup>34</sup> Paracellin-1 is in the tight junction of the loop of the Henle. That is, it has to do with the claudin family of tight junction proteins. An affinity for  $Mg^{2+}$  is bestowed by the negatively charged extracellular sphere. Numerous hormones, such as PTH, calcitonin, glucagons, and vasopressin, achieve simultaneously reabsorption of paracellular magnesium in the loop of Henle by way of their cell surface receptors, which increase paracellular flux. But it is not clear if the effect on paracellin-1 function come from serum magnesium or hormones. Magnesium reabsorption in the proximal convoluted tubule also is found against an electrochemical gradient, where the transport may be active and energy dependent. And so magnesium transport in the proximal convoluted tubule is stimulated by hormones; PTH, etc., as same as in the loop of Henle.<sup>35</sup>

Magnesium in urine decreases to less than 20 mg/day within 3 to 4 days in experimental humans magnesium deficiency.<sup>36</sup> Parathyroid hormone (PTH) may be not important for regulation of magnesium homeostasis in patients, for usually with primary hyper- or hypoparathyroidism, normal serum magnesium concentrations and a normal tubular maximum for magnesium are shown.<sup>37</sup> Glucagons, calcitonin, and ADH affect magnesium transport in the loop of Henle in a similar system to PTH, with no clear evidence of physiological action mechanisms.<sup>38</sup>

Excess alcohol intake may cause renal magnesium loss. All chronic alcoholics have been shown to have symptoms of magnesium depletion. But the evidence is not clear.<sup>39</sup> Hypermagnesuria may be caused by medical treatment using diuretics (commonly used for treatment of hypertension, heart disease, and other edematous states).<sup>40</sup>

## Magnesium Deficiency

### *Experimental Animal Magnesium Deficiency*

Magnesium depletion leads to specific biochemical abnormalities and clinical symptoms. McCollum and coworkers in 1931 reported at first the characteristic syndrome of magnesium deficiency in rats eating a diet of only 2 mg percentage magnesium.<sup>1</sup> After, the Johns Hopkins investigators clarified some changes in animal organism under dietary magnesium insufficiency, and also

magnesium deficiency in rabbits,<sup>41</sup> dogs,<sup>42</sup> and calves,<sup>43</sup> etc., were found. In mice eating the same diet as rats hyperemia did not appear, but hypocalcemia followed by hypomagnesemia did, and the mice frequently died under sudden and strong convulsion.<sup>44-46</sup>

The dilatation of cutaneous vessels, hyperirritability, and convulsive seizures are shown in the specific syndrome of magnesium depletion developed. The convulsive seizures in almost every animal appeared for first attack. The following syndrome in magnesium depletion appears: the excitable animals startle by sound and rush at rapid speed, and usual acutely fatal, tonic-clonic conversion. The entire body of animal stiffens as head stretched back, forelimbs extend to upper joints and flex to the metacarpophalangeal joint, and hind limbs extend backward. Often the teeth clench and perforate the tongue. Next, waxy skin appears. The respiratory movements suspend during the attack and come back with the relaxation of the musculature. Additionally, other changes in animals under a magnesium-deficient diet are reduced growth rate, alopecia, and more edema, hypertrophic gums, leukocytosis, and splenomegaly. Thymic abnormalities also appear, that is, malignant lymphosarcoma, disseminated lymphoblastic leukemia, or atrophy.<sup>47</sup> Usually serum in magnesium deficiency is with high calcium level and low PTH.

### ***Experimental Human Magnesium Deficiency***

The experimental diet included 9.6 mg magnesium per day, that is, following a baseline period with complete diet including adequate magnesium can induce signs and symptoms in magnesium depletion. Plasma magnesium fell gradually to 10% to 30% of those during control periods. Erythrocyte magnesium decreased more slowly and to lesser levels. Urine and fecal magnesium went down to very low level within 7 days. In all patients with these symptoms, hypomagnesium, hypocalcemia, and hypokalemia were shown. Positive calcium balance was found in good intestinal absorption of calcium and low urinary excretion. For increase of urinary losses, hypokalemia and negative potassium balance resulted in almost subjects. These subjects keep in positive sodium balance. These symptoms and signs reversed with re-addition of magnesium. In repletion of magnesium, serum magnesium concentration quickly return to normal range but serum calcium and potassium do not rapidly rise, and to return to baseline levels, a week or more is needed. Potassium balances are positive and sodium balances are negative.

### ***Magnesium Deficiency in Humans***

Clinical conditions contributing to magnesium depletion are shown in Table 7.2. The symptoms of magnesium deficiency in human may relate to disease states. These will depend on declined intake and intestinal or renal malabsorptive conditions.<sup>48,49</sup>

In clinical magnesium deficiency, the symptoms and signs are the same as in above experimental deficient cases of hypomagnesemia. The clinical signs for magnesium depletion are, for example, tremor, muscle spasm, muscle fasciculations, normal or depressed deep tendon reflexes, personality changes,

**TABLE 7.2.** Clinical conditions contributing to magnesium depletion.<sup>2</sup>**Malabsorption syndromes**

Inflammatory bowel disease  
 Gluten enteropathy; sprue  
 Intestinal fistulas, bypass, or resection  
 Bile insufficiency states, e.g., ileal dysfunction with steatorrhea  
 Immune diseases with villous atrophy  
 Radiation enteritis  
 Lymphangiectasia; other fat absorptive defects  
 Primary idiopathic hypomagnesemia  
 Gastrointestinal infections

**Renal dysfunction with excessive losses**

Tubular diseases  
 Metabolic disorders  
 Hormonal effects  
 Nephrotoxic drugs

**Endocrine disorders**

Hyperaldosteronism  
 Hyperparathyroidism with hypercalcemia  
 Postparathyroidectomy ("hungry bone" syndrome)  
 Hyperthyroidism

**Pediatric genetic and familial disorders**

Primary idiopathic hypomagnesemia  
 Renal wasting syndrome  
 Bartter's syndrome  
 Infants born of diabetic or hyperparathyroid mothers  
 Transient neonatal hypomagnesemic hypocalcemia

**Inadequate intake, provision, and/or retention of magnesium**

Alcoholism  
 Protein-calorie malnutrition (usually with infection)  
 Prolonged infusion or ingestion of low-magnesium nutrient solutions or diets  
 Hypercatabolic states (burns, trauma), usually associated with above entry  
 Excessive lactation

anorexia, nausea, vomiting, Frank tetany, myoclonic jerks, athetoid movements, convulsions, etc.<sup>50</sup> Magnesium supply was recovered tetany or convulsions with hypomagnesemia, hypomagnesuria, hypocalcemia, and hypokalemia. Only calcium and vitamin D supplies were not affected on improvement of calcemia without magnesium supply.

On the other hand, magnesium depletion in clinical practices can be found. A hypomagnesemia is generally led by surgery, and more severe cardiac surgery. This reason is not clear now. Mass blood transfusions, administration of large volumes of magnesium-free fluids, binding to free fatty acids, and catecholamine-induced intracellular magnesium shifts may be some of the causes.

Magnesium imbalance based clinical treatments may also lead magnesium depletion. For example, cellulose phosphate is used to limit calcium absorption, also bind to magnesium and limit to its absorption or re-absorption. Many common procedures—infusion of normal saline, the use of osmotic diuretics, treatment loop diuretics (furosemide, bumetanide, ethacrynic acid, etc.)—inhibit liminal Na-Cl-K channels in the loop of Henle, causing increas-



ing magnesium excretion. More pharmacological agents may also lead to renal magnesium excretion, for example, cisplatin causes hypokalemic metabolic alkalosis, aminoglycosides promote renal magnesium losses in consequence of secondary hyperaldosteronism, cyclosporine causes a mild and asymptomatic hypomagnesemia. The patients under severe burns also have magnesium losses through skin.

A number of genetic disease are associated with magnesium deficiency: (1) hypomagnesemia with secondary hypocalcemia causes reduced magnesium absorption from intestine; (2) primary hypomagnesemia with hypercalciuria causes increased renal magnesium loss and paracellin-1 mutation; (3) renal hypomagnesemia 2 causes excess renal magnesium loss; (4) Barter syndrome causes mutations in K channel ROMK or Na-K-Cl cotransporter SLC12A1; (5) Gitelman syndrome causes mutations in thiazide-sensitive Na-Cl cotransporter.<sup>51</sup> Metabolic acidosis increases renal magnesium excretion. Both primary and secondary hyperaldosteronism bring augmentation of the extracellular fluid volume that increase renal magnesium loss. Inappropriate antidiuretic hormone secretion also brings high renal magnesium excretion. Parathyroidectomy and diffuse osteoblastic metastasis show hungry bone syndrome, that is, excess bone formation was done drawing magnesium from serum. For example, under Crohn's disease, Whipple's disease, ulcerative colitis, celiac disease, and short bowel syndrome, magnesium absorption is reduced by diarrhea or steatorrhea, etc.<sup>52</sup>

Hypomagnesemia is found in alcoholics.<sup>53</sup> The reasons may depend on generally reduced food intake and poor nutritional status in alcoholics. Malabsorption of magnesium in alcoholics is often found under diarrhea, vomiting, and steatorrhea caused by liver disease and chronic pancreatitis, and renal magnesium excretion may be led by alcoholic ketoacidosis, phosphophosphate-mia, and hyperaldosteronism.

## ***Marginal Deficiency with Various Diseases***

### **Cardiovascular Disease**

Many reports for possible biochemical roles of magnesium in cardiovascular function are shown.<sup>54–56</sup> Kobayashi and colleagues<sup>57</sup> reported that coronary artery disease was frequent in populations living in hard water (i.e., calcium- and magnesium-rich) areas, compared with those in soft water areas, based epidemiological survey.<sup>58–61</sup>

It is reported that after an acute myocardial infarction serum, magnesium level is quick to decline. But these facts may occur under marginal deficiency by low magnesium intake. And as these subjects have also been given many clinical treatments in which hypomagnesemia may be caused, its cause and result are not clear. Seelig and coworkers reviewed the results of a large number of studies for magnesium in acute myocardial infarction, which still show this as an open question.<sup>62–64</sup>

Magnesium deficiency has been associated with significantly increased serum/plasma triglycerides, phospholipids, variable total cholesterol, low-

density cholesterol, and oleic and linoleic acids, and decreased stearic and arachidonic acids. The effects of magnesium deficiency on tissue levels of total lipids and fatty acids were evaluated. Serum cholesterol and total phospholipids were significantly higher in the magnesium-deficient rats than in the controls. Edema and polycystic degeneration of the kidneys were present in the magnesium-deficient rats. The main change in tissue fatty acid composition in magnesium deficiency was the higher docosahexaenoic acid in serum, liver, and aorta than in controls.<sup>65</sup>

In the magnesium-deficient rats, the percentage composition of triglycerides in very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) was elevated and that of protein was reduced. Although the proportion of cholesterol was reduced in LDL and HDL, that of phospholipid was decreased only in HDL. Magnesium deficiency induced a decrease in the percentage composition of apolipoprotein E (apo E) and a relative increase in the apo C for VLDL. In HDL from magnesium-deficient rats, the percentage composition of oleic and linoleic acids was increased.<sup>66</sup>

The mean prostanoids levels in plasma and tissue were significantly higher in magnesium-deficient rats than in controls. The increased synthesis of prostanoids is apparently linked to enhanced influx and translocation of  $\text{Ca}^{2+}$  into the cells. It is possible that the changes in prostaglandin synthesis in magnesium deficiency are linked to the development of different diseases.<sup>67</sup> Deficiency of magnesium with cardiovascular effects tend to be related to alterations in the biosynthesis of prostaglandins in the vasculature.<sup>68,69</sup>

Extensive experimental and clinical studies in animals and humans during the past 25 years have yielded considerable data regarding the availability of internal magnesium stores for the maintenance of extracellular and critical tissue concentrations of magnesium during periods of magnesium deprivation. The bulk of body magnesium is present in bone and skeletal muscle, and it is these two sites that provide essentially all the available magnesium. In the rat, approximately 15% of bone magnesium (equivalent to 1.5 mmol/kg body weight) can be lost, whereas less than one tenth of that amount is available from skeletal muscle. In humans, up to 35% of bone magnesium can be lost, but the mean loss in extant studies is 18% (equivalent to 1.2 mmol/kg body weight). In contrast to the rat, up to 40% human skeletal-muscle magnesium can be lost with an average loss of 15% (equivalent to 0.45 mmol/kg). In the human, the bone and skeletal-muscle magnesium pools can provide an average of 1.7 mmol/kg body weight, which is equivalent to 15% of total body magnesium. Release of magnesium from these stores appears to depend on the presence of hypomagnesemia, which may also result in small, significant, and potentially adverse magnesium losses from certain vital organs, such as the heart, kidney, and brain. The liver and other organs appear not to lose magnesium despite magnesium deprivation although intracellular magnesium shifts of importance cannot be ruled out. These data indicate that the body reserve to combat magnesium depletion is not designed to protect the extracellular magnesium pool and certain critical organs from magnesium deficiency. A continuous optimal intake of magnesium is needed for good nutrition and health.

## Blood Pressure

The relationship between blood pressure and magnesium intake was reported.<sup>70,71</sup> Serum concentrations of magnesium were similar between the hypertensive group and the control group, but urinary excretion of magnesium was significantly lower in hypertensive subjects and there was an inverse correlation between magnesium excretion and blood pressure.<sup>72</sup>

About 3 h after 200 mg Mg was infused into normal humans, serum magnesium level went on average from 0.83 mmol/L to 1.75 mmol/L, and systolic and diastolic blood pressure were in a drop. After 200 mg or Mg magnesium was infused into a normal human for about 3 hours, the serum magnesium level increased on average from 0.83 mmol/L to 1.75 mmol/L. Systolic and diastolic blood pressure dropped. The magnesium infusion also increased renal blood flow and urinary 6-keto-PGF1  $\alpha$  excretion. These data suggest that prostacyclin release via changes in Ca<sup>2+</sup> flux may be the mechanism of magnesium vasodilatory action. Magnesium loading blunted the rise in BP and the aldosterone-stimulating effect of AII, whereas magnesium depletion significantly enhanced these AII effects. These results support the hypothesis that magnesium may be an antagonist of the pressor and steroidogenic effects of AII.<sup>73,74</sup> Also, as a result of infusing MgCl<sub>2</sub> in healthy subjects for 1.5 hours, PTH concentration declined. Plasma rennin activity and rennin increased.<sup>75</sup> For patients with uncomplicated mild to moderate primary hypertension receiving MgO 3 times a day at a daily dose of 1.0 g, oral magnesium reduced significantly the systolic, diastolic, and mean blood pressure. After magnesium supplementation intraerythrocyte sodium concentration was reduced and intraerythrocyte magnesium concentration was increased. The diminution of the blood pressure correlated positively with the reduction in intraerythrocyte sodium after magnesium. However, our results have shown that blood pressure response to oral magnesium was not homogeneous. Intraerythrocyte potassium and calcium, serum aldosterone, plasma renin activity, and urinary sodium excretion remained unchanged after magnesium supplementation.<sup>76</sup> Touyz et al. studied the relationships between serum and erythrocyte magnesium and calcium concentrations and the plasma renin activity in black and white hypertensive patient. The relationship among the plasma renin activity, magnesium, and calcium may be more important in white than in black hypertensive patients.<sup>77</sup> Dyckner et al. suggested that the effect of magnesium on blood pressure may be direct or through influences on the internal balance of potassium, sodium, and calcium.<sup>78</sup> There is a report too that in magnesium-repleted hypertensive subjects, magnesium supplementation does not affect blood pressure or lipids, probably because magnesium has no effect on cellular cation metabolism in magnesium-replete individuals.<sup>79</sup> Dietary magnesium intake does not appear to play an important role in long-term regulation of blood pressure in rats; normotensive Wistar-Kyoto rats and spontaneously hypertensive rats.<sup>80,81</sup>

In a survey of 3,318 men and women who lived in rural China, it was reported that supplementation with a multivitamin/mineral combination may have reduced mortality from cerebrovascular disease and the prevalence of

hypertension in this rural population with a micronutrient-poor diet.<sup>82</sup> A “combination” diet that emphasized fruits, vegetables, and magnesium-rich and low-fat dairy products provides significant round-the-clock reduction in BP, especially in 354 hypertensive participants.<sup>83</sup>

### Diabetes

For many years magnesium loss in diabetes patients was well known. Type 2 diabetes patients are 25% to 38% hypomagnesemic,<sup>84</sup> and have associated with low serum magnesium and low magnesium intake.<sup>85</sup> A low magnesium concentration in nondiabetic subjects was associated with relative insulin resistance, glucose intolerance, and hyperinsulinemia.<sup>86</sup> Long-term (4 weeks) studies are needed to determine whether magnesium supplementation (15.8mmol/day) is useful in the management of type 2 diabetes.<sup>87</sup> Insulin-dependent diabetic pregnant women are at risk for magnesium deficiency, predominantly because of increased urinary magnesium losses.<sup>88</sup> A magnesium-supplemented diet did not reverse diabetes once already established in rats. An increased dietary magnesium intake in male obese Zucker diabetic fatty rats prevents deterioration of glucose tolerance, thus delaying the development of spontaneous NIDDM.<sup>89</sup>

### Osteoporosis and Bone

Recently, there is evidence that magnesium depletion plays a important role as a cause of osteoporosis in humans, and magnesium supplementation is beneficial in preventing and treatment of osteoporosis. It is evident that magnesium plays a major role in bone and mineral homeostasis, especially in its direct effects on bone-cell function and its influence on hydroxyapatite crystal formation and growth.<sup>90</sup> Magnesium effects mineral metabolism in hard and soft tissues indirectly through hormonal and other modulating factors, and directly effects the processes of bone formation and resorption and of crystallization (mineralization). Its causative and therapeutic relationships to calcium urolithiasis are controversial, despite an association between low urinary magnesium and urolithiasis. A tendency to low serum and/or lymphocyte magnesium levels exists in urolithiasis. There is an inhibitory effect of magnesium supplementation on experimental urolithiasis in animals and in spontaneous urolithiasis in humans. Small decreases in serum and/or erythrocyte magnesium in osteoporotic patients and improved bone mineral density with a multivitamin supplement rich in magnesium have been reported.<sup>91</sup> Magnesium depletion adversely affects many phases of skeletal metabolism and has been implicated as a risk factor in several forms of osteoporosis in the survey study for 60 patients. A highly significant decline in serum magnesium concentrations, associated with continued urinary magnesium losses, occurs after cardiac transplantation. Patients with low serum magnesium levels had significantly lower rates of bone loss, lower serum PTH concentrations, and lower bone turnover.<sup>92</sup>

The mean bone density on magnesium-deficient postmenopausal patients who received two to six tablets daily of 125mg each of magnesium hydroxide for 6 months and two tablets for another 18 months increased significantly after one year and remained unchanged after two years. The mean bone density of the

responders increased significantly both after one year and two years, while in untreated controls, the mean bone density decreased significantly. The disparity between the initial mean bone density and bone density after one year in all osteoporotic patients and in the responders differed significantly from that of the controls.<sup>93</sup> In a study of healthy premenopausal women with higher intakes of zinc, magnesium, potassium, and fiber, bone mineral density was significantly higher, and a significant difference in bone mineral density was also found between the lowest and highest quartiles for these nutrients and vitamin C intake. High, long-term intake of these nutrients may be important to bone health, possibly because of their beneficial effect on acid-base balance.<sup>94</sup> Osteoporosis, a major health problem in all Western countries, is a condition with many dietary factors. No significant correlation was found between current calcium intake and bone mass at any site, and iron, zinc, and magnesium intake were positively correlated with forearm bone mineral content in Caucasian premenopausal women. Iron and magnesium were significant predictors of forearm bone mineral content in premenopausal and postmenopausal women, respectively. Bone mass is influenced by dietary factors other than calcium.<sup>95</sup> It is supporting that alkaline-producing dietary components, specifically potassium and magnesium, fruit, and vegetables, contribute to maintenance of bone mineral density.<sup>96</sup> Increasing the mean magnesium intake in healthy young adult females above the usual dietary intake, which is currently above the U.S. estimated average requirement (EAR), but below the U.S. recommended dietary allowance (RDA) for magnesium, does not affect blood pressure or the rate of bone turnover.<sup>97</sup>

### **Parathyroid hormone**

PTH release and end-organ responsiveness to parathyroid extract were evaluated in women with magnesium deficiency associated with hypocalcemia and inappropriately low levels of serum immunoreactive PTH, which increase very rapidly with magnesium infusion. The evidence provided that the release of parathyroid hormone is impaired in magnesium deficiency and that the level of circulating calcium required for the suppression of parathyroid hormone secretion is lower than that in normal subjects.<sup>98</sup> Shils said that magnesium depletion was associated with a failure of parathyroid gland to either manufacture or secrete the hormone, so that both immunoreactive PTH secretion and bone reactivity to parathyroid extract were involved.<sup>99</sup> A response to PTH in magnesium deficiency are shown also in many reports. The hypercalcemic reaction in magnesium deficient animals has been noted, so that parathyroid hormone levels in rats responded to the rising calcium levels induced by the magnesium deficiency. In magnesium depleted rats, plasma magnesium levels were significantly decreased. A fall in plasma phosphate paralleled the decrease in plasma magnesium, and plasma calcium levels were significantly increased after 14 days of magnesium deficiency. A significant rise in plasma parathyroid hormone was observed on days 7 and 14 after magnesium deficiency in Rayssiguier's study.<sup>100</sup> The results over a 30-day period in rats maintained on a magnesium-deficient diet indicate that: (1) in the rat, an increase in parathyroid hormone secretion occurs early in the genesis of magnesium deficiency in the presence of a modest increase in serum

calcium; however, the subsequent further increase in serum calcium counteracts the stimulatory effect of hypomagnesemia on parathyroid hormone secretion; (2) unlike the human parathyroid gland, the rat parathyroid gland responds appropriately to both hypo- and hypercalcemia in magnesium deficiency; and (3) the hypercalcemia that occurs in the magnesium-deficient rat is not due to increased parathyroid hormone secretion and must be accounted for by another mechanism.<sup>101–103</sup>

The very rapid increase in serum immunoreactive parathyroid hormone produced by magnesium infusion suggests an effect of magnesium on hormone secretion rather than an effect on hormone synthesis, and the release of parathyroid hormone is impaired in magnesium deficiency and that the level of circulating calcium required for the suppression of parathyroid hormone secretion is lower than that in normal subjects.<sup>104</sup> Impairment of receptor responsiveness to parathyroid hormone of the osteoclasts follows with reduction of active bone resorption.<sup>105</sup> Despite increased levels of circulating parathyroid hormone, hypocalcemia progresses. An effect may play a role in the hypocalcemia associated with magnesium depletion *in vivo*.<sup>106</sup> Under progressed magnesium depletion, secretion of parathyroid hormone goes down to a very low level, but there are adequate intraparathyroid gland hormonal reserves.<sup>107</sup> Very low circulating parathyroid hormone, unresponsive bone, hypocalcemia, hypocalciuria, hypokalemia, sodium retention, neuromuscular, and other clinical symptoms and signs are indications of severe magnesium depletion.<sup>108</sup>

The calcemic effect of vitamin D—even in high dose—is blunted in the presence of magnesium depletion in human rickets, in human malabsorption, in vitamin-D-deficient animals, and in idiopathic or surgically induced hypoparathyroidism.<sup>109</sup>

### Other Diseases with Magnesium Depletion

Several other diseases with reduced serum magnesium level have been found, such as cerebral palsy, migraine, headache, asthma, and intrauterine fetal growth retardation. These situations will be improved mostly by supplementation with magnesium in at-risk populations. But this treatment has not been completed for all of them, and the link between cerebral palsy and magnesium status is shown on very low magnesium status, as little as 20% of normal body accrual.<sup>110</sup>

Magnesium was neuroprotective in many but not all of a variety of experimental studies and has a variety of biological effects that might account for its benefit.<sup>111</sup> Administration of magnesium sulfate during labor may protect against the development of neonatal brain lesions and cerebral palsy in low birth weight infants. Magnesium exposure may be associated with a reduction in the risk of cerebral palsy in low birth weight infants who have late-onset brain lesions, but this unpredicted observation requires confirmation in another data set.<sup>112</sup> The importance of magnesium in the pathogenesis of migraine headaches has been clearly established by a large number of clinical and experimental studies. Magnesium concentration has an effect on serotonin receptors, nitric oxide synthesis and release, NMDA receptors, and a variety of other migraine related receptors and neurotransmitters. Refractory patients can sometimes benefit from intravenous infusions of magnesium sulfate.<sup>113</sup> Infusion of magnesium

into patients with documented low-ionized magnesium results in rapid and sustained relief of acute migraines in human.<sup>114</sup> There is some evidence that magnesium, when infused into asthmatic patients, can produce bronchodilation in addition to that obtained from standard treatments. Current evidence does not clearly support routine use of intravenous magnesium sulfate in all patients with acute asthma. Magnesium sulfate appears to be safe and beneficial for patients who present with severe acute asthma.<sup>115</sup>

There were 12 interventions, including protein-energy, vitamin, mineral, and fish oil supplementation, as well as the prevention and treatment of anemia and hypertensive disorders. A primary concern is the limited data supporting the effectiveness of recommended nutritional interventions during pregnancy, some of which are widely used even in women without nutritional deficiencies. Magnesium is one of many nutrients.<sup>116</sup> The patients with malabsorption and magnesium depletion had subnormal levels of serum citrate and magnesium, decreasing 24-hour levels of urinary citrate, magnesium, and calcium, and excessive levels of urinary oxalate. Daily citrate excretion averaged only 15% normal. The hypocitraturia in the patients resulted from a subnormal filtered load of citrate and abnormally high net tubular reabsorption of the anion. An oral citrate supplement raised both the serum concentration and the filtered load of citrate to normal fasting values, but net tubular reabsorption remained abnormally high and urinary excretion abnormally low.<sup>117</sup>

## Assessment of Magnesium Nutritional Status

### *Serum Magnesium*

Magnesium plays an essential role in both extracellular and intracellular metabolism. Some clinicians recommend routine measurement of magnesium when other electrolyte analyses are required for the care of patients. The assessment of magnesium status is difficult because of the distribution of magnesium within the intracellular compartment. The serum magnesium level may not reflect intracellular magnesium availability. For assessment of magnesium status, measurement of serum magnesium is most easy and commonly adopted. The serum magnesium concentration may be influenced by changes in serum pH, serum albumin, other anionic ligands. Serum magnesium level is held within a tight range (1.7–2.2 mg/dL) by homeostasis mechanism. A serum magnesium concentration under 1.7 mg/dL indicates usually a limit of magnesium depletion.<sup>118</sup>

The magnesium in adult human body is mostly in bone and soft tissue. Only about 0.3% of the total body magnesium is present in serum, yet the majority of analytical data obtained is from this body fluid. Assessing the magnesium status of an individual is difficult, as present, no simple, rapid, and accurate test determines intracellular magnesium, but determination of total and free magnesium in tissues and physiological tests provide some information. Changes in magnesium status have been linked to cardiac arrhythmias, coronary heart disease, hypertension, and premenstrual syndrome. A better understanding of magnesium transport and of factors controlling magnesium



metabolism is needed to elucidate the role of magnesium in disease processes.<sup>119</sup> Dietary magnesium depletion induced experimentally leads to decreased serum magnesium values in healthy subjects. Under certain circumstances, serum magnesium is a sensitive indicator of magnesium nutritional status.<sup>120</sup> Hypomagnesemia usually follows with severe magnesium depletion (serum magnesium under 1.7 mg/dL). In clinical conditions such as in diabetes mellitus,<sup>121</sup> in alcoholism,<sup>122</sup> or in malabsorption,<sup>123</sup> etc., magnesium concentration in blood cells, bone cells, and muscle cells are very low, but serum magnesium concentration is within normal range. Thus, for magnesium nutritional assessment of humans serum magnesium concentration is not suitable and intracellular magnesium may be a better indicator.<sup>124,125</sup> Ryzen and colleagues also said that magnesium deficiency might play a role in the pathogenesis of atherosclerosis, cardiac arrhythmias, and coronary spasm. Because less than 1% of magnesium (Mg) is extracellular, the serum magnesium (sMg) does not always accurately reflect intracellular magnesium stores.<sup>126</sup>

The 8251-subject, 10-year followup in the National Health and Nutrition Examination Survey (NHANES I) was to assess the important roles of modifiable dietary and behavioral characteristics in the causation and prevention of coronary heart disease. Serum magnesium levels in 492 coronary heart disease patients selected from this group were under 1.9 mg/dL with risk factor for cardiovascular disease.<sup>127</sup> Based these results, we must discuss whether it is suitable or not for the normal range of serum magnesium level to be 1.8 to 2.3 mg/dL.<sup>128</sup>

### ***Serum Ionized Magnesium***

Ion-specific electrodes were coming available for determining of ionized magnesium in serum or plasma that is physiologically active. Ionized magnesium value in serum may be a better index for magnesium status than the total magnesium concentration, but may not to be most suitable to assess magnesium status and more studies are needed.<sup>129–131</sup>

### ***Intracellular Magnesium***

The total magnesium content of tissues such as red blood cells, skeletal muscle, bone, blood cells, and lymphocytes have been pointed out for index of magnesium nutritional status.<sup>132–134</sup> Determination of intracellular magnesium level should be a physiologically adequate measurement of magnesium status, that is, it plays a critical role in enzyme activation within the cell. But tissue and cell samples are not routinely collected in clinical spots. Between serum magnesium concentration and intracellular magnesium level a good correlation was generally not found.<sup>135–138</sup>

Lymphocyte magnesium concentration does not good agreement with serum or red blood cell magnesium levels and also does not reflect muscle magnesium.<sup>139,140</sup> Lymphocyte and skeletal muscle magnesium correlated well in healthy subjects, but not in patients with congestive heart failure.<sup>141,142</sup> For magnesium assessment, lymphocyte or muscle magnesium content may be best, but more studies will be needed. The blood cells provide the most accessible source of cells for determining intracellular magnesium. Magnesium



content of lymphocytes correlates with skeletal and cardiac muscle magnesium content<sup>143</sup> and intracellular ionized magnesium level in patients with high risk for magnesium depletion are lower than in healthy subjects.<sup>125,144</sup>

### ***Magnesium Tolerance Test***

A more intensive intervention test is the magnesium load or tolerance test.<sup>145</sup> This assessment tool is proper for adults but not for infants and children.<sup>146</sup> In this test, magnesium content in 24-h urine after magnesium loading by intravenous infusion is measured. In normal subjects, 80% of loaded magnesium excretes in urine within 24h, and retention under 30% of loaded magnesium suggests magnesium depletion. Its application has limits because this test needs a prolonged intravenous infusion.

### ***Magnesium Balance Studies***

In the past, the measure of adequate dietary magnesium has been the dietary balance study.<sup>147–151</sup> The best available method for estimating magnesium requirements is the dietary balance approach.<sup>125</sup> The balance study is based on individuals who consume a diet with known magnesium content. The quality of data depends upon both methodological aspects, such as selection of test diet, total collection urine, feces, sweat, etc., and physiological features of the test subjects, such as age, health status, prior nutrient intake, etc. To control the errors and variability associated with a balance study, the Food and Nutrition Board established minimum criteria for balance studies to be included for the Recommended Dietary Allowances in each country. The important items for a balance study are an adaptation period of at least 12 days before the balance study and preparing two dietary-magnesium-level diets in the studies. Magnesium intake is related to energy intake, so that magnesium needs a cofactor of energy metabolism, and in magnesium balance studies energy requirements must be considered.<sup>152,153</sup>

### ***Dietary Requirements and Food Sources of Magnesium***

The U.S. Recommended Dietary Allowances (RDA) for magnesium was revised in 1997.<sup>125</sup> The U.S. magnesium RDA for adult women is 320 mg/day and for adult men is 420 mg/day.<sup>154</sup> In 2005, the Japanese RDA was also revised; for adult women it is 240 mg/day and for adult men it is 310 mg/day.<sup>155</sup> The RDA for magnesium in various countries are: women, 270 mg/day, men, 300 mg/day in England; women, 270 mg/day, men, 320 mg/day in Australia; women and men, 400 mg/day in Russian; and women, 300 mg/day, men, 350 mg/day in Thailand.

Under increasing of death by cardiovascular diseases, magnesium status (high magnesium intake) is very important. For high magnesium intake, food containing high levels of magnesium and meals with low cooking loss must be prepared. Food with high magnesium content are whole grains, fish, algae, pulses (especially tofu), green leafy vegetables, etc. The poor magnesium foods are refined foods such as grains and wheat flour, etc., and may include meat.

Food with high levels of fiber, calcium, or phosphate may induce lower bio-availability of magnesium.

## Toxicity of Magnesium

There may be no evidence generally in healthy people with natural meals to oversupplement magnesium. High magnesium intake with toxicity is only found while taking supplements. Upper limits for magnesium supplements are defined to include oversupplementation of magnesium into consideration by the Food and Nutrition Board's report.<sup>124</sup> Severe magnesium toxicity is rare. Upper limits for magnesium are not set up in the RDA for Japan.<sup>155</sup>

There are some reports that much daily magnesium intake of 30 g/day results in symptoms of metabolic alkalosis and hypokalemia,<sup>6</sup> and by taking a single dose of up to 400 g, cardiorespiratory arrest and paralytic ileus can occur. When serum magnesium concentration are up to 4.8 to 8.4 mg/dL, cardiac and neurological symptoms will appear.<sup>156</sup>

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## 8

# Magnesium Requirement and Affecting Factors

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We performed 11 balance studies to learn the estimated average requirement (EAR) of magnesium (Mg). Magnesium intake was not correlated with Mg balance when all data was used ( $n = 109$ ). However, Mg intake was correlated with calcium (Ca) and phosphorus (P) balances. During the analysis, we found a correlation between sodium intake and Ca and Mg balances. After excluding the data of the highest sodium (Na) intake study, Mg intake turned out to be correlated with Mg balance.

We also studied the effects of some lifestyles on urinary excretion of Ca and Mg. Ingestions of an energy source increased in urinary Ca and Mg. Risk factors for chronic degenerative diseases also increased in urinary Ca and Mg.

Usually, Ca/Mg molar ratio in urine is isomolar or less than 1. To keep plasma Ca and Mg constant, Ca is derived from the bone. If all Mg was released from the bone, where there is major pool of Mg, by the osteophagocytosis, more than 10 times of the amount of Ca must be released at the same time, because the contents of Ca in the bone is far higher than that of Mg. So, Mg in the cell is concluded to be the major source of the compensation.

It is generally believed that there are three levels of dietary intake of a nutrient: excess, adequate, and deficit. The border between excess and adequate is recognized as the upper limit, while the border between adequate and deficit is termed the *requirement*.<sup>1,2</sup>

To determine an upper limit and a requirement for a nutrient, it is necessary to understand the scientific evidence giving quantitative information about the dietary intake and the signs and symptoms of excess or deficit of the nutrient.<sup>1,2</sup>

For the minerals Na, Ca, Mg, and P, whose physiological pools include bone<sup>3,4</sup> and for which the signs and symptoms of deficit or excess are poor, determination of the requirement and the upper limit is difficult because of an absence of evidence.

In such cases, results of balance studies that show an EAR, that is, a balance between intake and output, are the sole sources of information available to determine the requirement.

## Mineral Balance Studies

### Subjects and Methods

From 1986 to 2000, 109 volunteers (23 males, 86 females), ranging from 18 to 28 years old, took part in mineral balance studies after written informed consent was obtained. The ethical committee, established by the National Institute of Health and Nutrition in 1990, approved the studies. All studies were carried out in the Humanities Ward of the National Institute of Health and Nutrition.

The duration of the balance study periods ranged from 5 to 12 days, with 2 to 4 days of adaptation period. Body temperature and weight in the morning were measured throughout the experiment in all subjects. However, menstrual cycle for female subjects was not taken into consideration in these studies.

In each study, the same quantity of the diet, which varied in each experiment, was supplied to each of the subjects during the balance period without consideration of body weight.

However, small changes to the diet were carried out during the adaptation period so as to ensure consumption of all food supplied.

The subjects ingested a coloring marker for their feces (Carmine, 0.3 g; Merk KgaA, Germany) just before breakfast in the morning at the beginning and the end of the balance period. In one subject (no. 8, Table 8.1), magnesium oxide was added to the diet.<sup>5</sup> In six subjects (nos. 4–7, 9, and 10; Table 8.1;

**TABLE 8.1.** Subjects and dietary intake of energy and minerals.

Exp. No.	sex	Subjects n	Duration day	Energy kcal/d	Protein g/d	Fat % of energy	Intake of minerals			
							Na (g/d)	K (g/d)	Ca (mg/d)	Mg (mg/d)
1	m	5	10	2150	71	24	3.20	1.96	676	154
1	m	5	10	2150	71	24	3.20	1.96	676	334*
2	f	11	8	1900	66	35	3.40	1.86	347	186\$
3	f	12	8	1850	64	33	3.45	1.83	294	188\$
4	f	12	10	1650	65	35	3.27	2.06	495	194
5	f	7	8	1800	76	26	3.06	2.20	653	216#
5	f	2	8	1800	76	26	3.06	2.20	653	216
6	f	8	8	1700	69	25	3.08	2.20	671	243#
7	f	8	12	1950	87	27	4.06	2.68	629	261#
8	f	12	8	1550	75	38	3.90	2.55	672	261#
9	f	8	12	1750	78	25	3.69	2.47	719	279#
10	f	6	10	1950	89	25	2.21	2.71	802	283#
11	m	13	5	3250	136	28	6.87	3.61	1131	379
Total		109								

Energy, protein and fat are calculated value, while minerals are measured ones.

\*Mg (180 mg/d) was added to the diet as magnesium oxide (MgO).

\$Low calcium study.

#Mineral lost during exercise was estimated (n = 49).

$n = 49$ ), sweat from the arm during exercise on a bicycle ergometer (intensity, 1–1.5 kp; velocity, 50–60 rpm; duration, 30–60 min/trial; frequency, once or twice a day; room temperature, 22–29°C; relative humidity, 40%–65%) was collected to estimate element loss through sweat (Table 8.1).

The foodstuffs used in each study were selected from those commercially available. Some foodstuffs were avoided because of a heterogeneous content of nutrients revealed through chemical measurements taken before the studies. Both processed and nonperishable foodstuffs were purchased at the same time from the same lot before the experiments so as to ensure the same content of nutrients. Fresh foodstuffs were obtained from the same district by way of the same market.

Dietary menus were designed by a registered dietician so as to meet dietary allowances in Japan,<sup>6</sup> except for the low-calcium studies (subject nos. 1 and 2, Table 8.1) for which food composition tables were used.<sup>7</sup>

All foodstuffs were washed with ion-free water (passed through an ion-exchange resin) if necessary, weighed, cooked separately, and distributed uniformly to dishes for the subjects and diet sample(s).

The subjects were required to consume all of the diet. They were allowed no other food, but could drink as much ion-free water as they wanted. The weight of the water consumed was measured and recorded.

Statistics were obtained by StatView-J5.0.

Some indicators in this chapter are defined as follows:

Apparent Absorption = (Intake) – (Fecal Output) mg/kg body weight (BW)/day

Apparent Absorption (%) =  $\frac{[\text{Intake}] - [\text{Fecal Output}]}{[\text{Intake}]} \times 100\%$

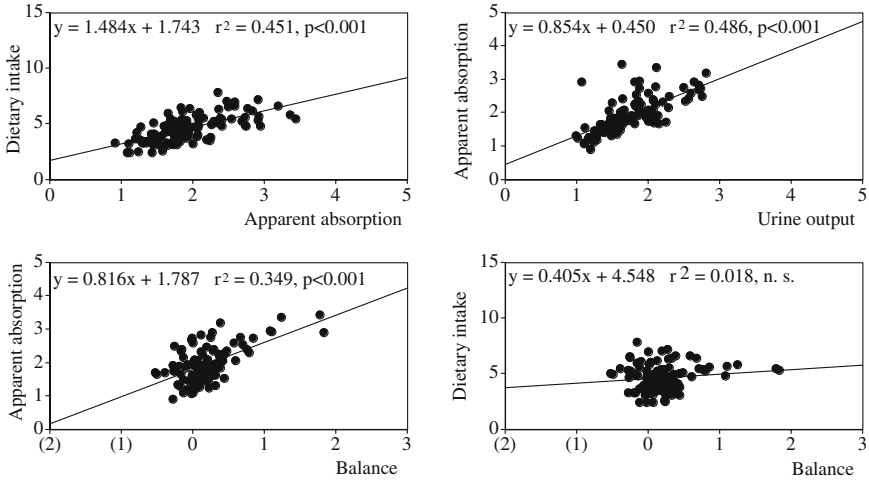
Balance = (Intake) – ([Fecal Output] + [Urine Output] + [Sweat loss]\*) mg/kg BW/day

## Results

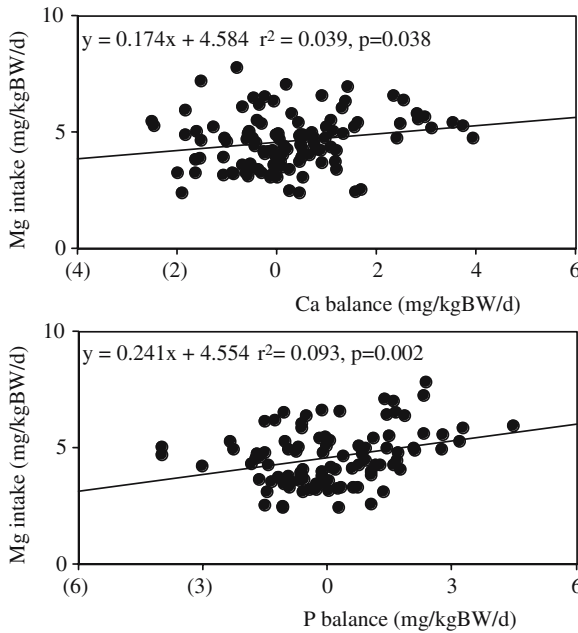
The dietary intake of Ca, Mg, and P ranged from 294 (1), 131, 154 to 379, and 807 (2), 198 mg/day, respectively (Table 8.1). In terms of body weight, the dietary intakes of Ca, Mg, and P were 4.83 to 23.58, 2.44 to 7.83, and 13.46 to 45.69 mg/kg BW/day, respectively. The relationships between dietary intakes, apparent absorption, urine output, and balances for Mg are shown in Figure 8.1.

Dietary intake (Intake) of Mg was positively correlated with apparent absorption (AA;  $r^2 = 0.451$ ), which was also correlated with both urine output (Urine;  $r^2 = 0.486$ ) and balance (Balance;  $r^2 = 0.349$ ). However, the Intake of Mg was not correlated with Balance. Using the above regression equations (Intake vs. AA and AA vs. Balance), the mean value of Mg Intake when balance is equal to 0 was indirectly calculated to be 4.395 mg/kg BW/day.<sup>1</sup>

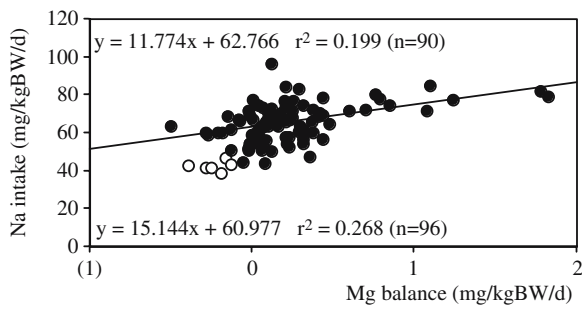
However, intake of Mg was positively correlated, not only with balance of Ca, but also with balance of P. The mean value and upper limit of the 95% confidence interval (95% CI) for the regression equation between Mg intake and balances of Ca and P (when each balance was equal to zero) were 4.584 and 4.802 ( $p = 0.0383$ , against Ca balance), 4.554, and 4.785 ( $p = 0.0024$ , against P balance) mg/kg BW/day, respectively (Figure 8.2).<sup>8</sup>



**FIGURE 8.1.** Dietary intake, apparent absorption, urine output, and balance of Mg (mg/kg BW/day;  $n = 109$ ). Dietary intake of Mg was not correlated with Mg balance, although Mg absorption was positively correlated with both balance and dietary intake of Mg. Using relations both between the intake versus apparent absorption, and apparent absorption versus balance, the mean value of dietary intake of Mg at balance = 0 was calculated to be 4.395 mg/kg BW/day ( $= 1.484 \times 1.787 + 1.743$ ).



**FIGURE 8.2.** Correlations between Mg intake and Ca and P balances. Estimated average requirement (EAR) of Mg can be obtained the above equations.



**FIGURE 8.3.** Relations between Na intake and Mg balance in Japanese young adults ( $n = 96$  or  $90$ ). Data of the highest Na intake study (no. 11, Table 8.1) were omitted in this figure. Data of the lowest Na intake study (no. 10, Table 8.1) are shown as open circles. Regression lines were obtained with and without the lowest Na intake study ( $n = 96$  and  $90$ , both  $p < 0.0001$ ).

On the other hand, Na intake influenced Mg balance. In the data of the combined studies ( $n = 109$ ), the balance of Mg was not correlated positively with Na intake, whereas in the data that excluded the highest Na study (no. 11, Table 8.1;  $n = 96$ )<sup>9</sup> or the highest and lowest Na studies (no. 10, Table 8.1;  $n = 90$ ),<sup>10</sup> the balance of Mg was correlated positively with Na intake ( $p < 0.0001$ )<sup>11</sup> as well as with Mg intake ( $p = 0.003$ ; Figure 8.3).<sup>12</sup> The mean value and upper limit of the 95% CI for the regression equation between Mg intake and Mg balance when the balance is equal to 0 were 4.078 and 4.287 mg/kg BW/day,

**TABLE 8.2.** The mean value and upper limit of the 95% confidence interval for the regression equation between Mg intake and balance of minerals (Na, Ca, Mg and P).

(n = 109)					
Against	Intake range (mg/kgBW/d)	$r^2$	Mean	UL95%CI*	p
Na balance	38.56–142.23	0.129	4.143	4.458	0.0001
Ca balance	4.83–23.58	0.039	4.584	4.802	0.0383
Mg balance	2.44–7.83	0.018			NS
P balance	13.46–45.69	0.093	4.554	4.785	0.0024

\*The upper limit of the 95% confidence interval for the regression equation.

(n = 90)					
Against	Intake range (mg/kgBW/d)	$r^2$	Mean	UL95%CI*	p
Na balance	43.71–96.40	0.001			NS
Ca balance	4.83–23.58	0.213	4.165	4.342	<.0001
Mg balance	2.44–6.42	0.141	4.078	4.287	0.003
P balance	13.46–45.69	0.027			NS

\*The upper limit of the 95% confidence interval for the regression equation.

respectively ( $n = 90$ ).<sup>12</sup> All EARs and the upper limit of the 95% CI for the regression equation between Mg intake and balances of Na, Ca, Mg, and P when the balance is equal to 0 are shown in Table 8.2.

## Factors Affecting Urinary Excretion and/or Intestinal Absorption of Magnesium

It was reported that various nutrients and hormones influenced urinary divalent cation excretion.<sup>13</sup> Nishimuta and colleagues also demonstrated Mg uresis by risk factors for chronic degenerative diseases (Table 8.3).<sup>14,15</sup> In these experiments, Ca uresis is always associated with Mg uresis except for one study,<sup>16</sup> in which the subjects were poor in Mg.<sup>17</sup> These factors undoubtedly affect the balances of Ca and Mg.

Figure 8.4 shows circadian variations of urinary excretion for Ca and Mg under the normal diet (control) and at the butter (60g) and egg (300g) loading test in the morning. This suggested energy increases in urinary excretion of Ca and Mg. This concept is new and far different from that of Lindeman,<sup>13</sup> who thought that fat does not alter urinary calcium and magnesium excretions.

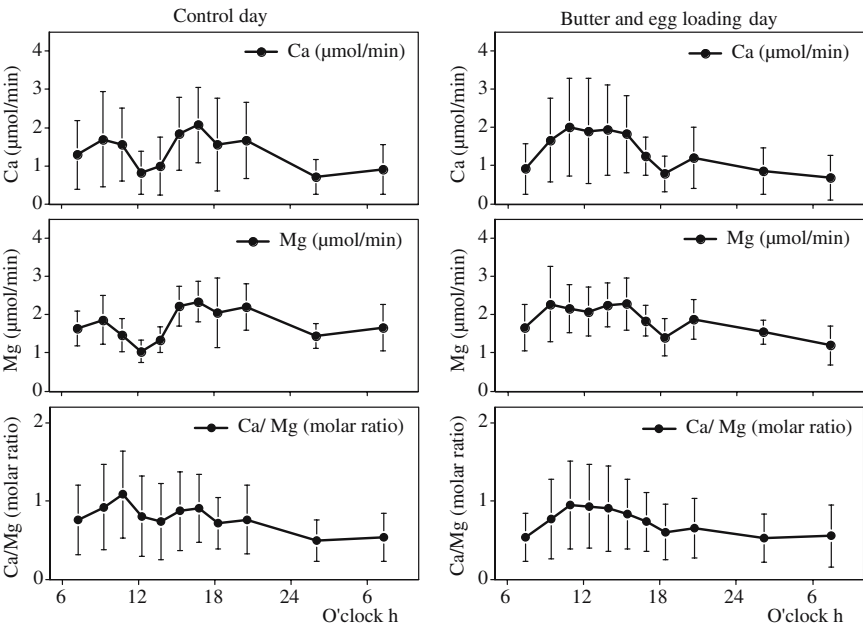
It was also suggested that urinary Ca/Mg ratio is stable and usually less than 1 (molar ratio).

**TABLE 8.3.** Effects of various nutrients, hormones, other agents and events on urinary divalent cation excretion.

A. Items that increase urinary calcium and magnesium		
1. Ethanol	6. Insulin	11. Heavy exercise
2. Glucose IV and oral	7. Vasopressin	12. Mental stress
3. Galactose	8. Sodium lactate and pyruvate	13. Cold exposure
4. Fructose	(vs. sodium bicarbonate)	14. Over eating
5. Protein (casein)	9. Tolbutamide	15. Mild exercise (increase absorption)
	10. Acid (NH <sub>4</sub> Cl) load	16. Sodium (increase absorption)
B. Items that decrease urinary calcium and magnesium		
1. Mild exercise		
2. Fasting		
3. Low energy diet		
C. Items that do not alter urinary calcium and magnesium		
1. Xylose		3. Aldosterone
2. Fat (Lipomul)(should be deleted in this table)*		4. Cortisone (with phosphate infusion)

\*Because this is contradict to overeating (A-14).





**FIGURE 8.4.** Circadian variation in urinary calcium (Ca) and magnesium (Mg) at control day (left) and butter-and-egg-loaded day. Eleven female students consumed an experimental diet (breakfast, 380 kcal; lunch, 730 kcal; supper, 390 kccal) at the control day (left). Next morning they consumed butter (60 g) and 5 boiled eggs (ca. 300 g) with a slice of bread (130 kcal), skip lunch, and eat same menu of supper as the control day (390 kcal) (right). Urinary Ca and Mg increased after diets, indicated by arrows.

Mechanism(s) to Maintain Plasma Levels of Calcium and Magnesium

To analyze the above-mentioned phenomena, characteristic of Mg and Ca as elements should be considered (Table 8.4).

**TABLE 8.4.** Speciation of the essential elements (20).

Major elements (4) Minerals		H, C, N, O		
Speciation of essential minerals (16)		All elements except major elements		
	Intake/d	Intra-cellular minerals	Extra-cellular minerals	Others
Major minerals (7)	more than 100 mg	K, Mg, P	Na, Cl, Ca	S
Trace elements (9)	less than 100 mg			
	Trace elements I (4)	Fe, Zn		Cu, Mn
	Trace elements II (5)	less than 1 mg		Co, Cr, I, Mo, Se
Bone minerals (5)		Mg, P, Zn	Na, Ca	

## Concept (Intracellular, Extracellular, and Bone Minerals)

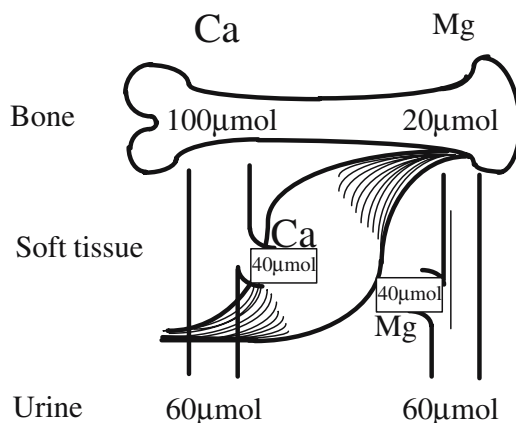
Minerals can be divided into two categories of intracellular and extracellular minerals based on physiological sites of accumulation. Essential minerals rich in intracellular space compared with in extracellular one, such as potassium (K), Mg, P, iron (Fe), and zinc (Zn), are proposed as *intracellular minerals* (ICMs). Those rich in extracellular space, such as sodium chloride (NaCl) and Ca, are proposed *extracellular minerals* (ECMs).<sup>3</sup> Essential minerals, which compose the bone, and whose physiological pool is including the bone, are proposed as *bone minerals* (BMs). Bone minerals consist of both intracellular (Mg, P, and Zn) and extracellular (Na and Ca) minerals.<sup>5</sup> In these definitions, Mg is belongs to both intracellular and bone minerals, and Ca belongs to extracellular and bone minerals.

## Homeostasis of Plasma Calcium and Magnesium after Isomolar Uresis of Calcium and Magnesium

Plasma contents in Mg and Ca are controlled within narrow ranges and have no circadian variations. Urine content in Mg and Ca are usually isomolar or high in Mg compared with that of Ca. Urinary excretion of both Mg and Ca have obvious circadian rhythm, higher in daytime and lower at night (Figure 8.4). Mechanism(s) to keep plasma Mg and Ca levels constant to compensate their urine loss may be important in considering both the metabolism of Mg and Ca and the etiology of chronic degenerative diseases. Under the condition of no food supply, Ca must be released from the bone, the sole Ca pool in the body. However, it is difficult to identify which organ, the bone or the cells, release Mg into the bloodstream to keep plasma Mg constant, because these two organs are both pools of Mg in the body. If all Mg was released from the bone, where there is major pool of Mg, by osteophagocytosis, more than 10 times the amount of Ca must be released at the same time from the bone, because the contents of Ca in the bone is far higher than that of Mg. So, Mg in the cell is concluded to be the major source of the compensation in case of enough Mg reservation. However, Mg in the bone may be released into bloodstream when Mg supply in the cell is short or when osteophagocytosis occurs by the unknown mechanism. In that case, excess Ca released from the bone enters into bloodstream, and then enters into the muscle, causing heart muscle infarction, skeletal muscle contracture (shoulder stiffness), and tonic contraction of blood vessels (hypertension). These are clearly illustrated the sign and symptoms of chronic degenerative diseases (Figure 8.5).

Excess Ca also forms stones outside of the bone as well as eminent Ca uresis without accompanied Mg uresis. In this case, molar ratio of Ca/Mg exceeds more than 1.

Molar ratio of Ca/Mg is a possible indicator for the Mg nutrition. If this value is more than 1, Mg in the bone is supposed to be resorped with large amount of Ca.



**FIGURE 8.5.** Trans-tissue transport of calcium (Ca) and magnesium (Mg), after isomolar uresis of Ca and Mg, induced by excess physical or mental load (stress). Here, isomolar ( $60\mu\text{mol}$ ) of Ca and Mg are supposed to be excreted. Those in the bone cannot explain all loss of the minerals, because Ca content is five times or much higher than that of Mg in the bone. Consequently, it concluded that some Mg is derived from the soft tissue. Actually, more Ca than that excreted are absorbed from the bone, and the rest of Ca enters into the soft tissue where Mg was deprived.

## Lifestyle against Magnesium Deficit

Because most of foodstuffs come from the cells of animals and vegetables, dietary intake of Mg is also adequate unless there is refined food (refined cereals, sugar, alcohol) and high fat consumption, when dietary intake of energy is adequate. However, overeating (excess energy intake) is a risk factor for Mg deficit.

Moderate physical exercise as well as adequate rest and sleep under comfortable circumstances are important for the cells to accumulate intracellular minerals, including Mg.

Further, freedom from any kind of stress is beneficial for maintaining Mg status.

All studies reported in this article generally believe that a good lifestyle is important for good health. However, we reported them based on the evidence obtained from our experiments.

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## 9

# Experimental Data on Chronic Magnesium Deficiency

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Many studies have shown that magnesium ions play an important role in the regulation of cellular and physiological metabolism in most systems within the organism. Moreover, epidemiological studies indicate that the amounts of magnesium consumed by considerable percentages of the population are below the recommended intakes, and that hypomagnesemia is present in 10% to 15% of the western population. However, the clinical manifestations of magnesium deficiency are difficult to define because depletion of this cation is associated with considerable abnormalities in the metabolism of many elements and enzymes. If prolonged, insufficient magnesium intake may be responsible for symptoms attributed to other causes, or whose causes are unknown. Many experimental models in laboratory animals have been used to investigate the alterations caused by magnesium deficiency, and these models can be divided into two main types: acute deficiency lasting a few days, and chronic deficiency in which a diet deficient in magnesium is given during a period of several weeks or for up to 1 year. Chronic studies aim to mimic the situation observed in western countries, that is, moderately low intakes of magnesium sustained over long periods. In this review we will summarize the results reported from long-term studies in experimental animals.

Magnesium (Mg) plays a structural and regulatory role in the organism. Among its functions, one of the most important is to act as the ion that activates a large number of enzyme systems, making Mg essential for the metabolism of many nutrients. Thus, Mg serves as an important link between transport systems and metabolism, and its concentration in the cytoplasm is probably regulated in a highly precise manner. Maguire<sup>1</sup> notes that “Ca<sup>2+</sup> is more of an ‘acute’ signal, performing a type of all-or-none function. Perhaps Mg<sup>2+</sup> fills the complementary role of a ‘chronic’ regulatory agent, setting the ‘gain’ or sensitivity of the overall response system.”

From a physiological standpoint, the functions of Mg are well known and have been amply described in many publications.<sup>2</sup> In another area of research, most epidemiological studies have reported that in western countries, Mg intake is below the recommended allowances in considerable percentages of the population. According to data published to date, the amounts of Mg

consumed are below 80% of the recommended intake in approximately 20% of the population.<sup>2-4</sup> In addition, hypomagnesemia ( $\text{Mg} < 0.75 \text{ mmol/L}$ ) has been found in substantial proportions of the adult population in Europe: 12.6% in Switzerland,<sup>5</sup> 21% in Austria,<sup>6</sup> and 14.5% in Germany.<sup>7</sup>

The clinical manifestations of Mg deficiency are difficult to define because depletion of this cation is associated with considerable abnormalities in the metabolism of many elements and enzymes.<sup>8-11</sup> However, if suboptimal intake is prolonged it can facilitate or cause the appearance of symptoms currently attributed to other causes, or whose causes are unknown. Some examples of this situation are the relationships between low intraerythrocyte levels of Mg and chronic fatigue,<sup>12</sup> migraine,<sup>13</sup> sleep alterations,<sup>14,15</sup> Alzheimer's disease,<sup>16</sup> and depression.<sup>17,18</sup>

## Models Used in Chronic Deficiency Studies

A number of animal models have been used to study the alterations caused by nutritional Mg deficiency. Most such studies have centered on acute deficiency in rats or mice fed a diet deficient in Mg. However, studies of chronic deficiency, in which rats or mice (usually) are given an Mg-deficient diet for relatively prolonged periods, are less numerous. The duration of such studies has varied widely from several weeks<sup>19</sup> to 1 year.<sup>20</sup> These experimental models attempt to mimic, as far as possible, the situation observed in western countries: moderately deficient Mg intakes sustained over long periods.

In the experimental model we have used at the University of Granada, adult Wistar rats are given the diet recommended by the American Institute of Nutrition (AIN) without magnesium oxide in the mineral supplement for a period of 70 days. The results are compared with those in a group of pair-fed animals given the same diet adjusted to meet the requirements of this species for Mg intake.<sup>21</sup>

Our model has been used in a series of studies designed mainly to elucidate the digestive and metabolic alterations caused by nutritional Mg deficiency, and to study the effects of recovery from nutritional deficiency.

## Effect of Chronic Magnesium Deficiency on Growth

Although an Mg-deficient diet is widely assumed to impair growth rate,<sup>2</sup> our research has led to different conclusions depending on the type of diet tested. When rats consumed the AIN-76 diet lacking in magnesium oxide, we found that rats given the deficient diet gained less weight, a finding attributable to the reduced Mg intake rather than to reduced food intake.<sup>22</sup> This finding was also reported by others.<sup>23</sup> However, when the dietary recommendations for this species were changed<sup>21</sup> and the new diet recommended by the AIN was tested (AIN-93G and AIN-93M), we found that the growth curves for control

and Mg-deficient rats did not differ significantly during 10 weeks, regardless of whether the AIN-93G or AIN-93M diet was used (unpublished data). The likely explanation for this finding lies in the composition of the Mg-deficient diet, prepared without magnesium oxide in the mineral supplement. The resulting diet contains Mg from other ingredients, mainly macronutrients. The changes in the proportions of macronutrients and modifications in the mineral and vitamin supplements appear to be responsible for the reported changes in growth rate. However, it should be recalled that the degree of hypomagnesemia after feeding with an Mg-deficient diet for 70 days was similar in rats fed with the AIN-76 diet<sup>22</sup> and the AIN-93 diet.<sup>24</sup> Another finding of note was the clear decrease in mortality among animals fed with the Mg-deficient diet. In rats given the AIN-76 diet, mortality was 27%,<sup>22</sup> whereas in rats given the AIN-93 diet the mortality rate was similar to that for the control group (unpublished data).

## Effect of Chronic Magnesium Deficiency on Mineral Absorption

Feeding with a low-Mg diet led to a clear increase in percentage absorption, although during the first few days of the experiment percentage Mg cation absorption decreased because of the decline in food intake. However, this situation was rapidly reversed, the changes in absorption reflecting the adaptation of the digestive system under conditions of Mg depletion.<sup>22</sup>

The Mg-deficient diet also affected the absorption of other nutrients, especially proteins and minerals. In this connection, earlier research has documented the interactions between calcium (Ca) and Mg in the intestine,<sup>25</sup> and decreased dietary Mg intake has been shown to lead to increased Ca absorption<sup>26</sup> despite alterations in 1,25-dihydroxyvitamin D synthesis.<sup>27</sup> Contradictory findings have been published regarding the intestinal interactions between phosphorus and Mg.<sup>28,29</sup> In our experiments with chronic Mg deficiency, we found that although absorption tended to decrease initially during the first few weeks, it later recovered and reached values approaching those in the control group.<sup>30</sup>

Although interactions in the intestine between Mg and zinc (Zn) were hypothesized,<sup>31</sup> the results obtained with our experimental model showed that short-term consumption of an Mg-deficient diet increased Zn absorption, and that the increase was sustained throughout the remainder of the study period.<sup>32</sup> Similar findings have been reported for iron (Fe)<sup>33</sup> and manganese (Mn) absorption.<sup>34</sup> However, no increase in copper (Cu) absorption was seen until the 10th week of Mg deficiency.<sup>35</sup>

We also investigated the effect on selenium (Se),<sup>36</sup> supplied in the diet in the form of tetravalent sodium selenite; we hypothesized that the digestive behavior of this element would differ from that of the divalent cations. Our findings showed that prolonged Mg deficiency decreased the absorption of Se.<sup>36</sup>

In general, deficient levels of Mg in the intestinal lumen, therefore, led to increases in the absorption of the divalent cations we investigated. The effects of Mg deficiency on digestive utilization may also be influenced by modifications in endogenous mineral excretion. It should be borne in mind that the method we used to measure absorption is indirect, and is based on determinations of mineral contents in the feed and in feces. The concentrations of these metals in enterocytes from the duodenum and proximal-most segment of the jejunum might provide a more accurate picture of the phenomena that occur in the digestive tract during Mg deficiency. These methodological considerations notwithstanding, in rats fed the deficient diet for 70 days the increases we found in enterocyte concentrations of all elements except  $\text{Se}^{24}$  confirmed the findings obtained with indirect methods.

Digestive interactions have been reported between Mg–Ca, Fe–Ca, Fe–Mn, Zn–Cu, and other elements. Antagonisms may occur between elements whose electronic structure and states are similar.<sup>37</sup> However, our results show that the Mg-deficient diet changed the absorption of the divalent elements without causing interactions between them. This finding suggests that the enterocyte Mg carrier may be able to transport other cations with similar electronic structures, or that the increase in divalent cation absorption may be related to changes in the behavior of paracellular pathways.<sup>38</sup>

## Effect of Chronic Magnesium Deficiency on Nutrient Metabolism

Intake of an Mg-deficient diet leads to mobilization of tissue Mg aimed at maintaining cation homeostasis. Under our experimental conditions, erythrocytes were the first cells to mobilize Mg cations in response to the dietary deficiency, and later studies have confirmed that both ionized and total intraerythrocyte Mg are good markers of Mg status,<sup>39</sup> although other authors have proposed that other markers able to more accurately reflect nutritional status for this cation should be sought.<sup>40</sup> After Mg from erythrocytes is used, Mg is mobilized from other stores in the body. After 35 days on an Mg-deficient diet, the rats showed evidence of mobilization from skeletal muscle, and after 49 days the cation was mobilized from bone and from other tissues.<sup>22</sup>

This situation not only affects the tissue distribution of Mg; in addition these changes in Mg concentrations in different tissues are accompanied by alterations in macronutrient and micronutrient metabolism, as summarized below.

### Macronutrients

#### Proteins

An interesting observation documented in several countries was that the most common types of protein-energy malnutrition involve Mg deficiency.<sup>41</sup>



The addition of Mg to a perfusion solution was found to improve the absorption of amino acids.<sup>42</sup> In our experimental model we found that Mg deficiency reduces the nutritive utilization of protein (absorption and balance), apparently as a result of decreased protein absorption and synthesis.<sup>43</sup> The lower digestive utilization of protein may be related with a decrease in sodium (Na<sup>+</sup>)—potassium (K<sup>+</sup>) adenosine triphosphatase (ATPase) activity in the enterocyte, together with alterations in various membrane transport systems.<sup>44</sup> In addition, Mg deficiency reduces mesenteric circulation, which may also impede the absorption of peptides and aminoacids.<sup>45</sup>

## **Lipids**

Substantial epidemiological and animal experimental evidence indicates that Mg deficiency is an important factor in the origin of cardiovascular diseases.<sup>46–52</sup> Studies with short-term experimental Mg deficiency in rodents have found hyperlipidemia, mainly due to hypertriglyceridemia, together with increased concentrations of free cholesterol and alterations in the content and distribution of plasma lipoproteins.<sup>48,51,52</sup> Experiments with chronic Mg deficiency confirmed the findings of acute deficiency studies with regard to the appearance of hypertriglyceridemia and increased free cholesterol and phospholipids, and also documented an increase in the levels of total cholesterol.<sup>23,53</sup>

Magnesium deficiency is thought to produce these results by reducing lecithin–cholesterol acyltransferase<sup>48</sup> and lipoproteinlipase<sup>49</sup> activity. This effect, together with a complex pattern of alterations in apolipoprotein gene expression, suggest a defect in catabolism as the major factor underlying alterations in the plasma lipoprotein profile.<sup>52</sup>

Studies of chronic Mg deficiency also confirmed that changes occurred in the fatty acid composition of tissues and erythrocyte membranes. The most notable change in tissue profiles was the increase in docosahexaenoic acid,<sup>54</sup> whereas in the erythrocyte membrane the main findings were a reduction in oleic acid and arachidonic acid content.<sup>53</sup> The decrease in arachidonic acid appears to be a result of impaired phospholipid incorporation into the membrane as a result of Mg deficiency,<sup>55</sup> a situation that allows the formation of greater amounts of eicosanoids derived from arachidonic acid, including thromboxane A<sub>2</sub>, and thus in turn favors platelet aggregation and increases the susceptibility to vascular lesions.<sup>47</sup>

## **Glucose Homeostasis**

Many studies have shown that Mg deficiency is linked to insulin resistance and type 2 diabetes mellitus.<sup>56</sup> Experiments with chronic Mg deficiency in rats

have confirmed the relationship between Mg and alterations in glucose homeostasis.<sup>57,58</sup>

## ***Micronutrients***

### **Minerals**

In rats fed an Mg-deficient diet for 70 days, the increase in mineral absorption described in the previous section is reflected as greater ion retention both in plasma and in various tissues. Plasma levels of all ions studied except Zn were significantly higher after feeding with an Mg-deficient diet.<sup>32–36,59</sup>

To investigate tissue changes in mineral concentrations we analyzed skeletal muscle (longissimus dorsi), heart, kidney, bone (femur), and liver. In rats fed for 10 weeks with an Mg-deficient diet, skeletal muscle tissue showed increases in the concentration of Ca,<sup>59</sup> Cu,<sup>35</sup> and Mn,<sup>34</sup> whereas Zn<sup>32</sup> and Fe<sup>33</sup> showed a tendency to increase and phosphorus (P) concentrations decreased.<sup>59</sup> Heart tissues showed an increase in weight<sup>32</sup> and increases in Ca,<sup>59</sup> Fe,<sup>33</sup> and Mn concentrations,<sup>34</sup> but decreases in Zn<sup>32</sup> and Cu.<sup>35</sup> In a separate set of chronic deficiency studies, Laurant and coworkers<sup>23</sup> also found increases in weight and Ca content in the heart. The larger Ca, Fe, and Mn stores may facilitate the appearance of alterations in contractility and favor the formation of reactive oxygen species, which would account for the cardiac symptoms that appear in connection with Mg deficiency, as described in many published studies.<sup>60–63</sup>

The kidney is one of the organs that is most seriously affected by Mg deficiency. Mineral concentrations begin to increase clearly soon after the animal begins to consume an Mg-deficient diet.<sup>59</sup> The changes are most notable for calcium phosphate, because when Mg concentrations in the filtrate decrease, Ca tends to bind to phosphate and precipitation ensues.<sup>59,64</sup> We also found that Mg deficiency caused a clear increase in phosphate concentration in urine as a result of the decrease in resorption,<sup>30</sup> which in turn facilitates the formation of calcium salts. The formation of these precipitates may favor Fe,<sup>33</sup> Zn,<sup>32</sup> and Cu<sup>35</sup> retention in the kidney.

Bone tissue is one of the largest reservoirs of Mg, and its mobilization from bone because of nutritional deficiency can alter the structure of this tissue. Our research showed that dietary Mg deficiency increased bone Ca concentrations<sup>59</sup> and decreased P concentrations,<sup>59</sup> a finding which in our experimental model was apparently independent of circulating levels of parathyroid hormone (PTH). Although PTH initially decreases, it tends to recover normal values after 5 weeks of feeding with an Mg-deficient diet.<sup>59</sup> The changes thus appear to be the result of modifications in the functioning of osteoblasts, osteocytes, and osteoclasts rather than a result of hypercalcemia.<sup>65</sup>

Chronic Mg deficiency for 16 weeks led in rats to increased calcemia and bone Ca content, and to decreases in PTH and 1,25-dihydroxyvitamin D. Histomorphometric studies revealed alterations in bone formation and loss of bone mass [secondary to the increased release of substance P and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ )], and the authors concluded that Mg deficiency constituted a risk for osteoporosis.<sup>66,67</sup> In another study, rats were made Mg deficient for 1 year, after which no changes were found in calcemia or bone Ca and P content, although plasma concentrations of PTH were markedly increased. In these Mg-deficient animals there was a decrease in bone density, an increase in fragility, and alterations in bone architecture, and as in the study cited previously, the authors concluded that prolonged consumption of an Mg-deficient diet increased the risk of osteoporosis.<sup>20</sup> Results obtained with our own experimental model showed that after 10 weeks of feeding with an Mg-deficient diet, femoral concentrations of Zn<sup>32,68</sup> and Mn<sup>34</sup> had increased.

In the liver, Mg deficiency leads to clear increases in Fe<sup>33,68</sup> and Cu<sup>35</sup> content, whereas in the spleen only Fe content was increased.<sup>33</sup> The high Fe and Cu content in the liver may be responsible for the activation of hepatic oxidative stress and for alterations in lipid metabolism.<sup>69,70</sup>

Our experimental model also made it possible to analyze the effect of Mg deficiency on mineral content in brain tissue. Initially we measured the concentration of Mg,<sup>22</sup> Zn,<sup>32</sup> Ca, and P<sup>59</sup> in brain homogenates, and found no differences between pair-fed control rats and animals fed the Mg-deficient diet. In later analyses we separated the brain into striatum, hippocampus, cerebellum, and cortex, and found that feeding with an Mg-deficient diet for 10 weeks led to increases in mitochondrial Zn,<sup>71</sup> Cu,<sup>72</sup> and Se<sup>73</sup> content in the striatum and hippocampus. In both the striatum and hippocampus, we also found a significant increase in glutathione peroxidase activity.<sup>73</sup> However, we found no changes as a result of Mg deficiency in the cortex and cerebellum. These changes in mineral content in certain brain structures may be related to alterations in the nervous system caused by Mg deficiency.<sup>14,15,74–76</sup>

The overall picture to emerge from these findings is one that suggests that consumption of an Mg-deficient diet tends to increase the content of divalent cations in tissues, but not all tissues show the same sensitivity to this dietary deficiency.

## Vitamins

Magnesium is known to be necessary for the correct metabolism of vitamins such as thiamin,<sup>77</sup> pyridoxine,<sup>2</sup> and vitamins D<sup>27,78</sup> and E.<sup>69</sup> In long-term experiments with rats, our group has shown that Mg deficiency leads to impaired pyridoxine status, possibly as a consequence of the inhibition of alkaline phosphatase activity.<sup>79</sup> Studies with this same experimental model showed that Mg deficiency can also decrease plasma levels of vitamin C (control =  $0.41 \pm 0.01$  mg/dL,  $n = 10$ ; Mg deficient =  $0.3 \pm 0.03$  mg/dL,  $n = 10$ ;  $p < 0.05$ ; unpublished data). However, we found no alterations in riboflavin status in association with Mg deficiency.<sup>79</sup>

## Magnesium Deficiency and Carcinogenesis

Several authors have found that Mg deficiency increased free radical production, inflammatory processes, and apoptosis.<sup>62,68,69,80,81</sup> Because of the important role Mg plays in regulating cell cycles, enzymatic activity, and the process of DNA repair, low concentrations of Mg might facilitate the accumulation of mutations. There is clinical and epidemiological evidence that Mg deficiency can favor the presence of cancer.<sup>82,83</sup> Although little information is available regarding the influence of chronic Mg deficiency on cancer, we recently found that the skin tumor-promoting activity of *p*-hydroxybenzenediazonium (PDQ) is accelerated in Mg-deficient rats. These results support the idea that PDQ genotoxicity is related to peroxidative damage resulting from the formation in vivo of primary aryl radicals.<sup>84</sup>

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# 10

## Clinical Forms of Magnesium Depletion by Photosensitization and Treatment with Scototherapy

Jean Durlach

The clinical forms of magnesium depletion due to dysregulation biorhythms may result from the association of a low magnesium intake with a dysregulated biorhythm: either hyperfunction or hypofunction of the biological clock. Their main biological marker is the production of melatonin.<sup>1</sup> The biological dysfunction of the biological clock may be primary (e.g., impaired maturation of photoneuroendocrine system in clinical forms of sudden infant death due to magnesium depletion by hypofunction of the biological clock)<sup>2,3</sup> or secondary to light hypersensitivity. The organism responds to the pathogenic effect of this hypersensitivity with a protective reactive photophobia whose mechanism is still unclear.<sup>1,4-6</sup>

The aim of this study is (1) to stress the importance of the main chronopathological form of magnesium depletion [with a decrease of melatonin (MT) product] secondary to photosensitization: that is, the clinical forms of photosensitive magnesium depletion with photophobia; and (2) to define its treatment, involving diverse forms of scototherapy, appropriate in the control of specifically photosensitive magnesium depletion.

### Clinical Forms of Magnesium Depletion by Photosensitization

Biologically and clinically, this chronopathological form of magnesium depletion secondary to photic hypersensitivity associates stigma of magnesium depletion with stigma of hypofunction of the biological clock with decreased production of melatonin ( $\downarrow$ MT) and with central and peripheral nervous hyperexcitability (NHE).

The manifestations may be psychic, algic, dyssomniac, or neuromuscular.

### *Magnesium Depletion*<sup>4</sup>

Magnesium deficit involves two types of dysregulation, distinct from each other: magnesium depletion and magnesium deficiency.

Magnesium deficiency is linked to an insufficient intake that may be corrected, over a long period of time, through a physiological nutritional oral magnesium supplementation. Magnesium depletion is due to a dysregulation of the magnesium status that cannot be corrected through nutritional supplementation only, but requests the most specific correction of the dysregulating mechanisms.

There exist as many clinical forms of magnesium depletion as possibilities of dysregulation of the magnesium status. But in both clinical therapeutics and in animal experiments, the dysregulating mechanisms of magnesium depletion associate a reduced magnesium intake with various types of stress.<sup>2</sup> Among these, biological clock dysrhythmias are found.

### ***Clinical Forms of Photosensitive Magnesium Depletion***

Clinical forms of magnesium depletion by photosensitization may be algic, neuromuscular, dyssomniac, or psychic, but present the same stigma: biologically, markers of magnesium depletion and of decreased melatonin production, and clinically, photophobia.

#### **Headache Due to Photosensitive Magnesium Depletion<sup>4-11</sup>**

The association of the stigma of magnesium depletion with clinical and para-clinical data of photosensitivity (visual stress tests, electroencephalographic, and cerebrovascular photic driving) in primary headache patients demonstrates that the new concept of photosensitive magnesium depletion is fully justified and agrees with the so-called continuum severity theory: the lower limit of the continuum is tension type headache (TTH), which progressively evolves into migraine (M) without aura and finally into migraine with aura. The interictal hallmark of these cephalalgic patients is sensitization (or potentiation) instead of physiological habituation. Comorbidity with the other forms (neuromuscular, dyssomniac, and psychic) of photosensitive magnesium depletion may be observed.

#### **Neuromuscular Forms of Photosensitive Magnesium Depletion<sup>4,5,12-16</sup>**

The central and peripheral manifestations of neuromuscular photosensitive magnesium depletion are represented by photosensitive epilepsy which may be either generalized or focal, authenticated through electroencephalogram (EEG) with intermittent light stimulation (ILS), with its corresponding form observed among television (TV) viewers and videogame players. Some migraine equivalents are often associated in this context.

The neuromuscular form of photosensitive magnesium depletion may also clinically appear as chronic fatigue syndrome or as fibromyalgia.

#### **Dyssomnia Due to Photosensitive Magnesium Depletion<sup>4,17-22</sup>**

Dyssomnia due to photosensitive magnesium depletion is mainly represented by the delayed sleep phase syndrome (DSPS). It is observed, for example, in

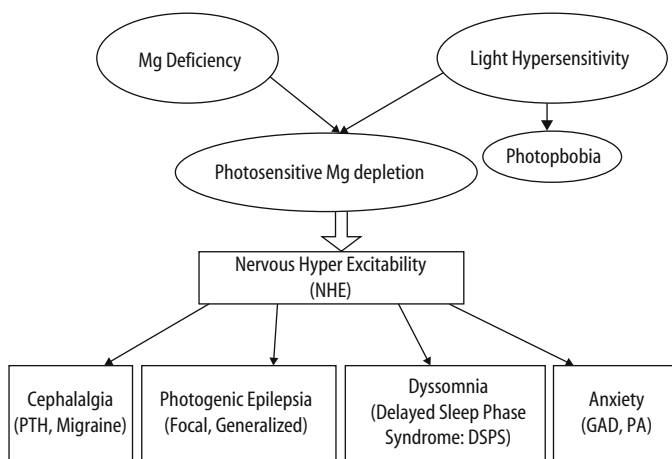
jet lag, night-work disorders, or insomnia of elderly patients. Some chronopathological forms of sudden infant death syndrome, of multiple sclerosis, and of asthma may be also associated here.<sup>2-4,22</sup>

### Psychic Forms Due to Photosensitive Magnesium Depletion

Psychic forms of photosensitive magnesium depletion essentially express themselves in the form of anxiety: either generalized anxiety disorder (GAD) or panic attacks (PA).<sup>4,5</sup>

These four clinical forms of photosensitive magnesium depletion may coexist (Figure 10.1) with the same association of clinical and biological characteristics: mainly, with aspecific clinical symptoms of NHE, with photophobia, and the tinted glass sign, the symptomatology being mainly diurnal and observed in spring and summer, particularly in sunny countries when light hyperstimulation is obviously maximum.<sup>4,5,23,24</sup>

This new concept of photosensitive magnesium depletion may induce therapeutic consequences through the association of an aspecific treatment of magnesium depletion with a specific treatment of photosensitivity through scototherapy.



**FIGURE 10.1.** The four main clinical forms of photosensitive magnesium depletion. The sum of a nutritional magnesium deficiency (deficient magnesium intake) and of a high light hypersensitivity (with reactive photophobia) induces four main clinical forms of photosensitive magnesium depletion: nervous hyperexcitability with stigma of magnesium deficit and of hypofunction of the biological clock. A large number of primary headache patients [currently classified as tension type headache (TTH) or migraine (M)] exhibit hypersensitivity to light associated with magnesium deficit. TTH and M agree with the so-called continuum severity theory. The interictal hallmark is sensitization (or potentiation) instead of habituation. Photosensitive magnesium depletion may also induce photogenic epilepsy (focal or generalized), delayed sleep phase syndrome (DSPS), and anxiety (generalized or attacks: GAD to PA). Comorbidity of these main clinical forms, cephalalgic, convulsive, dysomniac, and psychic, of photosensitive magnesium depletion is frequent.

## Treatment of Magnesium Depletion by Photosensitization

Symptomatic medications classically used for the treatment the various clinical forms of photosensitive magnesium depletion—antalgic drugs,  $\beta$  blockers, ergot derivatives, triptans, anxiolytic drugs, anti-convulsants, hypnotics, sedatives—although useful will not be considered in this study.

The following therapeutic approach only concerns the aspecific treatment of magnesium depletion and the specific treatment of photosensitivity through scototherapy (darkness therapy).

### *Aspecific Treatment of Magnesium Depletion*<sup>5,22–28</sup>

#### Preventive Treatment

Preventive treatment is more efficient than curative treatment. Because magnesium depletion is usually due to both primary chronic magnesium deficiency and stress, a prophylactic treatment must rely on a balanced magnesium status and the most specific possible anti-stress treatment.

To insure a balanced magnesium status, in case of chronic primary magnesium deficiency, atoxic nutritional magnesium supplementation will be administered via the diet or with supplemental magnesium salts. The dietetic supplement should have a high magnesium density with the greatest availability. Magnesium in drinking water is of particular interest as it associates a high bioavailability with the lowest nutritional density. The magnesium salt used should be hydrosoluble and the properties of the anion should be considered.

A specific anti-photosensitive drug that could be used as a preventive treatment of photosensitive magnesium depletion cannot be currently found.

#### Curative Treatment

The aspecific treatments of magnesium depletion which are available, such as pharmacological doses of parenteral magnesium, may be used. Several studies have shown that 1 g of intravenous magnesium sulfate has been considered as efficient, safe, and well tolerated in migraine headache, but its efficiency as an antalgic drug and as an anti-migraine treatment remains controversial.

Pharmacological doses of magnesium salts may induce a toxicity that varies according to the nature of anions. For example, the effects of  $\text{MgCl}_2$  and  $\text{MgSO}_4$  on the ionic transfer components through isolated amniotic membrane have been studied and revealed major differences.  $\text{MgCl}_2$  interacts with all the exchangers, whereas the effects of  $\text{MgSO}_4$  are limited to paracellular components.  $\text{MgCl}_2$  mainly increases the ionic flux ratio of this asymmetric human membrane while  $\text{MgSO}_4$  decreases it, with many deleterious fetal consequences.

It seems, therefore, necessary to determine the therapeutic index (LD 50/ED 50) of the various available magnesium salts before pharmacological use. The selection of one magnesium salt among others should take into account reli-

able pharmacological and toxicological data and the comparative therapeutic index of the various salts: the larger its value, the greater the safety margin. This logical prerequisite is lacking in most protocols:  $\text{MgSO}_4$  is just routinely used without justification.

### ***Specific Treatment of Photosensitivity: Scototherapy***<sup>5,22,29–34</sup>

The reactive response to photosensitivity induces hypofunction of the biological clock (hBC). But, in case of photosensitive headache, hBC is aggravated as the repetitive stimulating effects of light induce potentiation (sensitization)—sometimes with generalization—instead of habituation.<sup>3</sup>

Treatment of photosensitivity—so-called scototherapy (darkness therapy)—mirrors phototherapy, the treatment of hyperfunction of the biological clock.

Scototherapy aims either at stimulating the biological clock, or at palliating its hypofunction.

Stimulation of the biological clock may be obtained through physiological, psychotherapeutic, physiotherapeutic, or pharmacological strategies.

Palliative treatments of hBC are currently dependent on melatonin, its analogs, or its precursors.

### **Stimulating Scototherapies**

#### ***Physiological Scototherapy (Darkness Therapy)***

The best physiological stimulation of the biological clock is induced by light deprivation. It may be obtained by placing the patient in a closed room, in a totally dark environment, with an eye mask on. This genuine scototherapy may be used in acute indications, but should be of short duration. It is not compatible with any activity and is frequently associated with induction of bed rest, inactivity, and sleep. Prolonged exposure to darkness may induce darkness adaptation with a gain in photosensitivity, the reverse of the scototherapeutic effect.

Relative scototherapy may be obtained by wearing dark goggles or dark sunglasses but the number of lux passing through is not negligible. This relative darkness therapy may be used as an accessory treatment in the restoration of a light/dark schedule: a transition before a totally dark environment. A successful double-blind study demonstrated a significant difference between placebo and salicoside (salicin) in association with a photoprotective mask in treating the two main clinical forms of photosensitive headaches: M and TTH. The good results of this controlled clinical trial have not been confirmed.

Chromatotherapy uses a short exposure (4 min) to a precise yellow wavelength, once a week, for the treatment of hBC. This method, even though successfully used in practice, has not been validated yet.

Some studies have reported benefit from using colored filters in headache patients, in childhood migraine particularly. A double-masked randomized

controlled study with crossover design compared the effectiveness of precision ophthalmic tints (optimal tint) or glasses that provided a slightly different color (control tint). Using individually prescribed colored filters selected by each migraineur seems more helpful than the conventional practice of using a neutral grey or sometimes brown tint, but the effect is statistically marginal; it is suggestive rather than conclusive.

### ***Psychotherapeutic Scototherapies***

Cognitive behavioral strategies have been efficient for the treatment of photosensitivity. The treatment was to gradually increase exposure to computer monitor and TV screen photostimulation. This desensitization procedure resulted in a complete removal of the patient's phobic anxiety of photostimulation and of avoidant behavior. This behavioral therapy has been used in photosensitive epilepsy. It is akin to deconditioning techniques used as a nonpharmacological approach to prevent photosensitive headache. For example, variable frequency photostimulation (VFP) goggles, that is, a portable stroboscope using red light-emitting diodes (LED) to illuminate the right eye and the left eye alternately were used with limited efficacy. Various bio-feedback treatments for migraine were disappointing because a reduction in the number of migraine headaches was observed, but with no change in the intensity, duration, or disability of the headaches.

The concept of headache due to photosensitive magnesium depletion places this clinical form of headache among the indications of psychotherapeutic scototherapies.

### ***Physiotherapeutic Scototherapies***

Magnetic fields may be used to stimulate the biological clock in a variety of treatment methods using very weak (picotesla), extremely low frequency (2–7 Hz) electromagnetic fields. Transcranial treatment with alternative current pulsed electromagnetic fields of picotesla flux density may stimulate various brain areas (the hypothalamus particularly) and the pineal gland (which functions as a magneto receptor). Several studies concern its use for treatment of headaches. A double-blind placebo controlled trial has shown that this physiotherapy can alleviate symptoms of M but not of TTH. Electromagnetic fields for at least 3 weeks may be considered as an effective, short-term intervention for migraine, although the clinical effects were small.

### ***Pharmacological Scototherapies***

Three pharmacological agents may stimulate the biological clock: magnesium, L-tryptophan, and taurine.

To stimulate the BC, it seems well advised to facilitate the neural function of suprachiasmatic nuclei ( $\uparrow$ SCN) and of the hormonal pineal production ( $\uparrow$ MT). The deleterious effects of light and those of magnesium deficiency are often found together and may be partly palliated by nutritional magnesium supply ( $\uparrow$ Mg), providing the best possible link between photoperiod and mag-

nesium status. Palliative nutritional magnesium supplementation is efficient and atoxic when magnesium deficiency is present, but when there is a balanced magnesium status, it is illogical and inefficient. Pharmacological use of magnesium (high oral doses or parenteral administration) is uncertain and susceptible of inducing toxicity. Many data remain imprecise, such as nature and doses of the magnesium salts, oral or parenteral routes, association with magnesium fixing agents.

L-tryptophan (or 5OH-tryptophan) may stimulate the tryptophan pathway. But it is unspecific as it does not only concern melatonin production, but also serotonin synthesis. L-tryptophan supplementation may induce toxicity, eosinophilia–myalgia syndrome particularly,<sup>35</sup> and the more so as the occurrence may be facilitated by magnesium deficiency.<sup>36</sup>

Taurine is a sulphonated aminoacid that is present in the whole body in high concentrations, particularly in the brain. It has multiple functions in cell homeostasis, such as membrane stabilization, buffering, osmoregulation, and antioxidant activities together with effects on neurotransmitter release and on receptor modulation.

Taurine may act as a protective inhibitory neuromodulator that participates in the functional quality of the neural apparatus and in melatonin production and action. It plays a role in the maintenance of homeostasis in the central nervous system, particularly during central nervous hyperexcitability. Taurine, a volume-regulating amino acid, is released upon excitotoxicity-induced cell swelling. It has an established function as an osmolyte in the central nervous system.

In the course of magnesium deficit, the organism appears to stimulate taurine mobilization into playing the role of a magnesium vicarious agent. This compensatory action is usually rather limited. However, it allows us to observe the latent form of the least severe form of magnesium deficiency.<sup>31–34</sup> During migraine, the typical form of headache due to photosensitive magnesium depletion, taurine mobilization may be considered as a defensive reaction but it is less effective than in case of magnesium deficiency.<sup>5,34</sup>

Magnesium, tryptophan, and taurine may be used to stimulate the biological clock, but their efficiency seems limited. Palliative treatments of hypofunction of the biological clock may be necessary.

### **Substitutive Scototherapy (Darkness Mimicking Agents)**

#### ***Mechanisms of the Action of Darkness***

The mechanisms of action of darkness appear to be the reverse of those described with bright light, where direct cellular effects (membraneous and redox) and neural-mediated effects intervene.

Increased production of melatonin ( $\uparrow$ MT) constitutes the best marker of darkness, but it is only an accessory mechanism in its action.

The main central neural mechanisms of darkness therapy associate decreased serotoninergy ( $\downarrow$  5HT), which could account for the anti-migraine



effect, with stimulation of inhibitory neuromodulators (gamma-aminobutyric acid, taurine, kappa opioids:  $\uparrow$ GABA,  $\uparrow$ TA,  $\uparrow$ kO) and of anti-inflammatory and anti-oxidative processes. These effects may induce neural hypoexcitability, that is, sedative and anti-convulsant effects.

Humoral transduction may reinforce these last effects by decreasing neuro-active gases ( $\downarrow$ CO,  $\downarrow$ NO) through binding of CO with hemoglobine (Hb) and by increasing melatonin, bilirubin, and biliverdin, three antioxidants which are able to quench NO.

Apart from the exception of decreased serotonergy, these systemic effects of darkness are similar to those of magnesium.

Substitutive scototherapy should palliate all the mechanisms of action of darkness, but the only available scotherapeutic mimicking agents are at present melatonin (its analogs and its precursors, L-tryptophan, 5 hydroxytryptophan).

### ***Melatonin: An Accessory Scototherapeutic Agent***

Melatonin is the prototype of scotherapeutic agents. But, although its production is the best marker of photoperiod, melatonin appears to be only an accessory factor among the mechanisms of photoperiod actions. Most of the other mechanisms of the effects of darkness have been overlooked, which may account for the controversy around the therapeutic efficiency of MT. Its posology varies from physiological doses (around 3 mg per day) to pharmacological doses, usually 3 mg/per dose and even up to 300 mg as a contraceptive, which testifies to the weak toxicity of the hormone. In case of chronopathology with decreased MT production, MT constitutes a partial substitutive treatment of its deficiency.<sup>5-22</sup>

At the present time, substitutive scototherapy using melatonin or its analogs and its precursors as a partial substitutive treatment of hBC is possible, though melatonin is only an accessory mechanism of the action of darkness.

## **Conclusion**

The treatment of photosensitive magnesium depletion must, at present, associate the classical symptomatic treatments; a balanced magnesium status (through nutritional or carefully checked pharmacological magnesium supplementation); control of photosensitivity through the various types of scototherapy: physiological, psychotherapeutic, physiotherapeutic, or pharmacological, stimulating scototherapy or palliative scototherapy, today through MT, the only available partial scotherapeutic-mimicking agents.

Further research should take into consideration other agents with more efficient darkness-mimicking properties. A new model of photosensitive magnesium depletion with potentiation is currently described.<sup>37</sup> This test should be a useful tool for discriminating the most efficient scotherapeutic agent in photosensitive magnesium-depleted mice.

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# 11

## New Data on Pharmacological Properties and Indications of Magnesium

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Metabolic therapy involves the administration of a substance normally found in the organism in order to enhance a metabolic reaction within the cell. This may be achieved in two ways: First, for some systems, a substance can be given to achieve greater-than-normal levels in the body so as to drive an enzymatic reaction in a preferred direction. Second, metabolic therapy may be used to correct an absolute or relative deficiency of a cellular component. Thus, metabolic therapy differs greatly from most standard pharmacological therapy.<sup>1</sup>

Due to the fact that magnesium is an essential mineral in human nutrition, involved in over 300 metabolic reactions, the difficulty in ascertaining the underlying mechanisms of the metabolic therapy of this element may be evident. Magnesium is necessary for every major biological process, including the production of cellular energy and the synthesis of nucleic acids and proteins. Moreover, it is important for the electrical stability of cells, the maintenance of membrane integrity, muscle contraction, nerve conduction, and the regulation of vascular tone, among others.<sup>2</sup>

However, it must be borne in mind that magnesium is biologically tightly interlocked with calcium. In some reactions, such as the synthesis of nucleic acids and proteins, calcium and magnesium are antagonistic. Magnesium is necessary for these processes, while calcium may inhibit them. In others, the production of adenosine triphosphate or ATP, magnesium and calcium cooperate. Magnesium has been called nature's physiological calcium channel blocker because it appears to regulate the intracellular flow of calcium ions.

### Alterations at Subcellular Level

In order to establish the bases that may explain the pharmacological action and properties of magnesium in the different cases in which it is used, it is necessary to ascertain the alterations generated or the physiological processes altered in the cases in which it is administered or the processes or mechanisms restored with its administration.

## ***Intracellular Concentrations of Calcium and Magnesium***

In the 1980s, a considerable number of clinical, epidemiological, and experimental studies were published in which the important role of magnesium in cardiovascular pathophysiology was underlined.<sup>3</sup> The period in which the role of intracellular ions in hypertension was revealed began in 1984 with the study of Erne and coworkers,<sup>4</sup> which showed that the free cytosolic calcium of the platelets correlated tightly and positively with the levels of blood pressure in normotense subjects and in patients with hypertension. In the same year, Resnick<sup>5</sup> reported that the levels of free intracellular magnesium in the hypertense patients were lower than those of normotense subjects and, in 1993,<sup>6</sup> he indicated that the levels of free intracellular magnesium in hypertension were inversely related to those of free calcium. In 1991, our group<sup>7</sup> reported that different anti-hypertensive treatments provoked increases of intralymphocytary magnesium as their most consistent manifestation.

The cellular bases of a large amount of syndromes, such as hypertension, seem to be found in modifications of the levels of free intracellular ionic calcium and magnesium, as reported by Lawrence and Resnick.<sup>8</sup> Since then the majority of cases in which magnesium administration is recommended have presented these alterations.

Different mechanisms may be altered by this imbalance in the concentration of these intracellular ions. Thus, the amount of acetylcholine released has been shown to be dependent upon  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations. While  $\text{Ca}^{2+}$  increases acetylcholine release during neuronal depolarization,  $\text{Mg}^{2+}$  decreases acetylcholine release by stabilizing the membranes of presynaptic vesicles.<sup>9</sup>

In relation to the blockage of the calcium channel, magnesium blocks the voltage-gated calcium channels<sup>10,11</sup> and, consequently, may modulate post-traumatic neurochemical changes mediated by these elevated intracellular calcium levels.

Elevations in intracellular calcium concentration not only occur through ionotropic receptors, but also through second-messenger-linked receptors. Neurotransmitters, hormones, and even mechanical damage also increase intracellular calcium concentration through the activation of phospholipase C and the subsequent hydrolysis of phosphatidyl inositol 4,5-bisphosphate into inositol 1,4,5-triphosphate ( $\text{IP}_3$ ).<sup>12</sup>

## ***Receptors (Mediators/Modulators)***

Not only do the membrane-stabilizing properties of magnesium affect lipid peroxidation and the generation of reactive oxygen species, but they also impact upon the release of neurotransmitters and other mediators/modulators.<sup>13</sup> In addition to the actions controlled by the  $\text{IP}_3$  as intracellular messenger, magnesium participates in the regulation of other mediators and/or second messengers. Glutamate release, like acetylcholine release, is also reduced by the administration of magnesium. Both of these transmitters are

increased after trauma.<sup>14</sup> It is possible that the effect of magnesium on glutamate release indirectly arises largely due to the cation's membrane-stabilizing properties. Indeed, the NMDA channel has been implicated as a critical factor in the development of cellular injury following neurotrauma.<sup>15</sup> Some studies have shown that the magnesium cation is the endogenous regulator of NMDA-channel activity.<sup>16,17</sup> The specific effects of the ion on the activity of the NMDA channel have recently been reported to be critical to outcome following central nervous system trauma. This study demonstrates that the magnesium block of the NMDA channel is reduced after neural injury and that this reduction may be linked to either a decline in magnesium levels, or a change in the structure of the NMDA channel.<sup>18</sup>

### ***Membrane Enzymes***

There is now strong evidence that magnesium has an important role in the regulation of the metabolism of arachidonic acid.<sup>19</sup> Depletion of intracellular  $Mg^{2+}$  has been shown to reduce exogenous arachidonic acid incorporation into tissue phospholipids, perhaps by reducing arachidonyl CoA synthesis and thus modifying the first phase in the incorporation of exogenous fatty acids into membrane phospholipids.<sup>20</sup>

Moreover,  $Mg^{2+}$  binding to its specific binding site of protein kinase C (PKC) causes reduced activity of this enzyme, suggesting that some of the effects of intracellular  $Mg^{2+}$  depletion may be mediated by PKC activation. Increased PKC-mediated phosphorylation of enzymes that are involved in arachidonic acid incorporation (CoA synthetase, lysophosphatidylcholine acyl transferase) reduces their activities and subsequently increases the concentration of the free arachidonic acid and its metabolites.<sup>19</sup> The fact that phospholipase-induced products may also act to chelate magnesium<sup>21</sup> suggests a possible role of phospholipase activity in the post-traumatic depletion of free magnesium and impairments of the cellular bioenergetic state.

In the sphingomyelin pathway, an extracellular stimuli [vitamin D<sub>3</sub>, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), endotoxin, interferon, IL-1] causes the activation of sphingomyelinase, which hydrolyzes plasma membrane sphingomyelin to produce ceramide and phosphorylcholine. Ceramide appears to act as a lipid second messenger, playing important roles in a variety of fundamental biological processes, such as cell proliferation, cell differentiation, apoptosis, receptor functions, oncogenesis, immune functions, and inflammatory responses.<sup>22</sup> Sphingomyelinases are a group of phospholipases. Five types of sphingomyelinases have been identified. These sphingomyelinases differ in tissue distribution, cofactor dependence, mechanism of regulation, and involvement in diverse cellular responses.<sup>23</sup> The neutral  $Mg^{2+}$ -dependent sphingomyelinase is a plasma membrane-bound enzyme that requires magnesium ions and a neutral pH environment<sup>23</sup> and plays an important regulatory role with a vasorelaxant response in vascular smooth muscle.

## ***Membrane Stabilizing Agent***

One of the consequences of calcium influx is the initiation of pathways involved in the breakdown of lipid membrane constituents and the subsequent accumulation of free fatty acids, in particular arachidonic acid.<sup>19</sup> Magnesium depletion has been reported to increase membrane turnover and fluidity and to increase lipid peroxidation.<sup>24</sup> Increased lipid peroxidation is indicative of oxidative stress, suggesting that magnesium may also play a central role in this process. The reactive oxygen species initiate further cell damage, in part through the peroxidation of membrane components. These free radicals, along with other damaging neurochemical events, have been shown to play an important role in the pathophysiology of CNS injury.<sup>25,26</sup>

Thus, the magnesium ion is able to bind electrostatically to the negatively charged groups in the membranes, proteins, and nucleic acids. Binding to the polar heads of the membrane phospholipids may change the local configurations and produce a general effect of electric “screening.” Consequently, magnesium may influence the binding of other cations such as calcium and the polyamines which, depending on their concentration, may have antagonistic or collaborative effects. Generally, magnesium has a protective and stabilizing effect on the membrane that may be attributed to the electric effects and to the inhibition of phospholipase A<sub>2</sub>.

## ***Conclusion***

It is probable that the therapeutic effects of magnesium have a common base through the phenomena of the membrane which, according to the specificities of the cells of each tissue, will be manifested in one way or another. We think that the final tendency of the metabolic therapy of magnesium should be oriented to the restoration of the intracellular ionic balance.

## **Therapeutic Indications of Magnesium**

### ***Magnesium Deficits: Deficiency and Depletion***

The therapeutics of magnesium supplementation do take into account the basic distinction between two types of magnesium deficit: magnesium deficiency and magnesium depletion.<sup>2</sup> Magnesium deficiency is a type of magnesium deficit due to insufficient magnesium intake. It responds to simple magnesium supplementation. Magnesium depletion is due to a dysregulation in the mechanisms that control or disturb magnesium metabolism. This type of magnesium deficit responds to the correction of the pathogenic dysregulation. Thus, magnesium can be used preventively in order to avoid deficiencies in high-risk populations (competition sports, diuretic use, etc.) and therapeutically to treat deficiencies (nutritional magnesium therapy).

In isolated deficiencies of magnesium, up to 50 mmol of magnesium can be administered, according to individual requirements. A variety of magnesium salts can be used in clinical practice, although the most widely used is magnesium sulphate. In acute or severe hypomagnesemia, magnesium can be administered parenterally, usually as chloride or sulphate.<sup>27</sup> As an alternative, magnesium sulphate can be administered in an intramuscular or slow intravenous injection.

## ***Magnesium Use in Cardiology***

### **Myocardial Infarction**

Magnesium may have an anti-arrhythmic effect on myocardium and may protect myocardium from reperfusion arrhythmia and myocardial spasms, given the physiological role of magnesium in the homeostasis of ions in the myocardial muscle cells.

Although intravenous magnesium salts have been used for cardiac arrhythmia during acute coronary syndromes, the clinical trials assessing this use present inconclusive results. Based on the promising previous experience in small trials,<sup>28</sup> the second Leicester Intravenous Magnesium Intervention Trial LIMIT-2 study<sup>29,30</sup> was designed to assess the usefulness of magnesium in suspected myocardial infarction. In this trial it was observed an early and long-term (mean follow up of 2.7 years) beneficial effect on mortality, administering 8 mmol of intravenous magnesium prior to thrombolysis, followed by a perfusion of 65 mmol during the following 24h. On the other hand, the Fourth International Study of Infarct Survival Collaborative Group (ISIS-4) trial, with a larger sample of patients (58,050 patients) did not find a significant benefit of magnesium, although the way of administering magnesium was slightly different.<sup>31</sup> Finally, the Magnesium in Coronaries (MAGIC) trial<sup>32</sup> was designed in an attempt to solve the controversy, administering magnesium sulphate in similar doses to the LIMIT-2 trial, but the treatment did not show any benefit. In addition, the study by Galloe<sup>33</sup> did not show any benefit of the use of oral magnesium among survivors of an acute myocardial infarction. Currently, the guideline of the European Society of Cardiology does not recommend the use of magnesium in ST-elevation myocardial infarction.<sup>34</sup> However, the American College of Cardiology/American Heart Association (ACC/AHA) guideline<sup>35</sup> recommends analyzing electrolytes and magnesium in the early laboratory evaluation of these patients. In this guideline, ACC/AHA recommends magnesium administration exclusively in two situations (class IIa recommendations): (1) In magnesium deficits, especially in patients receiving diuretics before the onset of STEMI (level of evidence C); (2) In patients with episodes of torsade de pointes-type ventricular tachycardia associated with a prolonged QT interval (level of evidence C), the AHA recommends administering 1 to 2 g of magnesium as an intravenous bolus over 5 min.



## **Arrhythmia**

Low serum levels of magnesium have not been clearly shown to be associated with an increased risk of ventricular fibrillation, although tissue depletion of magnesium remains a potential risk factor. The ACC/AHA guideline<sup>35</sup> also recommends maintaining serum potassium levels at greater than 4.0 mEq/L and magnesium levels at greater than 2.0 mEq/L in patients with myocardial infarction (class IIa, level of evidence C), especially in those with refractory polymorphic ventricular tachycardia. Magnesium sulphate is indicated in some arrhythmia, such as torsade de pointes and arrhythmia associated to hypokaliemia. The current dose is 2 g of magnesium sulphate (8 mmol of magnesium), administered intravenously over 10 to 15 min, and repeated once if necessary. Magnesium (8 mmol) is recommended for refractory ventricular fibrillation if there is a suspicion of hypomagnesaemia, for example, patients on potassium-losing diuretics.<sup>36</sup> The European Society of Cardiology indicates normalizing magnesium levels when treating supraventricular tachycardia in patients with acute heart failure, especially in those patients with ventricular arrhythmias.<sup>37</sup>

## **Heart Failure**

American College of Cardiology/American Heart Association<sup>38</sup> recommends for the initial assessment of patients with chronic heart failure measuring electrolytes (including calcium and magnesium), searching hypomagnesaemia as a potential cause of heart failure.

## **Hypertension**

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure<sup>39</sup> in the treatment of acute severe hypertension in pre-eclampsia recommends caution when using nifedipine with magnesium sulphate, as it can induce precipitous blood pressure drop. Neither this guide nor the 2003 World Health Organization (WHO)/International Society of Hypertension statement on management of hypertension<sup>40</sup> support the use of magnesium sulphate to lower blood pressure.

## ***Magnesium Use in Gynecology***

### **Pre-Eclampsia**

Magnesium sulphate is indicated to prevent eclampsia in patients with pre-eclampsia. It has been showed in several studies that it is more effective than placebo,<sup>41</sup> phenytoin,<sup>42</sup> and nimodipine.<sup>43</sup> Magnesium sulphate decreases the risk of developing eclampsia around 50%, and also decreases maternal mortality. The WHO considers that magnesium sulphate is the elective drug for the prevention of eclampsia in patients suffering from pre-eclampsia.<sup>44</sup>

## Eclampsia

Magnesium sulphate induces a fast effect preventing seizures, but also a lack of sedation in mother and child treated for eclampsia. It has been demonstrated that it is more efficacious than phenytoin,<sup>45,46</sup> diazepam,<sup>45,47</sup> and lytic cocktail.<sup>48</sup> In addition to a wide security therapeutic margin, calcium gluconate is an effective antidote in case of overdose. The WHO considers that magnesium sulphate is the elective drug for the treatment of eclampsia.<sup>44</sup>

## Preterm Labor

Magnesium sulphate has been administered either intravenous or orally as acute tocolytic therapy for preventing premature birth in threatened preterm labor. It has a similar efficacy to the adrenergic B2 agonists as tocolytic agent,<sup>49,50</sup> but a systematic study concluded that it is not effective to delay or prevent premature birth.<sup>51</sup> On the other hand, it has been found that the use of magnesium sulphate is associated to cerebral palsy, mental retardation, and a higher neonatal and pediatric mortality,<sup>51</sup> especially in very low birthweight infants whose mothers received doses over 48 g of magnesium sulphate.<sup>52–54</sup>

## *Magnesium Use in Neumology*

### Asthma

The Global Initiative for Asthma (GINA)<sup>55</sup> of the National Heart, Lung, and Blood Institute (NHLBI) says that regular dietary supplementation with magnesium adds no clinical benefit to current standard asthma therapy. However, they recommend to consider magnesium as adjuvant therapy for severe episodes of asthma exacerbations. Moreover, they affirm that intravenous magnesium can help reduce hospital admission rates in selected groups of patients: adults with FEV1 25% to 30% predicted at presentation; adults and children who fail to respond to initial treatment; and children whose FEV1 fails to improve above 60% predicted after 1 h of care<sup>56,57</sup> (evidence A). The usual dose is 2 g given intravenously over 20 min. In addition, magnesium sulphate should be infused early in the resuscitation phase of severe asthma episodes, and these patients should have at least daily monitoring of metabolic parameters, especially serum potassium (evidence D).<sup>55</sup> On the other hand, one study<sup>58</sup> indicated that use of isotonic magnesium as an adjuvant to nebulized salbutamol results in an enhanced bronchodilator response in treatment of severe asthma. The National Asthma Education and Prevention Program<sup>59</sup> recommends magnesium sulphate to treat preterm labor in patients with asthma during labor and delivery.

### Chronic Obstructive Pulmonary Disease

Intravenous magnesium sulphate has also been used with promising results in patients with severe worsening of chronic obstructive pulmonary disease receiving inhaled salbutamol.<sup>60</sup>

**Pulmonary Hypertension**

Promising results have been published recently for newborn patients with severe persistent pulmonary hypertension. In a recent clinical trial,<sup>61</sup> it has been treated successfully with a loading dose of 200 mg/kg magnesium sulphate was given over a period of 20 min, followed by a continuous infusion at the rate of 20 to 150 mg/kg/h to obtain a serum magnesium level between 3.5 and 5.5 mmol/L. Oxygen index (OI) and alveolar–arterial oxygen gradient (A-aDO<sub>2</sub>) showed significant improvement within 24 h of treatment, proving that magnesium sulphate was found may be a safe and effective pulmonary vasodilator.

***Magnesium Use in Gastroenterology*****Antacids**

Some magnesium salts, such as carbonate, hydroxide, oxide, and trisilicate are widely used due to their antacid properties.

**Constipation**

Magnesium salts present with a laxative effect. The most widely used for this purpose is magnesium sulphate at oral doses of 5 to 10 g in 250 mL of water. This provides a rapid evacuation of intestine. Magnesium citrate, magnesium phosphate, and magnesium hydroxide are also used. The American Gastroenterology Association recommends milk of magnesia for the management of constipation<sup>62</sup> as one of the initial therapeutic options. However, the Rehabilitation Nursing Foundation<sup>63</sup> discourages the routine use of saline magnesium laxatives due to possible side effects such as abdominal cramping, water stools, and potential for dehydration and hypermagnesium. They only indicate the use of these laxatives in end-stage patients when other options have failed, and with an adequate prospective evaluation of magnesium levels.

**Acute Colonic Pseudo-Obstruction**

The American Society for Gastrointestinal Endoscopy<sup>64</sup> indicates that if the conservative treatment option is preferred, magnesium levels should be evaluated and corrected parenterally.

**Acute Liver Failure**

The American Association for the Study of Liver Diseases recommends analyzing magnesium in the initial laboratory analysis of the acute liver failure and to monitor it closely, because it is frequently low and may require repeated supplementation throughout the hospital course.<sup>65</sup>

### Other Potential Indications of Magnesium

Magnesium's past and present indications are very diverse. Dehydrated magnesium sulphate has been used as a cream for the topic treatment of inflammatory diseases of the skin, such as anthrax and infection of the hair follicles, but the prolonged or repeated use may harm the surrounding skin.

Magnesium has been used or is in study for the treatment of a variety of conditions, including analgesia, headache, alcohol withdrawal delirium, chronic fatigue syndrome, fibromyalgia, osteoporosis, and many others.

### Conclusion

Metabolic therapy of magnesium is vastly used, although the basis of this therapy remain partly unknown. Future research will make them more easily understood, and it is likely that the preventive use of magnesium will increase in hospital environments as well as in specific risk groups (athletes, patients on diuretics, etc.).

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# **Epidemiology**

# 12

## Magnesium Intake and the Incidence of Type 2 Diabetes

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Low serum magnesium levels have been related to abnormalities in insulin action and decrease in insulin secretion, both involved in the pathophysiology of type 2 diabetes. In addition, hypomagnesemia is also related to the main risk factors for development of diabetes, such as obesity, low-grade chronic inflammatory syndrome, and aging.

Because customary diet is the main source of magnesium and because several prospective, interventional, and cross-sectional studies have demonstrated a strong association between low serum magnesium levels and metabolic disorders of glucose and insulin, has been hypothesized that low magnesium intake could be a risk factor for development of type 2 diabetes.

In this chapter, we review evidence from large prospective studies and cross-sectional and interventional clinical research to discuss the role of magnesium intake and the incidence of type 2 diabetes.

Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and is expected rise to 5.4% by the year 2025. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. Estimates indicate that diabetes will increase 0.42% in the developed countries and 1.7% in the developing countries,<sup>1</sup> imposing a substantial public health burden.

The main associated risk factors for development of diabetes, such as obesity, low-grade chronic inflammatory syndrome, aging, diet, and family phenotype<sup>2-10</sup> are also associated with hypomagnesemia.<sup>11-16</sup> Additionally, low serum magnesium levels has been related with abnormalities in insulin action and insulin secretion, both involved in the pathophysiology of type 2 diabetes.<sup>12</sup> Furthermore, evidence from prospective, interventional, and cross-sectional studies<sup>17-24</sup> have demonstrated an association between low serum magnesium levels and disorders of glucose and insulin metabolism, suggesting that low serum magnesium intake could be a risk factor for development of type 2 diabetes.

In addition to clinical evidence emerged from clinical research, the background for supporting the hypothesis that hypomagnesemia is associated with incidence of type 2 diabetes derives from the role that magnesium exerts on

energetic metabolism. In this regard, magnesium, the fourth most abundant cation in the body and the second most abundant intracellular cation,<sup>25</sup> is an essential cofactor crucial in multiple physiological processes in humans.<sup>26,27</sup> It is involved in more than 300 enzymatic reactions, including glycogen breakdown, fat oxidation, and adenosine triphosphate (ATP) synthesis,<sup>28</sup> and plays an essential role for maintaining insulin sensitivity in target tissues.<sup>29</sup> In addition, magnesium is also involved in the immune and hormonal function.<sup>30</sup> Intracellular abnormalities of magnesium homeostasis may decrease tyrosine kinase activity at insulin receptors, increasing insulin resistance,<sup>31</sup> and influence glucose-stimulated insulin secretion,<sup>32</sup> decreasing  $\beta$  cell insulin secretion, the main process involved in the genesis of type 2 diabetes. Furthermore, we have found that low serum magnesium levels are strongly related with elevated serum concentrations of both tumor necrosis factor  $\alpha$  (TNF $\alpha$ )<sup>33</sup> and C-reactive protein,<sup>14</sup> suggesting that magnesium deficiency may also be involved in the triggering of low-grade inflammatory response and, through this pathway, with the development of diabetes.<sup>14,33</sup>

Magnesium is an important component of whole grains, nuts, green leafy vegetables, dried fruit, and shellfish, and is largely lost during the processing of foods.<sup>25,34</sup> In 1967 it was stated that magnesium intake of 0.30 to 0.35 mEq/kg per day in healthy people regularly produced positive magnesium balance.<sup>35</sup> The Recommended Dietary Allowance (RDA) is 400 mg for men aged 19 to 30 years and 420 mg for men more than 31 years old. The RDA is 310 mg for women aged 19 to 30 years and 320 mg for women more than 31 years old.

Although small amounts of magnesium are required in the diet in order to ensure the capacity for energy expenditure and to maintain optimal physiological function, usually dietary intake of magnesium is inadequate.<sup>36–39</sup> Recently, analyzing data from the National Health and Nutrition Examination Survey (NHANES III; 1999–2000), Ford<sup>39</sup> reported that mean magnesium intake was  $290 \pm 4$  mg per day, and that the intake of magnesium decreased with increasing age. Ford<sup>39</sup> concluded that during 1999 to 2000, the diet of a large proportion of the U.S. population was inadequate in magnesium intake. The mean intake of magnesium estimated from the first phase of NHANES III, conducted from 1988 to 1991, was 361 mg and 291 for Caucasian and African American men, and 256 mg and 215 mg for Caucasian and African American women.<sup>39</sup> In addition, extensive analysis by Kawano and colleagues<sup>40</sup> showed that dietary magnesium intake has declined in the U.S. population, from 475 to 500 mg/day in 1900 to 215 to 283 mg/day in 1990.<sup>40</sup>

Because dietary sources accounted for approximately 96% of total intake of magnesium<sup>16</sup> and individuals in westernized societies largely consume processed foods, it is difficult to reach the RDA through customary diet, and as consequence deficiency of magnesium is becoming more common.<sup>41</sup>

Magnesium deficiency has been associated with cardiovascular abnormalities, including hypertension, acute myocardial infarction, arrhythmia, dyslipidemia, and coronary artery disease<sup>25,42,43</sup> and macrovascular diabetic complications.<sup>21,44–46</sup> In addition, several large prospective cohorts<sup>16–19,47</sup>

provide evidence to support an inverse association between dietary magnesium intake and incident type 2 diabetes (Table 12.1). However, results from these studies are controversial.

Salmeron and colleagues<sup>48,49</sup> analyzed, during 6 years of follow up, the relationship between glycemic diets, low fiber intake, and risk of type 2 diabetes in two large cohorts of 65,173 women 40 to 65 years of age and 42,759 men 40 to 75 years of age, free from cardiovascular disease, cancer, and diabetes at baseline. They reported an inverse relationship between dietary intake of magnesium and incident type 2 diabetes and concluded that their finding support the hypothesis that diets with high glycemic load and lower fiber content increase the risk of diabetes. These data are partially in accordance with previous reports by Colditz,<sup>18</sup> who analyzed, also during 6 years of follow up, data of 84,360 women from the Nurse's Health Study Cohort and found that intakes of fiber and carbohydrate were not related to risk of diabetes. Both Salmeron and colleagues<sup>48,49</sup> and Colditz and colleagues<sup>18</sup> reported that magnesium intake was inversely related to risk of diabetes. However, Kao and coworkers,<sup>47</sup> who conducted a prospective study of 15,792 individuals aged 45 to 64 years to examine the association between serum magnesium level and dietary magnesium intake and the subsequent risk for type 2 diabetes, found that individuals in the highest quartile of dietary magnesium intake appeared to be at lower risk than those in the lower quartiles; however, this relationship was not statistically significant and showed no evidences of gradedness.

Recently, the association between magnesium intake and the risk for diabetes has been examined in only sparse prospective studies.<sup>16,17,19,50</sup> Lopez-Ridaura and coworkers<sup>17</sup> followed, over 18 years, 85,060 women and 42,872 men free from diabetes, cardiovascular disease, and cancer at baseline. After adjusting for age Body Mass Index (BMI), physical activity, family history of diabetes, smoking, alcohol consumption, history of hypertension, and hypercholesterolemia, they found a relative risk (RR) for type diabetes of 0.66 (95% confidence interval (CI), 0.60–0.73;  $p$  for trend <0.001) in women, and 0.67 (95% CI, 0.56–0.80;  $p$  for trend <0.001) in men, comparing the highest with the lowest quintile of magnesium intake. Additionally, because the high proportion of subjects taking vitamins containing magnesium, Lopez-Ridaura and colleagues<sup>17</sup> also assessed the association between magnesium supplements and risk of diabetes, which was not statistical significant.

Meyer and colleagues<sup>19</sup> followed, over 6 years, 35,988 older Iowa women initially free of diabetes. Adjusted for age, total energy intake, BMI, waist-to-hip ratio (WHR), education, smoking, alcohol intake, and physical activity, the multivariate analyses RR was 1.0, 0.81, 0.82, 0.81, and 0.67 ( $p$  for trend = 0.0003) across quintiles of dietary magnesium intake.

Song and colleagues<sup>16</sup> followed over 6 years a cohort of 39,345 American women over 45 years old without previous diagnosis of diabetes, cardiovascular disease, and cancer in the Women's Health Study, found a significant inverse association between magnesium intake and risk of type 2 diabetes that was independent for age and BMI ( $p$  for trend = 0.007), but significantly

**TABLE 12.1.** Relative risk (95% CI) of incident type 2 diabetes across quintiles of dietary magnesium intake.

Reference	Quintile of Intake					<i>p</i> for Trend
	1	2	3	4	5	
Lopez-Ridaura <sup>17,a</sup>	1.0	0.80 (0.69–0.94)	0.84 (0.71–0.98)	0.56 (0.54–0.76)	0.56 (0.47–0.67)	<0.001
Song <sup>16,b</sup>	1.0	1.06 (0.88–1.28)	0.81 (0.66–1.0)	0.86 (0.70–1.06)	0.89 (0.71–1.10)	0.05
Colditz <sup>18,c,d</sup>	1.0	1.25	1.03	0.81	0.73	0.008
Meyer <sup>19,e,f</sup>	1.0	0.81 (0.68–0.96)	0.82 (0.68–0.98)	0.81 (0.67–0.97)	0.67 (0.55–0.82)	0.0003
Kao <sup>47,g,h</sup>	0.95 (0.52–1.74)	1.19 (0.72–1.96)	1.11 (0.74–1.67)	1.0	—	ns
Kao <sup>47,h,i</sup>	0.80 (0.56–1.14)	0.96 (0.70–1.31)	0.98 (0.75–1.29)	1.0	—	ns

Abbreviation: ns, not significant.

<sup>a</sup>Adjusted by age and energy intake.

<sup>b</sup>Adjusted by age, smoking, BMI, exercise, alcohol use, family history of diabetes, and total calories intake.

<sup>c</sup>Distribution by quintiles intake of energy.

<sup>d</sup>Adjusted for age, BMI, alcohol intake, family history of diabetes, prior weight change, and time period.

<sup>e</sup>Distribution by quintiles intake of grain, dietary fiber, and dietary magnesium.

<sup>f</sup>Adjusted for age, total energy intake, BMI, WHR, education, pack-years of smoking, alcohol intake, and physical activity.

<sup>g</sup>Distribution for quartiles of dietary magnesium intake, comparing the lowest with the highest quartile of dietary magnesium intake in African American individuals.

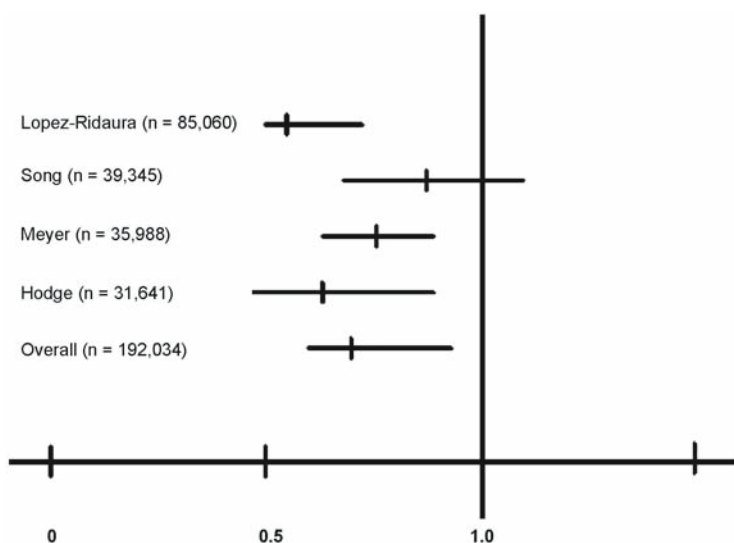
<sup>h</sup>Adjusted for age, sex, education, family history of diabetes, BMI, WHR, exercise, alcohol consumption, diuretic use, dietary calcium intake, potassium dietary intake, fasting insulin, and fasting glucose levels.

<sup>i</sup>Distribution for quartiles of dietary magnesium intake, comparing the lowest with the highest quartile of dietary magnesium intake in Caucasian individuals.

attenuated after adjustment for physical activity, alcohol intake, smoking, family history of diabetes, and total calorie intake at 1.0, 0.96, 0.76, 0.84, and 0.78 ( $p$  for trend = 0.05). Among overweight and obese women, the inverse trend in the multivariate-adjusted model was statistically significant ( $p$  for trend = 0.02).

Hodge and coworkers,<sup>50</sup> analyzing data from The Melbourne Collaborative Cohort Study, reported that the most consistent patterns among nutrients were observed for sugars, fiber, and magnesium, which decreased with increasing glycemic index. The highest quartile of magnesium intake compared with the lowest was inversely associated with incidence of diabetes [odds ratio (OR) 0.62; 95% CI, 0.43–0.90], a relationship that was attenuated after adjustment for BMI and WHR.

Although dietary information was collected using different questionnaires (a 131-item semiquantitative food frequency questionnaire by Song et al.; a 61-item semiquantitative food frequency questionnaire by Lopez-Ridaura et al.; and 127-item food frequency questionnaire by Meyer et al.), we analyzed data on magnesium intake and the risk for incidence of type 2 diabetes in women included in the Women's Health Study, the Iowa Women's Health Study, the Nurses' Health Study, and the Health Professionals' Follow-Up Study.<sup>16,17,19</sup> Comparing the highest with the lowest quintile of total magnesium intake the RR that estimates the risk for incident diabetes remained significant (0.69; 95% CI, 0.56–0.98; Figure 12.1), suggesting a protective role of dietary magnesium in the development of type 2 diabetes.



**FIGURE 12.1.** Relative risk (RR; 95% CI) showing the association between magnesium dietary intake and risk for type 2 diabetes among women.

Despite the high number of subjects included in these studies,<sup>16,17,19</sup> the estimation on the underlying association between magnesium dietary intake and risk of incident diabetes in women should be conservative. Errors in the measurement of exposition to risk (dietary magnesium intake) and outcome (incident diabetes) limit the reliability of estimated RRs. On this concern, although the prospective design reduce recall bias, the well-known limitation of food-frequency questionnaires, mainly their limitation to evaluate dietary changes during large follow-up periods, could be an important source of bias and misinterpretation. Furthermore, although the vast majority of dietary factors related to diabetes risk were considered, several additional nutrients that may influence diabetes risk were not included. On the other hand, because serum glucose levels were not measured, incident cases of diabetes were ascertained by self-reported questionnaire. Although diagnostic algorithm for type 2 diabetes was validated by review of medical records reporting a high rate of true-positive response, the true-negative and false-negative rates are not reported. Thus, the RR could be overestimated.

In addition to follow-up studies, Balon and colleagues<sup>51</sup> analyzed the effects of a magnesium-supplemented diet in the male obese Zucker fatty rat. The rats maintained on the magnesium diet had markedly lower fasting and fed-state blood glucose concentrations and an improved glucose disposal. At the end of 6-weeks follow-up, all of the eight animals on the control diet group became diabetic, whereas diabetes developed in only one of eight animals on the magnesium diet. These data indicate that an increased dietary magnesium intake in male obese rats prevents deterioration of glucose tolerance, delaying the development of type 2 diabetes.<sup>51</sup>

Although experimental studies in humans have shown benefits of magnesium supplements on glucose metabolism and/or insulin sensitivity, the vast majority of these studies have been conducted in subjects with diabetes and their results are controversial.<sup>24,52-59</sup>

Whether magnesium supplementation decreases the risk for type 2 diabetes in nondiabetic subjects is unclear. Studies for testing the hypothesis that magnesium supplementation improves insulin secretion or insulin actions in healthy subjects are scarce (Table 12.2)<sup>23,60-64</sup>

Fung and colleagues,<sup>61</sup> after adjustment for age, obesity, total energy intake, physical activity, hours per week spent sitting outside work, alcohol intake, smoking, and family history of diabetes, showed that magnesium intake was inversely associated with fasting insulin concentration among nondiabetic women. Nadler and colleagues<sup>60</sup> found that dietary-induced isolated magnesium deficiency in lean subjects leads to a significant reduction in insulin sensitivity, suggesting that magnesium deficiency may be a common factor associated with insulin resistance. Humphries and coworkers,<sup>64</sup> in a sample of nondiabetic, young adult black Americans (aged  $30 \pm 3.4$  years), showed a significant negative correlation of total dietary magnesium intake with the sum of insulin levels measured during an oral glucose tolerance test. Rosolova and colleagues,<sup>62,63</sup> assessing the relationship between the plasma magnesium

**TABLE 12.2.** Low serum magnesium levels and glucose-insulin changes in nondiabetic subjects.

Reference	n	Population	Design	Conclusion
Fung <sup>61</sup>	219	Women subsample of the Nurses' Health Study	Nested case-control study	Higher magnesium intake is associated with lower fasting insulin concentrations.
Nadler <sup>60</sup>	16	Normal subjects	Cross-sectional	Magnesium deficiency is associated with insulin resistance.
Humphries <sup>64</sup>	179	Young adult black Americans	Longitudinal	Total dietary magnesium intake is inversely related with insulin levels.
Rosolova <sup>62</sup>	18	7 mild hypertension, 7 no apparent disease 2 chronic bronchitis 1 vascular disease	Cross-sectional	Magnesium concentration is associated with insulin resistance, glucose intolerance, and hyperinsulinemia.
Rosolova <sup>63</sup>	98	1 stable angina Healthy subjects	Cross-sectional	Serum magnesium have a significant effect on insulin-mediated glucose disposal in insulin resistant subjects.
Guerrero-Romero <sup>23</sup>	63	Healthy Mexican subjects	Randomized double-blind placebo-controlled trial	Oral magnesium supplementation improves insulin sensitivity.



concentration and steady-state plasma insulin and glucose concentrations at the end of a 180-min infusion of octreotide, showed that a low magnesium concentration in nondiabetic subjects is associated with insulin resistance, glucose intolerance, and hyperinsulinemia, suggesting that variations in the serum magnesium concentration have a significant effect on insulin-mediated glucose disposal in healthy subjects, and that lower serum magnesium levels are associated with increased insulin resistance. These findings support the hypothesis that higher dietary magnesium intake may reduce the risk of developing type 2 diabetes mellitus.

Recently, we conducted a randomized double-blind placebo controlled trial<sup>23</sup> to test the hypothesis that oral magnesium supplementation modify insulin sensitivity in nondiabetic subjects. The causes of magnesium depletion linked to metabolic dysregulation, such as chronic diarrhea, alcohol intake, smoking, diabetes, high blood pressure, surgical stress, chronic diseases, diuretic therapy, and reduced renal function, were exclusion criteria. In addition, subjects who received magnesium supplementation previous to randomization were not included. At end of 3-month follow up, magnesium-supplemented subjects significantly reduced their fasting serum glucose, insulin levels, HOMA-IR index, serum triglycerides, total and low-density lipoprotein (LDL) cholesterol, and increased high-density lipoprotein (HDL) cholesterol levels, whereas in the control subjects there were no significant changes. These data supports the hypothesis that oral magnesium supplementation increases insulin sensitivity in nondiabetic subjects with decreased serum magnesium levels, but it clinical significance in the reduction of incidence of type 2 diabetes remain to be established.<sup>23</sup>

Taking into account the bias involved in measuring magnesium intake, dietary change during follow up, and diagnostic criteria of diabetes used in the large cohorts, the limitation by small numbers of subjects in cross-sectional studies, and the short-term of follow up in clinical trials, we can conclude that currently there is no sufficient accumulated clinical-based evidence for supporting that low serum magnesium intake is associated with the risk for developing type 2 diabetes.

Because the most frequent source of magnesium deficiency is an inadequate diet, healthcare providers should invest more effort in providing an appropriate dietary counsel with the aim of people reach the RDA of magnesium intake. On the other hand, results from long-term trials are needed in order to evaluate the safety and beneficial role of magnesium supplements for reducing the incidence of type 2 diabetes.<sup>27</sup>

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# 13

## Magnesium Intake and Hepatic Cancer

Andrzej Tukiendorf

Epidemiological studies conducted around the world have primarily focused on the negative correlation of magnesium in drinking water, coupled with differing sample sizes (states, provinces, towns), multiplications of observed morbidity variables (geochemical and geographical factors, analytical data on water), and different causes of mortality (vascular, cardiac, general). The majority of studies carried out have confirmed the existence of this inverse relationship; those on the largest scale have been rather conclusive on this point. However, there are cofactors that prevent us from considering water magnesium as a constant risk. Its effect may not be present when atmospheric and etiological variables are taken into account. We cannot underestimate the influence, especially statistical significance, of such factors when we are telling the water story. That said, the primary goal of this research must be to define and clarify the quantities of magnesium in drinking water that provides nutrition the body needs so that its organs are maintained in a satisfactory condition.<sup>1</sup>

The focus of epidemiological research has expanded from an initial concentration on infectious disease. A wide spectrum of effects, such as mortality and chronic ailments, acute ailments, and psychological factors, as well as environmental ones have been incorporated into study designs and cover what is usually referred to as effect on health.<sup>2</sup>

Regarding magnesium levels in drinking water, the question has been posed as to how a proportionally small intake of magnesium via water can influence the body's total magnesium content.<sup>3</sup> The problem is more complex, and the significance of magnesium intake is in fact both quantitative and qualitative.<sup>1</sup>

Quantitatively speaking, the amount of magnesium required to bridge a deficit in dietary magnesium can be represented by water magnesium. Allowing for differences in water intake via products such as tap water, bottled water, and drinks prepared with magnesium-containing water, water magnesium can represent a crucial contribution that allows for control of a relatively small intake.<sup>1</sup> Therefore a major source of magnesium is foodstuffs.<sup>4</sup>

A qualitative analysis of ingestion and uptake of metals, particularly magnesium, shows that such materials are better absorbed by the intestines when

ingested in a waterborne solution as opposed to in food. Recent studies have suggested that tap water intake is a more effective means of maintaining body magnesium levels than dietary supplementation. This results from greater bioavailability: either increased absorption (better biodisposability) and/or increased utilization, probably linked to the biological significance of the hexahydrated form of magnesium.<sup>1</sup>

Metabolic pathology can be influenced by water magnesium intake either in quantitative or qualitative properties. Because cancer cells have high metabolic requirements and the critical quantitative intake of water magnesium (which relieves marginal magnesium deficiency) may explain its importance as a possible protective cancer disease factor in the case of absolute magnesium deficit, the metal has been judged as likely to contribute to human carcinogenesis.<sup>5</sup>

This chapter will initially give a brief description about the epidemiological aspects of magnesium intake in humans. Some basics about liver cancer are given, followed by information about the consequences of a cancerous state on magnesium metabolism and the influence magnesium has on the status of the disease. Reviews of studies on the possible influence of water magnesium on cancer as well as about major factors enhancing hepatic cancer survival are placed in the following sections of the chapter. New personal data on the epidemiology of magnesium intake in the considered aspects will be presented. Finally, some research and public health conclusions will close the chapter.

## **Magnesium Intake**

Magnesium is a widely occurring element, and its primary paths of entry into the organism are through water and food. It is important to note that intestinal absorption of magnesium is very difficult to quantify despite extensive research on the subject.<sup>6</sup> The short half-life of radioactive magnesium, the type of magnesium most commonly used in studies of absorption, causes significant difficulty in research.<sup>4</sup> Due to this there is a paucity of knowledge available on magnesium uptake by the intestines from food.<sup>3</sup>

Apart from the above-mentioned problem, as previously mentioned the most significant proportion of magnesium intake comes from consumables. A great number of foodstuffs contain magnesium, especially vegetables and grains (i.e., leafy green vegetables, whole-grain cereals, beans, nuts, and shellfish). It is estimated that, on average, 30% of magnesium ingested through food is absorbed, but this figure is roughly dependent on the total amount of magnesium present in the diet as well as the amounts of other elements taken in such as calcium, phosphorous, fiber, and phytic acids.<sup>7</sup>

Another deciding factor in the level of magnesium intake is the volume of the element present in cooking water. It has been indirectly proven that an inverse correlation exists between magnesium loss in prepared food and its

presence in cooking water; if the level of magnesium in cooking water is high, the magnesium loss in food is lowered.<sup>1</sup>

Approximately 10% to 30% of magnesium intake can be accounted for by drinking water. There are, however, wide variations in the levels of magnesium in drinking water. Ground water in lime and other soft rock contains greater concentrations of magnesium than water from soil and harder rock (granite).<sup>4</sup> Changes in food preparation methods as well as a shift to using ground water with a low magnesium content have caused the relatively new problem of magnesium deficiency. The suggestion has been made that in individuals with a low intake of magnesium through their diet while consuming water with elevated levels of magnesium, the body's total magnesium status is crucially dependent on the contribution of magnesium from water.<sup>8</sup>

Due to a higher bioavailability of water magnesium hydrated ions than magnesium in food, the magnesium concentration in plants bred in regions with magnesium-rich water may be higher, especially in cases where the soil is rich in magnesium along with water of high magnesium content used in irrigation. Residents of such areas eating locally cultivated foodstuffs may, owing to this phenomenon, experience higher magnesium intake. Therefore, food composition tables may not be completely accurate when listing mineral content. It is, however, believed that plant magnesium composition is determined more by genetic factors than environmental ones.<sup>8</sup>

Examining evolutionary changes in intake levels of various nutrients can help us determine, from an ecological point of view, disease risks owing to changes in the diet of modern man. The magnesium intake of a man from the Neolithic era is believed to be greater than 1000 mg per day. In our times the trend is towards decidedly lower levels of magnesium consumption, mainly due to the wholesale removal of magnesium that occurs in the processing and refining of grain; some 70% to 80% of magnesium is lost in this way. From 1910 to 1970, daily magnesium intake fell markedly from 410 mg/daily to 340 mg/daily. In Japan, magnesium intake decreased from 351 mg/daily to 318 mg/daily in the period from 1971 to 1985.<sup>4</sup> Increased levels of industrial treatment and decreasing magnesium levels by as much as 80% to 95% cause most people to ingest lower than the daily recommended allowance of magnesium (6 mg/kg/day). Some sources recommend a magnesium intake level of about 350 mg/daily.<sup>7,9</sup> In drinking water, the level of magnesium content should be approximately 30 mg/L.<sup>1</sup>

Hypomagnesaemia can be attributed to a number of other factors apart from low intake levels. A loss in magnesium can be caused by alcohol consumption. A study of regular consumers of alcohol who were not alcoholics was performed by means of an oral loading test with magnesium. The level of magnesium and calcium expelled in urine was higher among those consuming alcohol and proportional to the amount of alcohol consumed.<sup>10</sup>

In any particular population, deficiencies of magnesium that cause disease can be accounted for by many different risk factors combined with low intake.



Cases such as this are characterized by an accompanying hypocalcaemia, which does not respond to calcium and vitamin D therapy. However, it has been shown that vitamin D plays a role in the regulation of the magnesium uptake.<sup>9</sup> Therefore, it is a question of several weeks required to reconstitute normal intracellular levels of magnesium in deficiency cases. This may be an explanation for the lack of direct success of magnesium-treated hypomagnesaemia-caused disease.<sup>4</sup>

Furthermore, it has been suggested that calcium decreases magnesium uptake, which may itself suggest a decrease in membrane permeability by calcium or a negative influence on the carrier. Optimal uptake probably requires sodium and phosphorous. High levels of fatty acid—magnesium soaps in the intestines, caused by fats consumption, decreases uptake. Protein may decrease or increase uptake.<sup>4,5</sup>

However, the exact proximate causes of differing magnesium intake levels in the body are not fully known. This is particularly the case with meals of mixed ingredients, and total intake cannot be seen as a proxy or relevant measure for uptake. Studies of the body balance of magnesium measured in subjects with controlled levels of magnesium intake in food and water should be done to explore the proportional uptake of magnesium from consumed foodstuffs and water.<sup>4</sup>

There is a sharp decline in the intake of general nutrients by the elderly, and this principle holds true for magnesium. Older persons are also less able to absorb magnesium intestinally. Moreover, older men exhibit lower amounts of magnesium discharged in urine, whereas young women excrete lesser amounts of magnesium than postmenopausal women. This difference was more pronounced in women taking oral contraceptives. Magnesium requirements are, in line with those of other minerals and nutrients, probably raised in the case of the elderly.<sup>11,12</sup>

## **Magnesium in the Body**

Ninety-nine percent of the total amount of magnesium in the body is located intracellularly.<sup>9</sup> More than half of this is found in bone and other tissues (approximately 60%), the greatest amount being located in skeletal muscle and the heart, but only 0.3% is present in the serum.<sup>4</sup>

Magnesium is a cofactor in all enzymes involved in phosphate transfer reactions that utilize adenosine triphosphate (ATP) and other nucleotide triphosphates and substrates. Magnesium plays a crucial role in most of the enzymes processing carbohydrate, lipid, nucleic acid, and protein metabolism (Magnesium is required in over 300 enzymes that aid the metabolic process of these reactions and ion transport.)<sup>13</sup>

Energy-reducing reactions (oxidative phosphorylation) and energy-consuming reactions (contraction) are also dependent on magnesium.<sup>4</sup> It is

also involved in the regulation of cellular permeability<sup>5</sup> and neuromuscular excitability.<sup>3</sup>

It is well known that magnesium plays an essential role in a wide range of fundamental cellular reactions and its deficiency in an organism may lead to serious biochemical and symptomatic changes. As regards the liver, it is known that higher collagen concentrations were found in the livers of magnesium-deficient alcohol-fed rats that were observed under control due to increased collagen production in magnesium deficiency.<sup>14</sup> Moreover, the increase in plasma triglycerides and cholesterol observed in magnesium-deficient rats may be the result of increased hepatic synthesis (thus cholesterol output is markedly reduced in deficient animals).<sup>15</sup>

Future investigations should clarify whether these facts have a strong influence on the condition of the liver, including carcinogenesis, that may be caused by the disturbance of magnesium levels in the human organism. Some basics on the possible influence of magnesium on cancer are given in the next section.

## Magnesium and Oncogenesis

The relationship between magnesium and cancer is complex and contradictory. Both magnesium load and deficit can produce carcinogenic and anti-carcinogenic effects.<sup>16</sup> Carcinogenesis modifies magnesium status, inducing magnesium distribution disturbances which may frequently associate a tumor magnesium load with magnesium depletion in non-neoplastic tissues.<sup>17</sup>

In chemical carcinogenesis, cellular magnesium deficit can be seen in both preneoplastic and neoplastic states. Structural and functional changes of the plasma membrane are caused by magnesium deficit, including a marked reduction of the intracellular cations Mg and K, with a raising of extracellular cations Ca and Na in neoplastic cells.<sup>18</sup> Such extreme changes are similar to those cellular alterations resulting from magnesium deficiency. One basis for the prophylactic use of magnesium in oncology is to avoid the facilitation of the effect of a carcinogenic agent by the triggering of this kind of cellular disturbance.<sup>17</sup>

Disorders in magnesium distribution in carcinogenesis are far more complex than what can be found in simple magnesium deficiency. In the neoplastic state, magnesium contained in intracellular structures is lower, whereas its occurrence in cytosol (as well as mitochondria) rises.<sup>5</sup> Such a variance in magnesium distribution, not found in ordinary magnesium deficiency, points to an alteration of intracellular magnesium regulation. In other words, a depletion of magnesium is starting at the beginning of carcinogenesis.<sup>5</sup>

Later on, variances in the distribution of magnesium are even more complex. At later stages of cancer in humans, the disturbance in magnesium distribution associates an increased magnesium level in the tumor and in blood cells,

including erythrocytes and lymphocytes, with some stigmata of magnesium depletion that vary according to the method of treatment.<sup>19</sup> Magnesium depletion occurs more often in patients with solid tumor malignancy than with hemopathic malignancy.<sup>5,17</sup>

Magnesium depletion does not occur equally in all forms of tissue. Experimental and clinical data indicates that the adverse effects of magnesium depletion in non-neoplastic tissue are reversible. It seems, therefore, that an established cancer induces magnesium disturbances that effect magnesium load in tumoral tissue, probably due to magnesium mobilization through blood cells, with magnesium lessening in non-neoplastic tissue.<sup>5,17</sup>

Such a process can be understood in this way—established carcinogenesis induces magnesium disturbances that accentuate tumor magnesium load due to magnesium mobilization through blood cells with magnesium depletion in non-neoplastic tissue. At a premalignant stage and in the case of some hemolymphoreticular malignancies, magnesium deficiency appears carcinogenic, but in the case of solid tumors it exerts a negative influence on tumor growth.<sup>5</sup> These disturbances lead to several logical consequences regarding oncological research and treatment. Indications for cancer therapy of magnesium supplementation or of induced magnesium deficiency are critical. It is possible to hypothesize on the importance of optimal treatment for magnesium disturbances as part of a general cancer treatment regime.<sup>5,16</sup>

There are several studies of magnesium experimental oncogenesis in animal laboratory subjects (mostly rats) that focus on the relation between the magnesium deficiency in diet and lymphomas and leukemias, bone tumors, intestinal tumorlike overgrowth, etc.<sup>20,21</sup> At this time, however, no such outcomes have been reported regarding a relationship between magnesium deficiency and hepatic cancer.

To familiarize the reader with the problem considered, some brief medical information on liver cancer and a concise epidemiological review of water magnesium studies on the disease, as well as hepatic cancer survival, are given in the following sections of the chapter.

## Hepatic Cancer

Hepatic cancer is a rare disease and it is many times more common in developing countries, such as Africa and East Asia, than in the United States or Europe.<sup>22</sup> There are four main types of liver cancer that appear in different parts of the organ. The hepatocellular carcinoma starts in the hepatocytes, the main liver cells, and according to the American Cancer Society accounts for 84% of cases. Cholangiocarcinoma appears in the small bile ducts of the liver and accounts for about 13% of liver cancers. Angiosarcoma is a rare cancer that starts in the blood vessels of the liver, accounting for less than 1% of cases according to National Cancer Institute. Finally, hepatoblas-

toma is the rarest type of liver cancer (in children) affecting usually the right lobe.<sup>23</sup>

It is necessary to stress that no one knows the exact etiology of liver cancer. However, scientists have found that people with certain risk factors are more likely than others to develop liver cancer. Different hypotheses are tested that focus on hepatocarcinogenic mechanisms as well.<sup>23</sup> The epidemiological approach may play an essential role in this scientific activity.

Besides the causes of the disease not being well established, the factors that may prolong survival have also not been recognized so far.<sup>24</sup> That is why the prognoses for patients with liver cancer are generally poor, as most patients have no symptoms until the tumor has grown or spread to other parts of the body. By the time a diagnosis is made, it is often no longer possible to remove all cancerous tissue surgically in order to prolong survival. Late diagnosis may be a reason why the average survival after diagnosis is often quoted as 3 to 6 months.<sup>25</sup> In advanced liver cancer, median survival is usually 2 to 4 months.<sup>24</sup>

## Epidemiology of Water Magnesium and Cancer

Although in dozens of epidemiological studies the effects of drinking-water magnesium on morbidity and mortality—predominantly from cardiovascular disease—have been recognized, until now not many reports have confirmed the epidemiology of water magnesium and cancer. Important findings in this field were provided recently by Taiwanese scientists. However, in most of their studies, these authors indicated negative statistical associations of various types of cancer morbidity/mortality but mostly with the hardness of water and calcium rather than magnesium.

In a review of these publications, it is worth noting the results concerning the possible association between the risk of gastric cancer and the levels of calcium and magnesium.<sup>26</sup> The present study suggests that there was a significant protective effect of calcium intake from drinking water on the risk of gastric cancer. Magnesium also exerted a protective effect against gastric cancer, but only for the group with the highest levels of magnesium exposure. In another earlier matched case-control study,<sup>27</sup> the authors found a possible association between the risk of colon cancer and hardness levels in drinking water from municipal supplies in Taiwan (obtained trend analyses showed an increasing odds ratio for the cancer with decreasing hardness in drinking water). Similar epidemiological trends were also achieved for the relations between hardness levels in drinking water and the risk for rectal cancer<sup>28</sup> and pancreatic cancer mortality,<sup>29</sup> however, the researchers did not find any association with magnesium levels (the adjusted odd ratios were not statistically significant for the relationship between magnesium concentrations in drinking water and rectal cancer).<sup>30</sup> One of the strongest epidemiological proofs of a significant protective effect of magnesium intake from drinking water was

that given for the risk for esophageal cancer<sup>31</sup> and ovarian cancer.<sup>32</sup> Unfortunately, these authors did not find any results pertaining to the similar trend between drinking-water magnesium and liver cancer.<sup>33</sup> The first strong evidence concerning the possible ecological relation between exposure to water magnesium and hepatic cancer was reported in Eastern Europe by this author.<sup>34,35</sup>

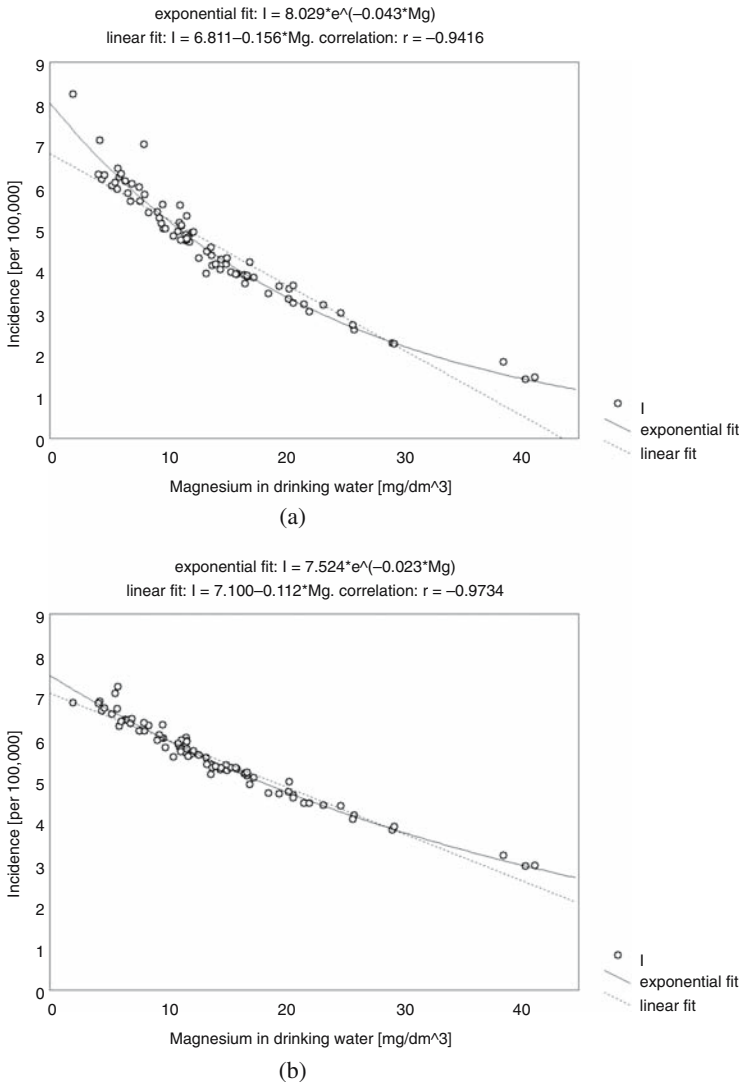
## Hepatic Cancer Survival

In patients with hepatic cancer the prediction of survival is difficult. This problem particularly concerns the used therapy.<sup>36</sup> These studies are mostly based on clinical trials and among the favorable prognostic factors that prolong the survival of patients with hepatic cancer. The following treatment methods are suggested: transarterial chemoembolization, partial resection, liver transplantation,<sup>36</sup> percutaneous ethanol injection,<sup>37</sup> cytoreductive surgery,<sup>38</sup> radiotherapy,<sup>39</sup> and a combination of some of these therapies<sup>40</sup> rather than anti-cancer drugs (chemotherapy),<sup>41</sup> although sometimes these theses are questioned.<sup>42</sup> In the cited studies it has been also reported that the survival on liver cancer strongly depends, for example, on an early detection of the disease, stage of disease, tumor size, association with viral infections or alcoholism, etc. Unfortunately, some skeptical opinions in the hepatological community are also claimed. One of these is that most of the cancer hepatic treatments are in fact curative and can palliate only.<sup>43</sup>

## New Personal Data on Drinking-Water Magnesium and Hepatic Cancer

Based on extensive ecological data and conducted Bayesian modeling,<sup>44</sup> these results report on epidemiological findings concerning a possible relationship between magnesium in drinking water and risk for liver cancer and its survival. The studies were performed using historical cancer morbidity registers and water quality was assessed by geographical area at the commune level. No information was available on other risk factors for hepatic cancer disease, such as dietary habits, occupational exposure, or smoking. Despite these methodological shortcomings, surprisingly large differences and very strong dose-response relationships were found. Relative estimated contributions of water quality to endemic waterborne disease for magnesium concentrations both for males and females using random effects logistic regression<sup>44</sup> are shown in Figure 13.1.

Both relations presented for males and females accordingly show that for the lowest magnesium concentration in drinking water, the cancer risk was significantly higher than it was for the highest magnesium content (approximately four times in males and three times in females, respectively). Thus, the



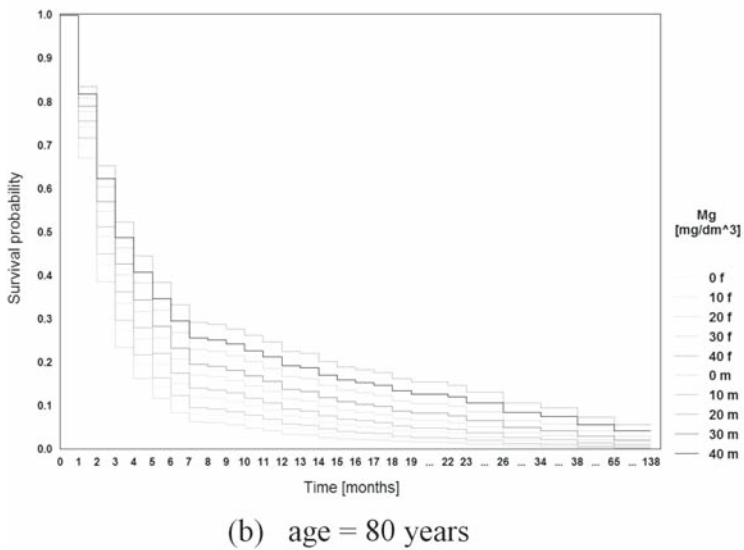
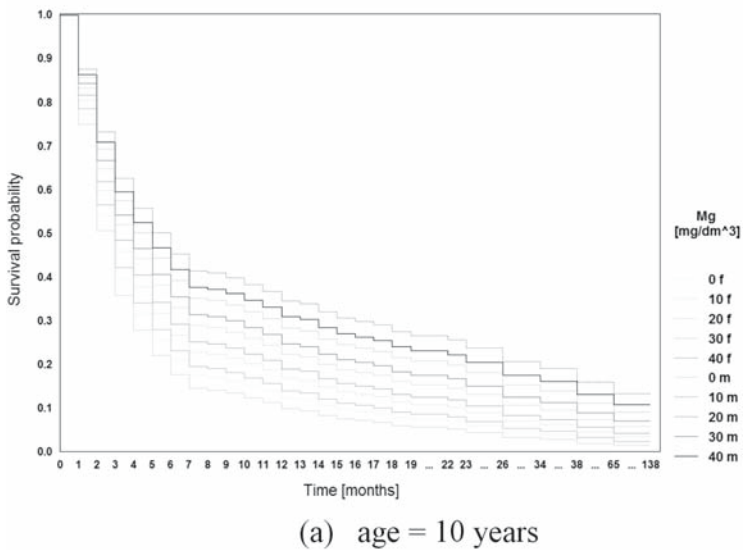
**FIGURE 13.1.** Liver cancer incidence ( $I$ ) in (a) males and (b) females (1985–1994) versus magnesium concentration in drinking water (1980–1985) in administrative units of the province of Opole, Poland.<sup>34</sup>

achieved outcomes provide evidence of possible positive health effects of magnesium water supplies on this lethal disease.

The question of how water magnesium exposure might influence survival of liver cancer patients with age using Cox's regression approach<sup>45</sup> is considered in Table 13.1 and Figure 13.2; the results were recently displayed during the scientific conference Magnesium In Biochemical Processes & Medicine

**TABLE 13.1.** Estimates of hazard ratios (Cox's regression).

Variable	Hazard Ratio	95% Confidence Interval
Gender (M, F)	0.873	(0.711, 1.072)
Age (year)	1.006	(0.997, 1.016)
Magnesium (mg/dm <sup>3</sup> )	0.979	(0.962, 0.997)



**FIGURE 13.2.** Estimated postdiagnosis survival probability in males (m) and females (f) with liver cancer for drinking-water magnesium exposure for patients aged 10 (a) and 80 (b) years old.

organized by the Gordon Research Conferences in Ventura, California (06–11.02.2005).

The values indicate that females have a lower chance of early mortality (over 12%) in comparison to males. Moreover, the mortality risk increased with the age of the patient at diagnosis (around 0.6% with every year of age). Finally, the estimated hazard ratio associated with magnesium supports the hypothesis of a beneficial effect of water magnesium consumption on the chance of early mortality from the disease (the risk of early mortality for the patients decreases about 2% with every unit magnesium concentration increment; see the hazard ratios in Table 13.1). Evidence for the relationship between survival in patients suffering from liver cancer and exposure of the magnesium content of drinking water (for the extreme age bands) can also be identified in the modeled survival curves (Figure 13.2).

The modeled survival curves presented in Figure 13.2 confirm the results set in Table 13.1. For different drinking-water magnesium concentrations for men and women aged 10 and 80, they illustrate the improved survival of patients suffering from liver cancer disease who were exposed to a higher magnesium concentration. Moreover, slightly longer prognoses are noticed for the younger patients.

## Conclusions

Speculation on and research into the cause of disease have always been an important focus of interest for man. Researchers in hospitals and medical centers around the world are working to learn more about what causes liver cancer. To the most important etiological factors of the disease, hitherto studies have included the chronic liver infection (hepatitis). Many scientific reports accordingly confirm that there is a strong association between chronic hepatitis B and C viral infection and the development of the disease. Moreover, people with fibrosis and cirrhosis also have an increased risk of liver cancer. Other possible hepatocarcinogens include aflatoxin, nitrosamines, oral estrogen compounds, and numerous other chemicals. The National Cancer Institute mentions risk factors such as family history, being male, and age.<sup>46</sup>

The last two factors seem to be confirmed by the results presented above. From the ecological and medical points of view, a possible impact of water magnesium intake on the risk for hepatic cancer as well as liver cancer survival is attention grabbing. No such relation, except for in the Opole province in Poland, has been reported before in scientific literature.<sup>34,35</sup> Therefore, these results should undergo scientific assessment and verification. Moreover, the creation of this type of epidemiological hypotheses is faced with theoretical problems.

The major problem is the contributions of magnesium in drinking water to the total amount ingested. However, at this stage of knowledge it is suggested that waterborne magnesium is better absorbed than dietary magnesium, and this fact is associated with the general properties of cellular membranes.



Because amnion is a particularly leaky membrane, the effect of water magnesium may be due to the hexahydrated form in connection with linked amnion. Water magnesium may antagonize lead and cadmium on the plasma membrane, particularly during intoxication.<sup>1</sup>

The next question regards the time of latency between the putative cause-specific factor and the follow-up neoplastic process. Intuitively, the period of the liver cancer morbidity should have followed the water magnesium consumption. Although the condition seems to be fulfilled in the personal study, no one knows how long this time should be at least approximately assumed.

Nevertheless, the present results and given theoretical background allow for some scientific speculations. One of these could be the reason why alcohol causes abnormalities in magnesium metabolism and results in severe liver diseases (including cirrhosis),<sup>47,48</sup> as well as why the most diseased group (both male and female) consisted of older patients. One of the hypothesized backgrounds for the association between the magnesium exposure and liver cancer could be direct/indirect effects of alcohol consumption. This hypothesis could be justified by the fact that alcohol decreases the magnesium concentration in body and it can be returned into the organism via water ingestion. Then, linking the magnesium higher intakes from the richer water supplies together with its preventive properties in neoplastic processes might be a reasonable justification why liver cancer morbidity is diminishing with the increase of drinking water magnesium exposure, and vice versa. If the hypothesis is true, the introduction of social drinking, a habit that is also spreading into the younger age groups, is thus a new phase of our adaptation to a lifestyle that implies severe risks for health effects, including cancer.

If in the light of the given facts concerning drinking-water magnesium and the risk for liver cancer turn more into consistency, the identified epidemiological relationship between survival in patients suffering from hepatic cancer and the element's exposure still remains unclear. The latter effect might be justified by the greater load of magnesium in body in people who were drinking magnesium-rich water. However, any attempts to explain the possible metabolic mechanism need collaborative consultations and discussions of a variety of specialists, that is, biochemists, physiologists, hepatologists, etc.

Further studies of the possible magnesium effects on the hepatic cancer and its survival will open new paths to cancer research. It is believed, however, that present epidemiological findings will be helpful in directing scientific concern towards proper research in the field of a possible relation between magnesium intake and hepatic cancer and to explain the observed evidence.

Accordingly, as concerns public health, it is important that such studies are undertaken. If a risk group can be identified, then public health concerns to restore magnesium deficiency should be targeted exclusively to that group, however if a risk group cannot be identified, the entire population must be protected, probably by adding magnesium to the drinking water in optimal amounts. However, before public health activities are undertaken, it is paramount to determine whether this risk is present but only after more precise

clinical or experimental research; thus, possible preventive actions can then be acted upon efficiently.

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## Exercise

# 14

## Exercise and Magnesium

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Magnesium is essential for the optimal function of a diversity of life-sustaining processes. It is cofactor of more than 300 enzymes, participating in the metabolism of carbohydrates, lipids, proteins, and nucleic acids, in the synthesis of hydrogen transporters, and particularly in all reactions involving the formation and use of adenosine triphosphate (ATP). Magnesium also serves as a regulator of many physiological functions, including neuromuscular, cardiovascular, immunological, and hormonal functions, as well as the maintenance of membrane stability.<sup>1,2</sup>

Magnesium can participate in the reactions involving the formation and use of ATP by two different mechanisms. Kinetic studies of several enzymes requiring both magnesium and ATP showed that enzyme activity depends on the ratio, as well as on the absolute concentrations, of the two cofactors and that magnesium chelates strongly with ATP, forming a Mg(ATP) complex, the active substrate for enzyme action. Similarly, a magnesium complex with adenosine diphosphate (ADP), Mg(ADP), appears to be the active substrate for some enzymes. The second general mechanism of magnesium action is the direct binding of free magnesium ( $Mg^{2+}$ ) to the enzyme protein and resultant allosteric activation. With some enzymes magnesium has a dual function, not only forming part of the reactive substrate but also activating the enzyme allosterically. However, in some cases Mg(ATP) and  $Mg^{2+}$  may have opposite actions. For example, the Mg(ATP) complex stimulates the type-L calcium channels and  $Mg^{2+}$  inhibits them.

Magnesium is also known to alter both receptor sites and ion movements across the cell membrane. By making complexes with phospholipids, magnesium stabilizes the membranes, reducing their fluidity and permeability. Thus, magnesium is an important modulator of intracellular ion concentrations. In magnesium deficit, intracellular concentrations of calcium and sodium increase, and concentrations of potassium and phosphorus decrease. Simultaneously, the membrane depolarizes. These alterations may be the result of magnesium's direct effect on sodium, calcium, or potassium channels or the indirect result of its effect on enzymes in the cell membrane that are involved in active transport, for example,

( $\text{Na}^+\text{K}^+$ )—ATPase. Magnesium also regulates lipid and phosphoinositide-derived second messengers.

Within the cell, magnesium affects the function of organelles such as sarcoplasmic reticulum, primarily by its ability to alter calcium flux, or mitochondria, by altering their membrane's permeability to protons, which leads to alterations in the coupling of oxidative phosphorylation and electron transport chains, thus affecting the efficiency of ATP production.<sup>1</sup> Magnesium also acts as a calcium antagonist. In the neuromuscular system it reduces the electric excitability of the neurons and inhibits the release of acetylcholine by the nerve endings at the neuromuscular junction, and blocks the effect of *N*-methyl-*D*-aspartate, an excitatory neurotransmitter of the central nervous system. In muscle contraction, both stimulation and the activity of the calcium transport system in the sarcoplasmic reticulum membranes depend on the presence of  $\text{Mg}^{2+}$ . Troponin contains four calcium-binding sites, two of which have a high affinity for calcium and bind  $\text{Mg}^{2+}$  competitively. These calcium-magnesium type sites do not seem directly involved in any rapid twitching mechanism, but play a structural role in muscle. Magnesium bound to these sites may maintain the protein permanently in a particular conformational state regardless of the fluctuation in calcium (assuming that both magnesium and calcium-induced structural changes are essentially the same). This conformation may be a prerequisite for calcium activation via binding at the calcium-specific sites.

These and many other functions make it easy to understand why performing exercise is highly dependent on the regulation of magnesium homeostasis. Additionally, there is evidence that exercise performance seems to be impaired under conditions of magnesium deficiency.<sup>3</sup> This is why individuals performing exercise should pay extra attention to magnesium status.

## Athletes and Magnesium Status

Frequently, physically active individuals fail to consume a diet that contains adequate amounts of minerals, including magnesium, which leads to marginal nutrient deficiency and results in substandard training and impaired performance.<sup>4–6</sup> Additionally, mineral losses in urine and sweat are more important during exercise than at rest.<sup>7</sup> These conditions may contribute to frequent mineral deficits among athletes.<sup>6,8</sup>

During exercise, compartmental shifts of magnesium have been observed, but data to demonstrate magnesium variations induced by exercise are inconsistent. Such heterogeneity can be partially attributed to differences in experimental designs and work intensity and duration. Moreover, the timing of blood sample and the different analytical protocols have to be taken into account.<sup>9</sup>

With respect to blood extracellular magnesium, various authors have indicated that high-intensity exercise leads to hypermagnesemia as a consequence of the decrease in plasma volume.<sup>10–12</sup> These changes may depend on the relative contribution of anaerobic metabolism to the total energy expended during exercise.<sup>13</sup>

On the other hand, submaximal exercise has been reported to be accompanied by hypomagnesemia.<sup>14-19</sup> Prolonged strenuous exercise, especially under hot conditions, may lead to hypomagnesemia.<sup>14,20-23</sup> Low plasma magnesium levels have been explained by several mechanisms: redistribution of magnesium to red blood cells, adipocytes or myocytes;<sup>24</sup> loss in urine due to increases in aldosterone, anti-diuretic hormone, thyroid hormone, and acidosis that reduce the tubular reabsorption of magnesium;<sup>13</sup> or increased lipolysis due to raises in catecholamines levels induced by exercise.<sup>25-27</sup> Hyperexcretion in sweat only acquire real importance in case of intense activity made in conditions of damp atmosphere and high temperature.<sup>28</sup>

A transient shift of magnesium to the intracellular space during exercise is a probable explanation for a large proportion of hypomagnesaemia. However, regarding red blood cell (RBC) magnesium variations with exercise, dissimilar findings are reported. Red blood cell magnesium levels were reported to be increased after several exercises<sup>8,10,13,29</sup> and were related to the increased metabolic activity during exercise, which would induce a shift of the cation from the plasmatic compartment.

Conversely, RBC magnesium levels were reported to be decreased.<sup>18,30-32</sup> Golf and coworkers<sup>32</sup> postulated that as exercise duration increases, magnesium shifts from the erythrocyte reservoir into the plasma and then into the working muscles. With prolonged exercise (more than 1 h) hypomagnesaemia may occur as a result of the depletion of the erythrocyte reservoir. Several studies suggest that low red blood cell magnesium levels may persists during a season of training.<sup>33-35</sup>

Mg<sup>2+</sup> concentration is supposed to be a more sensitive parameter than that of total magnesium and so it should give more reliable information about the status and regulation of major, mobilizable magnesium pools in the body. However, only limited information on the exercise effects on the metabolic and regulatory fraction of Mg<sup>2+</sup> is available.<sup>36</sup>

Recently, Mooren and coworkers<sup>37</sup> showed that, at the end of a treadmill ergometer test, both total blood and serum Mg<sup>2+</sup> and serum total magnesium decreased. In contrast, Mg<sup>2+</sup> increased in both thrombocytes and erythrocytes. Intracellular total magnesium was unchanged, making a Mg<sup>2+</sup> shift between the intra- and extracellular blood compartment unlikely. This study also showed opposite changes of the ratio [Mg<sup>2+</sup>]/[total Mg] in the intracellular and the extracellular compartment after anaerobic exercise. In *in vitro* experiments, similar changes of Mg<sup>2+</sup> in the two blood compartments could be mimicked by application of weak acids like lactic and propionic acid. These authors concluded that changes in the fraction of Mg<sup>2+</sup> should be enough to influence intracellular signaling and metabolic processes.

Although some explanations have been offered for the compartmental shifts of magnesium, the precise mechanism remains to be clarified. It is important to evaluate whether there is only a transient fall in plasma magnesium concentration, or if the participation in sustained exercise may induce permanent alterations. Several studies indicate that there is a sustained fall in plasma



magnesium after strenuous exercise and that hypomagnesaemia persists during a season of training.<sup>34,35,38–40</sup>

Exercise may increase the demand for magnesium and/or increase magnesium loss, potentially leading to magnesium deficit, which can result in muscle weakness, and neuromuscular dysfunction.<sup>20,41</sup>

Overt signs and symptoms of magnesium deficit, for example, hyperirritability, tetany, convulsions, and cardiac arrhythmias, may not be manifested until serum magnesium concentration has decrease below 0.5 mmol/L. Generally, exercise-induced magnesium decrease does not approach the level that would be of concern from a health point of view. Thus, the consequences of exercise-induced magnesium decreases on health status in healthy people with normal magnesium levels seem to be negligible.<sup>42</sup> However, they may be more relevant in people who already have low magnesium. This problem may be exacerbated in those who experience large increases in plasma free fatty acids during exercise, because of the inverse relation between serum magnesium and free fatty acids. It is also important to note that magnesium deficit plays a role in myocardial injury after prolonged sustained exercise, as it may lead to an increased potential for thrombus formation and/or coronary vasospasm.<sup>43</sup>

Reports on magnesium supplementation are also discordant. It has been reported to increase muscle strength and power.<sup>44</sup> Conversely, marathon runners with adequate magnesium status exhibited no improvement in running performance or skeletal muscle function and no increase in muscle magnesium concentrations.<sup>45</sup>

In spite of the fact that most studies lack information about magnesium status of the subjects previous to supplementation, which may justify some of the discordant results, the findings suggest that magnesium supplementation per se does not illicit beneficial effect on physiological function or performance when magnesium status is normal.<sup>5</sup> Doctors and athletes should be aware of the magnesium changes during exercise in order to avoid health risk.<sup>42</sup>

## **Exercise and Magnesium Deficiency Induce Oxidative Stress**

Strenuous physical exercise is capable of inducing oxidative stress, a state where the production of reactive oxygen species (ROS) in the body transcends the anti-oxidant defense capacity.<sup>46,47</sup> Several physiological processes are involved in the ROS generation and propagation during exercise: increased hemoglobin deoxygenation and reoxygenation rates; accelerated mitochondrial electron transport; acidosis and increased release of catecholamines, as during their oxidation to adrenochrome free radicals are formed. Strenuous exercise can also induce trauma, which leads to the extravasation of blood and to the introduction of free iron and copper into tissues, which can catalyze the propagation of ROS and consequently modify a wide range of biomolecules, including the polyunsaturated fatty acids of the cell's membrane.<sup>48</sup> Inflammatory injury, with the infiltration and activation of monocytes, produces a spectrum of free radicals.<sup>49</sup>

If the rise in oxygen free radicals exceeds the protective capacity of cell's anti-oxidant defense systems, it can lead to ROS-mediated injury and consequently to the loss of the cell's membrane integrity and to tissue damage. Several studies have shown that strenuous exercise promotes free-radical formation, resulting in a measurable degree of oxidative modifications to various molecules.<sup>47,50–52</sup>

The efficiency of the anti-oxidant defense systems relies on adequate dietary vitamin and micronutrient intake and on endogenous production of anti-oxidants, such as anti-oxidant enzymes and glutathione.<sup>53</sup>

Like the practice of exercise, magnesium deficiency is also prone to the increase of ROS generation as magnesium has an important role in the inhibition of ROS-induced cell injury. It inhibits catecholamine release and is a cofactor for their methylation that prevents oxidation.<sup>54</sup> It also participates in the *de novo* synthesis of Reduced glutathione (GSH), which may be important in the restoring of GSH cellular levels during exercise.<sup>55</sup>

In magnesium-deficient rodents, it has been demonstrated that ROS formation and the concentration of thiobarbituric acid reactive substances (TBARS), reflecting lipid peroxidation, are increased.<sup>56,57</sup> These changes were associated with structural damage in skeletal muscles affecting sarcoplasmic reticulum and mitochondria,<sup>56</sup> probably resulting from impairment of intracellular calcium homeostasis by altering the integrity of the sarcoplasmic reticulum membrane.<sup>58</sup> However, the mechanism by which magnesium deficiency potentiates injury remains unclear. Other explanations include increased cytokine concentrations, alterations in iron metabolism, or decreased endogenous anti-oxidant capacity.<sup>59</sup> The lack of magnesium was also associated with a depletion of selenium and reduced glutathione peroxidase activity.<sup>60</sup> Chugh and coworkers<sup>61</sup> supplemented patients with oxidative stress diseases with magnesium and suggested its anti-peroxidant effect when observing a decrease in lipid peroxidation.

In view of the above, during the practice of exercise, the conditions are prone to both the increase of reactive oxygen species generation and the decrease of magnesium status. As a consequence, individuals practicing exercise are more susceptible to free radical—mediated injury. We emphasize the importance of evaluating magnesium status in athletes, not only because its deficit may compromise performance, but also because the practice of exercise with a magnesium deficit may render the individual more susceptible to the occurrence of cellular damage, especially those associated with oxidative stress.

## **Immune Function Related to Exercise: Possible Roles of Magnesium**

Magnesium has a strong relation with the immune system in both nonspecific and specific immune response.<sup>62</sup>

The attempt of an infectious agent to enter the body immediately activates the innate system. The nonspecific line comprises the action of macrophages or natural killer (NK) lymphocytes. Natural killer cells are rapidly activated and capable of policing the host until the more specific immune competent cells are activated. It has become clear that NK cells are highly influenced by various types of stress, including acute physical exercise.<sup>63-65</sup>

Failure of the innate system activates the acquired system. Monocytes or macrophages process and present foreign material (antigens) to lymphocytes. This is followed by clonal proliferation of T and B lymphocytes that possess receptors able to recognize the antigen. Action and function of both branches of the immune system are governed by the action of cytokines, which include interleukin 1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF). Their excessive or insufficient production may contribute to many infections or immunoinflammatory diseases.<sup>66</sup> While TNF and IL-1 have pro-inflammatory effects, IL-6 has more restorative effects by being the main inducer of the acute phase response by the liver.<sup>67</sup>

Exercise, both high intensity and prolonged, is a stress to the body that is proportional to the intensity and duration of the exercise, relative to the maximal capacity of the athlete. Exercise stress leads to a proportional increase in stress hormone levels and concomitant changes in several aspects of immunity, including high cortisol; neutrophilia; lymphopenia; decreases in granulocyte oxidative burst, NK cell activity, lymphocyte proliferation, production of cytokines in response to mitogens, and in nasal and salivary immunoglobulin A (IgA) levels; and increases in blood granulocyte and monocyte phagocytosis, and pro- and anti-inflammatory cytokines.<sup>68</sup>

The acute effects of exercise on lymphocytes are mediated by adrenaline and noradrenaline, whereas neutrophils are mediated by cortisol.<sup>69</sup>

Many aspects of immune function can be depressed temporarily by either a single bout of very severe exercise or a longer period of excessive training.<sup>68,70</sup>

Although the disturbance is usually quite transient, it can be sufficient to allow a clinical episode of infection, particularly upper respiratory tract infections.<sup>71,72</sup> However, regular and moderate exercise has been reported to improve the ability of the immune system to protect the host from infection.<sup>73,74</sup> Resting levels of NK cells are enhanced as a result of training.<sup>69</sup> Leucocyte number is clinically normal and remains unchanged with training.<sup>73</sup>

The response of the immune system to exercise is varied, with different behaviors for each cell type and dependent on the intensity and duration of the exercise test and on the training of the subject. Besides these factors, nutritional deficiencies alter immunocompetence and increase the risk of infection. Both heavy exercise and nutrition exert separate influences on immune function, appearing to be greater when acting synergistically.<sup>62</sup> Exercise training increases the body requirement for most nutrients. However, some athletes adopt an unbalanced dietary regimen predisposing them to immunosuppression.<sup>75</sup>

Several elements are known to exert modulatory effects on immune function, including zinc, iron, selenium, calcium, copper, and magnesium.<sup>68,72,76-80</sup>

Several groups leading investigations in nutrition and immunology have shown evidence that magnesium plays a key role in the immune response: cofactor for immunoglobulin synthesis, C'3 convertase, immune cell adherence, antibody-dependent cytotoxicity, IgM lymphocyte binding, macrophage response to lymphokines, T helper—B cell adherence, binding of substance P to lymphoblasts, and antigen binding to macrophage RNA.<sup>81,82</sup> Most of these studies have been designed in animal models, focusing on what happens in magnesium-depleted animals.

The reason for assuming an association between magnesium and immune function was based on findings that magnesium deficiency leads to increased inflammation.<sup>83,84</sup> The appearance of clinical signs of inflammation is one of the early symptoms of magnesium deficiency in the rat.

The activation of immune cells, such as monocytes, macrophages, and polymorphonuclear neutrophils, which synthesize a variety of biological substances, some of which are powerful inducers of inflammatory events (cytokines, free radicals, eicosanoids), was also reported.<sup>85</sup> High levels of circulating cytokines, such as IL-6, could be detected early after initiating the magnesium-deficient diet, leading to the release of the acute phase proteins by the liver.<sup>59,84</sup>

In vitro assays showed that macrophages and neutrophils from magnesium – deficient rats were metabolically stimulated with a higher production of superoxide anion when compared with the control group. When increasing concentrations of magnesium in the diet, the rate of superoxide anion formation was decreased.<sup>85</sup>

There is increasing evidence that these mechanisms could be mediated by an increase in substance P during magnesium deficiency.<sup>59,84,86</sup> However, the magnitude of the induced increases of substance P was not similar between studies, probably due to different inflammatory responses.<sup>87</sup> The increase in substance P induces a neurogenic inflammatory response, including the activation of mast cells, macrophages, and both B and T lymphocytes. In addition, T lymphocytes from magnesium-deficient animals exhibit increased expression of substance P receptors associated with a marked increase in the release of histamine, PgE<sub>2</sub>, IL-1, IL-6, and TNF. When magnesium-deficient rats were treated with a specific substance P receptor blocker, a marked attenuation of the elevations of histamine, PgE<sub>2</sub> and TNF and of the oxidative-induced loss of blood glutathione was observed. The authors hypothesized that substance P may initiate a cascade of inflammatory/pro-oxidant events leading to the cardiomyopathic characteristics associated with severe dietary magnesium restriction.<sup>59</sup> It was also observed that substance P plays a direct role in promoting activation of neutrophils and endothelium as well as the induction of NO production.<sup>88</sup> These processes may participate in the oxidative stress that contributes to the depletion of blood glutathione and cardiac pathology. The association between the early release of substance P and the subsequent

inflammatory response is unclear. Studies conducted in human populations are less extended than those using animal models. Bussiere and coworkers<sup>89</sup> observed in vitro that high magnesium concentrations of the medium decrease human leukocyte activation and suggested that extracellular magnesium can diminish leukocyte activation by its calcium antagonism, as magnesium counteracts calcium in many physiological and pathological processes.

Mooren and coworkers<sup>90</sup> observed that, after a 2-month period of magnesium supplementation, an exercise test until exhaustion induced an activation of the immune system as indicated by an increase in granulocyte count and a postexercise lymphopenia. However, magnesium supplementation seems to have been unable to prevent any exercise-associated alterations in immune cell function in athletes with balanced magnesium status.

The similarities between exercise-induced alterations in immune function and the changes in immunity caused by the deficiency in magnesium are noteworthy.

Prolonged, exhaustive exercise has been shown to be associated with temporary immunosuppression. However, there is accumulating evidence that this type of exercise also leads to considerable magnesium decreases. Therefore, particularly if dietary intake is low, athletes may be prone to magnesium deficit. Magnesium deficit has been shown to be related to impaired cellular and humoral immune function.

Although considerably more work needs to be done in this area, there is evidence that immunoregulation during and after intense physical exercise is influenced by transient or manifest deficiencies in magnesium.

Further research is needed and special efforts should be made to establish the most adequate dose in nutritional supplements in athletes to reach benefits on health and performance.

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# **Metabolic Syndrome**

# 15

## Review of Magnesium and Metabolic Syndrome

Yasuro Kumeda and Masaaki Inaba

With the westernization of lifestyle in people all over the world, especially in advanced countries in recent years, dietary intake of magnesium via grain, barley, seaweed, vegetable, and nuts has remarkably diminished. As a result, people may more easily develop hypomagnesemia. Likewise, metabolic syndrome has been an increasing problem for health, probably resulting from various causes such as increased intake of animal fat, exercise insufficiency, and accumulation of various stresses. Metabolic syndrome is often complicated with obesity, hypertension, hyperglycemia, and hyperlipidemia, and thus people with the syndrome may be susceptible to cardiovascular events. Hypomagnesemia may cause an increase of vascular tonus by intracellular magnesium depletion, resulting in an increase of blood pressure. Furthermore, it might cause impaired insulin secretion, insulin resistance, and hyperlipidemia, and finally lead to the development of metabolic syndrome. Therefore, the importance of magnesium intake for the maintenance of health should be recognized.

Magnesium has been proven to be a very important for more than 300 kinds of enzyme activation *in vivo*. It is easy for the intake of cereals, including barley, beans, seaweed, and green vegetables to decrease along with the change in recent years to European and American lifestyles, causing hypomagnesia. Similarly, metabolic syndrome is recognized as an important problem along with the change of lifestyle. The increase of animal fat intake, the lack of physical activity, and the accumulation of life stress in recent years, perhaps causes an increase of metabolic syndrome frequency, and this brings about the diseases that cause blood vessel problems such as obesity, high blood pressure, hyperglycemia, and hyperlipidemia. As a result, the risk of the onset of cerebral infarction and cardiac infarction is very high. The relationship between magnesium intake shortage and the onset of metabolic syndrome has been recognized in recent years, and the relevance is outlined in this chapter.

## What Is Metabolic Syndrome?

Metabolic syndrome is a group of diseases with increasing tendency in recent years. Hyperlipidemia, diabetes (including the boundary type), high blood pressure, and smoking are indicated as the four largest risk factors of an arteriosclerotic disease. It is shown that the risk of ischemic heart disease increases rapidly, and takes the appearance of a cardiovascular disease when these risk factors overlap even when the extent is light, and this kind of recognition develops into the concept of metabolic syndrome. In metabolic syndrome, hyperinsulinemia continues chronically because the insulin level of the blood could not decrease easily due to insulin resistance, and if the hyperinsulinemia continues, it becomes easy to develop hypertension, hypertriglycemia, hypo high-density lipoproteinemia (HDL), the storage of visceral fat, and so on. The compound lifestyle disease that had been called metabolic syndrome, syndrome X<sup>1</sup>, deadly quartet<sup>2</sup>, insulin resistance syndrome<sup>3</sup>, and visceral fat syndrome up to now is thought to be deeply related to lifestyle, including obesity, eating habits, and exercise, in addition to hereditary predisposition and aging. Metabolic syndrome might develop more once obesity, hypertension, high blood sugar, and hyperlipemia are present, that is, the risk factors of arteriosclerosis, and develops easily into cardiac infarction and cerebral infarction. For obesity, visceral fat-type obesity is more harmful for health than subcutaneous fat-type obesity, and often complicates hyperinsulinemia.

In the investigation of 120,000 Japanese workers, it appeared that the person who has one risk factor of obesity, hypertension, high blood sugar, hypertriglycemia, or hypercholesterolemia, even if it is a slight illness, increases their risk factor for heart disease 5 times; the person who has two risk factors increases the risk of heart disease 10 times; and the person who has three to four factors increases 31 times the risk of heart disease. The diagnosis standard of metabolic syndrome is shown by the United States Hyperlipemia treatment guideline<sup>4</sup> and WHO.<sup>5</sup> In Japan, eight societies, such as the Japanese Society of Internal Medicine, defined the disease concept of metabolic syndrome and the diagnosis standard was set at the Japanese Internal Medicine Department Society general meeting in April 2005 (see Table 15.1). At that time, it was agreed that metabolic syndrome is the concept of screening for a high risk of an arteriosclerosis, and has aimed to improve diagnosis of the disease that presents as hyperlipidemia, hypertension, and hyperglycemia.

Recently, a report of the importance of the relationship between metabolic syndrome and magnesium was presented. In a cross-sectional population-based study comparing 192 individuals with metabolic syndrome and 384 disorder-free control subjects, matched by age and gender, low serum magnesium levels were identified in 126 (65.6%) and 19 (4.9%) individuals with and without metabolic syndrome ( $p < 0.00001$ ). The mean serum magnesium level among subjects with metabolic syndrome was  $1.8 \pm 0.3$  mg/dL, and among control subjects  $2.2 \pm 0.2$  mg/dL ( $p < 0.00001$ ). There was a strong independent

**TABLE 15.1.** Diagnosis standards of metabolic syndrome.**The United States hyperlipemia treatment guideline (ATPIII: Adult Treatment Panel III, NCEP National Cholesterol Education Program)**

More than three of the five following items:

1. The waist (abdominal circumference) is 102 cm or more (85 cm or more in the Japanese) in men and 88 cm or more in women (90 cm or more in Japanese).
2. Triglycerides are 150 mg/dL or more.
3. HDL cholesterol is less than 40 mg/dL in men and less than 50 mg/dL in women.
4. 130 mm Hg or more in the systolic blood pressure or 85 mm Hg or more in the diastolic blood pressure.
5. Fasting blood sugar level is 110 mg/dL or more.

**Diagnosis standard by WHO**

Two or more of the following conditions, in addition to hyperinsulinemia (high rank 25% of nondiabetic) or fasting blood sugar is 110 mg/dL or more.

1. Visceral fat obesity waist/hip ratio is  $>0.9$  (man),  $>0.85$  (woman), or BMI is 30 or more, or the abdominal circumference is 94 cm or more.
2. Lipid metabolic disorder: Triglycerides are 150 mg/dL or more, or HDL cholesterol is less than 35 mg/dL in men and less than 39 mg/dL in women.
3. Blood pressure is 140/90 mm Hg or more, or a hypotensive drug is being taken.
4. Microalbuminuria (urinary albumin excretion rate is  $20 \mu\text{g}/\text{min}$  or more), or urinary albumin/creatinine ratio is 30 mg/g.Cr or more.

**Diagnosis standard by eight-society Joint Committee in Japan<sup>a</sup>**

Item 1, plus two or more of items 2 through 4.

1. A waist diameter is 85 cm or more in men, 90 cm or more in women. (These waist diameters correspond to visceral fat area  $100 \text{ cm}^2$  in both men and women.)
2. Hyperlipidemia (triglycerides are 150 mg/dL or more, and/or the HDL cholesterol is less than 40 mg/dL).
3. Hypertension: Systolic blood pressure is 130 mm Hg or more, and/or diastolic blood pressure is 85 mm Hg or more.
4. Hyperglycemia: 110 mg/dL or more.

<sup>a</sup>Japan Atherosclerosis Society, Japan Diabetes Society, Japanese Society of Hypertension, Japan Society for the Study of Obesity, Japanese Circulation Society, Japanese Society of Nephrology, The Japanese Society on Thrombosis and Hemostasis, and Japanese Society of Internal Medicine.

relationship between low serum magnesium levels and metabolic syndrome [odds ratio (OR) = 6.8; 95% confidence interval (95% CI), 4.2–10.9]. Among the components of metabolic syndrome, dyslipidemia (OR 2.8; 95% CI, 1.3–2.9) and high blood pressure (OR 1.9; 95% CI, 1.4–2.8) were strongly related to low serum magnesium levels. This study revealed a strong relationship between decreased serum magnesium and metabolic syndrome.<sup>6</sup>

However, when we pay attention to each of the diseases that compose metabolic syndrome, it is revealed that the pathology in connection with magnesium deficiency is important. Moreover, it is recognized that the lifestyle that causes magnesium deficiency plays a major role in the appearance of the diseases of metabolic syndrome.

Next, we make a detailed explanation of the relation between each disease that composes metabolic syndrome and magnesium.

## Magnesium and Diabetes Mellitus

Urinary excretion of magnesium is increased in either type 1 or type 2 diabetes mellitus patients, resulting in the reduction of the levels of magnesium in both serum and intracellular components.<sup>7</sup> In patients with cerebrovascular attack, hypertension, and diabetes, serum magnesium level is decreased. Serum magnesium level correlated in a negative manner with fasting plasma glucose and insulin in the patients with cerebrovascular stroke,<sup>8</sup> indicating that magnesium deficiency causes the occurrence of insulin resistance in those patients.

The magnesium ion ( $\text{Mg}^{2+}$ ) plays a pivotal role in insulin secretion from pancreatic  $\beta$  cells. The first step to stimulate physiological insulin secretion is the intracellular uptake of glucose into pancreatic  $\beta$  cells via glucose transporter in plasma membrane. Glucose is metabolized via the Tricarboxylic acid (TCA) cycle to produce adenosine triphosphate (ATP). ATP then binds to  $\text{Mg}^{2+}$  to close ATP-sensitive K channels, resulting in the depolarization of plasma membrane. The depolarization opens up voltage-dependent calcium ion ( $\text{Ca}^{2+}$ ) channel to stimulate intracellular entry of  $\text{Ca}^{2+}$ . The resultant increase of intracellular  $\text{Ca}^{2+}$  level stimulates insulin secretory vesicle to secrete insulin outside the cells.  $\text{Mg}^{2+}$  deficiency inhibits glucose metabolism via the TCA cycle to suppress ATP production;  $\text{Mg}^{2+}$  deficiency and reduced ATP production both inhibit the synthesis of Mg-ATP, and, thus, the activation of ATP-sensitive K channel, resulting in the suppression of insulin secretion.

Although mice fed on  $\text{Mg}^{2+}$  deficiency showed an attenuated insulin secretory response to glucose, magnesium supplementation restored, although partially, its response.<sup>9</sup>

For intracellular uptake of glucose by insulin-sensitive cells, the translocation of glucose transporter (GLUT) into plasma membrane is a vital step, which insulin induces by autophosphorylating  $\beta$  subunits of insulin receptor after its specific binding.

$\text{Mg}^{2+}$  acts in a stimulative manner in the step of insulin binding to its receptor and autophosphorylation of  $\beta$  subunits. Furthermore, intracellular magnesium stimulates intracellular glucose transport and its oxidation step.<sup>10</sup> Because insulin by itself stimulates intracellular magnesium uptake in insulin-sensitive cells, magnesium deficiency forms a vicious cycle, inhibiting insulin action mainly by inhibiting insulin binding to its receptor and intracellular glucose metabolism. These mechanisms cause the occurrence of insulin resistance and glucose intolerance, and possibly leading to the development of diabetes.

Very recently, the evidence that hypomagnesemia and/or intracellular decrease of  $\text{Mg}^{2+}$  inhibit insulin receptor-associated tyrosine kinase activity may explain the development of insulin resistance in magnesium deficiency.<sup>11</sup>

In summary, magnesium deficiency inhibits insulin secretion from pancreatic  $\beta$  cells and insulin sensitivity in insulin-targeted cells, and possibly worsens glucose intolerance to cause the development of diabetes.

## Magnesium and Hyperlipidemia and Atherosclerosis

Magnesium deficiency inhibits lipoprotein lipase (LPL) activity to induce hypertriglyceridemia. Magnesium deficiency also impairs the metabolism of saturated fatty acid to polyunsaturated fatty acid by inhibiting desaturase activity. The resultant increase of saturated fatty acids in plasma membrane may accelerate atherosclerosis.<sup>12</sup>

Because the activation step of lecithin-cholesterol acyltransferase, which, as well as LPL, plays important role in increasing HDL cholesterol and degrading triglycerides, requires magnesium, magnesium deficiency leads to increases of very low low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and triglycerides, and a decrease of HDL cholesterol.<sup>13</sup>

In rabbits fed on a magnesium-deficient and cholesterol-enriched diet, arterial thickening in the intimal layer of aorta was accelerated by increased accumulation of lipid in arterial wall. Magnesium supplementation to those rabbits lowered serum cholesterol and triglycerides, and attenuated the progression of atherosclerosis in magnesium-deficient rabbits.<sup>14</sup>

Lal and colleagues reported the effect of magnesium supplementation on lipid metabolism in diabetes patients.<sup>15</sup> They found a significant decrease of serum magnesium levels in 40 type 2 diabetes patients, compared to 50 control subjects (diabetes,  $1.44 \pm 0.48$  mg/dL, vs. controls,  $2.29 \pm 0.33$  mg/dL;  $p < 0.001$ ). They administered magnesium oxide at the daily dose of 600 mg for 12 weeks to type 2 diabetes patients. Serum total, LDL, and triglycerides decreased and HDL cholesterol increased significantly by 4 weeks, without any change in fasting or postprandial plasma glucose levels, concluding that magnesium administration is effective in improving lipid metabolism.

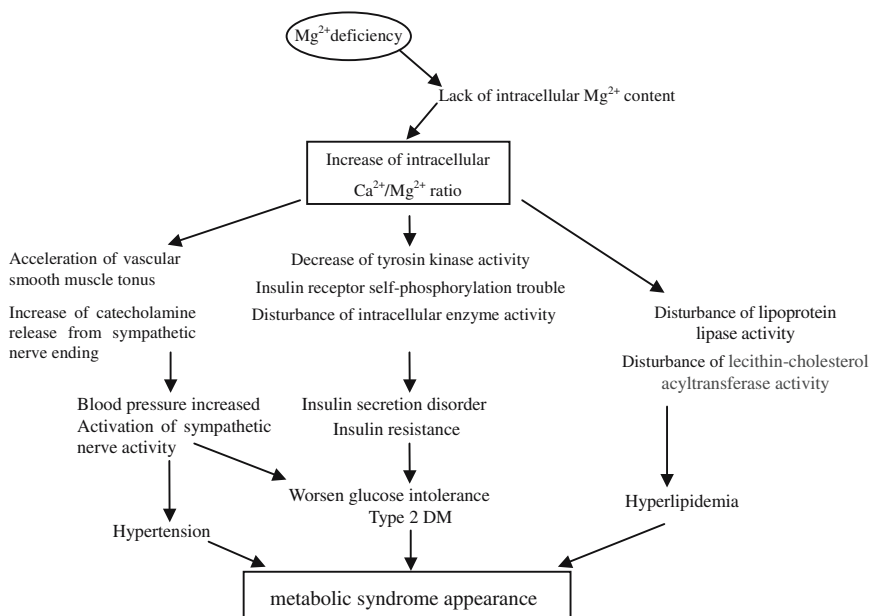
## Magnesium and Hypertension

Magnesium supplementation to patients with essential hypertension or alcohol-loaded hypertensive rats is known to suppress blood pressure, the mechanism by which is explained in relation to intracellular magnesium and calcium level.<sup>16</sup> The intimate mechanism is magnesium-induced inhibition of intracellular  $\text{Ca}^{2+}$  entry via L-type calcium channel of vascular smooth muscle cells.<sup>17</sup>

Magnesium deficiency suppresses intracellular  $\text{Mg}^{2+}$  levels in vascular smooth muscle cells, resulting in an increase of Ca/Mg ratio, thereby causing hypertension by constricting vascular smooth muscle cells.<sup>18</sup>

$\text{Mg}^{2+}$ , in addition to its direct effect on vascular smooth muscle cells, suppresses blood pressure by its effect via peripheral sympathetic nerves. Shimozawa and colleagues<sup>19</sup> reported that in the perfused mesenteric arteries system, norepinephrine release was significantly attenuated ( $51\% \pm 2\%$ ,





**FIGURE 15.1.** The outline of magnesium action on metabolic syndrome. The increase of intracellular  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratio due to magnesium shortage accelerates the vascular smooth muscle tonus and increases the catecholamine release from sympathetic nerve endings, consequentially becoming high blood pressure. Moreover, high intracellular  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratio causes the decrease of tyrosine kinase activity, self-phosphorylation disorder of the insulin receptor, and the disturbance of intracellular enzyme activity; as a result, insulin secretion disorder and insulin resistance is caused. As for the lipid metabolism, magnesium shortage causes the disturbance of both lipoprotein lipase activity and lecithin-cholesterol acyltransferase activity, and as a result, VLDL, LDL, and triglycerides increase. Therefore, hyperlipidemia is caused. Metabolic syndrome comes to develop as these diseases come in succession.

$p < 0.01$ ) by high  $\text{Mg}^{2+}$  concentration solution (4.8 mmol/L) compared with normal  $\text{Mg}^{2+}$  solution (1.2 mmol/L). They proved that  $\text{Mg}^{2+}$  blocked voltage-gated  $\text{Ca}^{2+}$  currents in a concentration-dependent manner by the perforated whole-cell patch clamp method to nerve growth factor-treated PC12 cells. The majority of the voltage-gated  $\text{Ca}^{2+}$  currents were carried through N-type channels, followed by L-type channels.  $\text{Mg}^{2+}$  blocked both of these channels. From these experimental results, they suggested that  $\text{Mg}^{2+}$  blocks mainly N-type  $\text{Ca}^{2+}$  channels at nerve endings, and thus inhibits norepinephrine release, which decreases blood pressure independent of its direct vasodilating action.

The action of magnesium on the appearance of disease of above-mentioned metabolic syndrome is shown in Figure 15.1.

## Magnesium and Obesity

The obese population of Europe and America has increased in recent years, and it is said that an increase of childhood obesity is a hotbed for the future increase of lifestyle diseases. Milagros and colleagues<sup>20</sup> made comparative study, for 24 obese nondiabetic children [body mass index (BMI)  $\geq$  85th percentile] and 24 sex- and puberty-matched lean control subjects (BMI < 85th percentile), of magnesium intake and body composition of serum magnesium, the insulin receptivity index, and meal. The following became clear as a result that serum magnesium was significantly lower in obese children ( $0.748 \pm 0.015$  mmol/L, mean  $\pm$  SE) compared with lean children ( $0.801 \pm 0.012$  mmol/L;  $p = 0.009$ ). Serum magnesium was inversely correlated with fasting insulin ( $r_s = -0.36$ ; 95% CI,  $-0.59$  to  $-0.08$ ;  $p = 0.011$ ) and positively correlated with quantitative insulin sensitivity ( $r_s = 0.35$ ; 95% CI,  $0.06$ – $0.58$ ;  $p = 0.015$ ). Dietary magnesium intake was significantly lower in obese children (obese,  $0.12 \pm 0.004$  vs. lean,  $0.14 \pm 0.004$  mg/kcal;  $p = 0.003$ ). Dietary magnesium intake was inversely associated with fasting insulin ( $r_s = -0.43$ ; 95% CI,  $-0.64$  to  $-0.16$ ;  $p = 0.002$ ) and directly correlated with insulin sensitivity ( $r_s = 0.43$ ; 95% CI,  $0.16$ – $0.64$ ;  $p = 0.002$ ). From this, it is clear that the association between magnesium deficiency and insulin resistance (IR) is present during childhood, and that serum magnesium deficiency in obese children may be secondary to decreased dietary magnesium intake. Therefore, magnesium supplementation or increased intake of magnesium-rich foods may be an important tool in the prevention of type 2 diabetes in obese children.

## Conclusion

In recent years, the diseases of modern people has changed remarkably due to the dramatic change in lifestyle, including dining habits. It is clear that intake shortage of dietary magnesium is related to the appearance of metabolic syndrome; this is a social issue. In addition, it is known that metabolic syndrome may take part in the honeshiti deterioration due to osteoporosis, one of the lifestyle diseases not mentioned in this text. It is assumed that the importance of the magnesium intake via food is acknowledged more widely, and a part of the prevention of lifestyle diseases, including metabolic syndrome, becomes possible by increasing dietary magnesium intake. Detailed clarification of magnesium participation in the process of metabolic syndrome is needed.

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# 16

## Diabetes Mellitus and Magnesium

Masanori Emoto and Yoshiki Nishizawa

The number of individuals with diabetes is now steadily increasing worldwide.<sup>1</sup> Research over 50 years has demonstrated that abnormal magnesium homeostasis exists in both type 1 and type 2 diabetes. In the 1980s and 1990s, the association between hyperglycemia and magnesium metabolism and the mechanisms of impairment of insulin sensitivity and secretion in magnesium deficiency were vigorously investigated. Later in the 1990s, epidemiological studies including large numbers of subjects established the close association of low dietary magnesium intake with development of type 2 diabetes. It is now of great interest to determine whether research on magnesium can provide new means of preventing diabetes. In this chapter, we review historical to contemporary findings concerning magnesium and diabetes in an attempt to obtain new insights in this field.

### Hypomagnesemia in Diabetes

More than 50 years ago, reduced blood levels of magnesium in diabetic individuals were documented in the literature.<sup>2</sup> In 1979, Mather and colleagues reported plasma magnesium levels measured by atomic absorption spectrophotometry in 582 unselected diabetic outpatients and 140 controls.<sup>3</sup> They found that mean plasma magnesium level in diabetic patients was lower than that in controls, and that 25% of diabetic patients had magnesium levels below those of all controls. Hypomagnesemia is frequently observed in both type 1 and type 2 diabetes.<sup>3-8</sup> The frequency of hypomagnesemia in type 2 diabetes is reported to vary from 25% to 48%.<sup>3,5,8-11</sup> Because magnesium is predominantly an intracellular ion, its plasma and serum levels do not necessarily reflect intracellular and tissue magnesium status. Serum and intracellular levels measured by nuclear magnetic resonance of erythrocytes are also reduced in stable type 2 diabetic patients, even when total serum magnesium level is comparable to that in control subjects.<sup>10</sup> Intracellular magnesium deficiency may occur even if plasma or serum magnesium level is normal. However, once decrease in plasma magnesium level becomes evident, it closely reflects

deficiency of intracellular magnesium.<sup>12,13</sup> Only a few studies have reported normal or even high levels of magnesium in serum in diabetic patients.<sup>14</sup> These variations in plasma and serum magnesium levels in individuals with diabetes may be attributed to differences in state of glycemic control, diabetic complications, and oral intake of magnesium, as well as other factors.

### ***Possible Mechanisms of Hypomagnesemia in Diabetes***

Magnesium is abundant in whole grains, nuts, legumes, green leafy vegetables, and reduced-fat dairy products. Magnesium homeostasis is maintained mainly by intestinal absorption and renal excretion.<sup>15</sup> Loss of magnesium in sweat is negligible except in special conditions such as marked sudation. Normally, 30% to 50% of dietary magnesium intake is absorbed by the small intestine. Net magnesium absorption is determined by the magnesium content of ingested foods and the retention of magnesium in digestive fluids. Approximately 70% to 80% of serum magnesium is filtered through the glomeruli, but only 20% to 30% of filtered magnesium is re-absorbed along the proximal tubules. On average, one third of dietary intake of magnesium is eliminated in the urine, as determined by analysis of magnesium intake and urinary losses.

Two possible mechanisms have been proposed for low levels of magnesium in plasma and blood cells in diabetic patients: (1) low magnesium intake from diet, and (2) increased urinary loss of magnesium. A few studies in the United States and Denmark found that dietary intake of magnesium was low in individuals with type 1 and type 2 diabetes.<sup>16</sup> The westernization of dietary habits has sometimes resulted in daily intake of magnesium below the recommended daily allowance. In fact, as described below, recent epidemiological studies have demonstrated a close association of low dietary magnesium intake with the development of type 2 diabetes.<sup>17–21</sup>

Most previous studies have demonstrated that urinary excretion of magnesium is increased in patients with diabetes<sup>6,22,23</sup> and that poor glycemic control is inversely correlated with hypomagnesemia.<sup>8,22,24–26</sup> Sjogren and colleagues found that glycosylated HbA1 level was inversely correlated with magnesium levels in plasma, mononuclear cells, and skeletal muscle, in a study of muscle biopsies obtained from type 1 diabetic patients.<sup>24</sup> Their subsequent study of 18 type 2 diabetic patients revealed lower levels of magnesium in skeletal muscle and higher excretion of urine of magnesium than in control subjects, but no correlation of plasma magnesium level with magnesium levels in skeletal muscle or circulating blood cells.<sup>22</sup> Another study of 12 type 1 diabetic patients reported that urinary magnesium excretion exhibited an inverse correlation with magnesium level in erythrocytes, but not with fasting plasma glucose or glycosylated hemoglobin levels.<sup>23</sup> In a large cohort study, the Atherosclerosis Risk in Communities (ARIC) Study including 15,248 participants aged 45 to 64 years, serum magnesium levels were found to be inversely associated with fasting serum insulin and glucose levels.<sup>26</sup> On the other hand, no correlations of plasma magnesium level with intracellular magnesium level or

glycemic control were found in 128 type 2 diabetic patients reported by the Lima Mde group.<sup>11</sup>

Several studies have attempted to determine whether improvement of glycemic control can improve magnesium deficiency in diabetes. Schnack and coworkers found in a study of 50 type 2 diabetic patients that neither initiation of insulin therapy nor intensification oral hypoglycemic agent treatment improved plasma magnesium level during a 3-month follow-up period despite marked improvement of glycemic control.<sup>27</sup> Interestingly, Djurhuus and colleagues, in an attempt to clarify the effect on magnesium deficiency of improvement of glycemic control in type 1 diabetic patients,<sup>28</sup> found that their intervention, which involved a mean increase in insulin dose of 10%, with resulting a 20% decrease in fasting plasma glucose, induced a 14% decrease in urinary excretion of magnesium concomitant with improvement of serum lipid profile, increase in high-density lipoprotein (HDL) cholesterol level, and decrease in triglyceride level. Recently, Walti and coworkers found that neither absorption nor retention of dietary magnesium was impaired in well-controlled type 2 diabetic patients compared with healthy controls, in a study using meals test-labeled with a magnesium isotope.<sup>29</sup>

## **Magnesium and Insulin Secretion**

### ***In Vitro and In Vivo Studies***

The role of intracellular magnesium in insulin secretion by pancreatic  $\beta$  cells remains unclear, and there have been few in vivo or in vitro studies of this subject. Magnesium is involved in more than 300 enzymatic reactions including those involving adenosine triphosphate (ATP), and also acts as an intracellular calcium blocker. It is thus likely that intracellular magnesium deficiency affects the insulin secretory capacity of pancreatic  $\beta$  cells, although the interaction of intracellular magnesium with insulin secretion signaling appears to be complex. Legrand and colleagues investigated the effect of a low-magnesium diet on insulin secretory capacity in the rat.<sup>30</sup> In their study, insulin responses during oral and intravenous and oral glucose tolerance tests were markedly reduced in rats fed a magnesium-deficient diet for 4 weeks, and these changes could be reversed by a high-magnesium diet. Recently, Palanivel and colleagues clarified the role of regulation of acetyl-CoA carboxylase by a magnesium-activated protein phosphatase in pancreatic  $\beta$  cells derived from the GK rat, a model of type 2 diabetes.<sup>31</sup> These findings suggest that magnesium plays a role in insulin secretion.

### ***Clinical Evidence Concerning the Relationship of Magnesium and Insulin Secretion***

Several studies have investigated the effects of oral supplementation of magnesium on insulin secretion in diabetic, elderly, and healthy subjects. Mentzel

and colleagues failed to detect an effect of serum magnesium level on residual insulin secretion assessed by an oral glucose tolerance test with glucagon stimulation in 33 type 1 diabetic and 10 control subjects.<sup>32</sup> Paolisso and coworkers evaluated insulin secretion carefully using an intravenous glucose test and the glucose clamp technique, the gold standard for measurement of insulin secretion and sensitivity in humans.<sup>33–35</sup> They reported that in eight elderly type 2 diabetic patients, oral supplementation of 3 g magnesium per day significantly increased acute insulin response after an intravenous glucose (0.33 g/kg for 3 min) and arginine (5 g) test, and that net increase in this response was correlated with increase in magnesium content in erythrocytes.<sup>33</sup> Subsequently, they also showed that chronic supplementation of 2 g magnesium per day increased both acute insulin response after glucose loading and the rate of disappearance of glucose during euglycemic hyperinsulinemic clamp, with a positive correlation of insulin response with net increase in magnesium level in erythrocytes in elderly type 2 diabetic patients<sup>34</sup> and elderly subjects.<sup>35</sup> Oral magnesium supplementation thus appears to stimulate insulin secretion in certain specific conditions, although clinical use of it for the purpose of glycemic control in diabetes will require further investigation.

## **Magnesium and Insulin Resistance**

### ***In Vitro Studies of Magnesium and Insulin Signaling***

Magnesium is a cofactor for multiple enzymes involved in carbohydrate metabolism.<sup>36</sup> The reason for the strong associations of magnesium deficiency with decrease in insulin sensitivity and insulin resistance has yet to be clearly determined. In vivo animal and in vitro studies have revealed that magnesium deficiency strongly reduces insulin-mediated glucose uptake in muscle and adipocytes.

In rat adipocytes cultured in low-magnesium media, insulin-mediated glucose uptake, oxidation, and incorporation into triglycerides were significantly reduced, but could be restored to normal levels by re-incubation in high-magnesium medium.<sup>37</sup> They found a significant decrease of 60% in intracellular magnesium level in adipocytes cultured in low-magnesium medium compared with that in adipocytes cultured in medium with physiological magnesium level.<sup>37</sup>

In rats made hypomagnesemic by feeding of a low-magnesium diet for 4 days, 40% reduction of rate of disappearance of glucose after glucose intravenous administration and 45% reduction of glucose-stimulated insulin secretion were found compared with control rats.<sup>38</sup> Furthermore, muscle perfusion experiments and receptor analyses revealed a 50% reduction in insulin sensitivity (ED50) without changes in basal or maximum glucose uptake, 50% reduction of autophosphorylation of the  $\beta$ -subunit of the insulin receptor, and

reduction of tyrosine kinase activity of insulin receptors without changes in insulin binding to insulin receptors or abundance of glucose transporter protein (GLUT 4). Similar findings have been reported in fructose-fed insulin-resistant rats.<sup>39</sup> This impairment of glucose uptake in magnesium deficiency is at least partly reversible by improvement of intracellular magnesium level in vitro<sup>37</sup> and of serum magnesium level in vivo.<sup>39</sup> Magnesium supplementation has also been reported to delay the development of diabetes in spontaneously obese diabetic rats.<sup>40</sup> These findings indicate that intracellular magnesium deficiency causes up to 50% decrease in insulin-mediated glucose disposal in muscle and adipocytes, mainly due to impairment of autophosphorylation and tyrosine kinase activity of insulin receptors at the postreceptor level.

Other intracellular relationships between the insulin signaling and magnesium homeostasis are complex and not yet fully elucidated.<sup>16,41</sup> Intracellular ionic magnesium exerts various effects by interacting with intracellular ionic calcium as a natural physiological calcium blocker.<sup>15,41</sup> It is known that magnesium regulates the influx and efflux of ionic calcium in cells. Protein kinase C (PKC), a constitutive negative regulator of the insulin receptor, is activated by increase in intracellular ionic calcium secondary to a deficiency of intracellular ionic magnesium.<sup>41,42</sup> Another possible mechanism of insulin resistance is an interaction of magnesium with PPAR- $\gamma$  receptor, a member of the steroid/thyroid hormone nuclear receptor superfamily of transcription factors. This receptor plays an important role in glucose/lipid metabolism and adipocyte differentiation. Intracellular ionic magnesium acts as an important cofactor in the phosphorylation of PPAR- $\gamma$  and its coactivator, PGC-1 $\alpha$ , which regulate enzymes participating in liver gluconeogenesis such as glucose-6-phosphatase and phosphoenolpyruvate carboxylase.<sup>43</sup> Interestingly, Guerrero-Romero and colleagues recently found that treatment with pioglitazone 30 mg per day for 12 weeks increased serum magnesium level by 112% above pretreatment level, in a randomized, controlled trial with 60 subjects.<sup>44</sup> This finding suggests the possibility of a new link between PPARs and magnesium.

## ***Epidemiological and Clinical Evidence Concerning the Relationship between Magnesium and Insulin Resistance***

### **Epidemiological Evidence**

Noninvasive indirect simple indexes of insulin sensitivity can be used in a larger number of subjects in epidemiological studies, such as fasting insulin, homeostasis model assessment (HOMA-IR),<sup>45,46</sup> and the quantitative insulin check index (QUICKI).<sup>47,48</sup> In the Atherosclerosis Risk in Communities (ARIC) Study including 15,248 participants aged 45 to 64 years, serum magnesium level and dietary magnesium intake were each inversely associated with fasting serum insulin level, suggesting an association of magnesium with fasting hyperinsulinemia and insulin resistance.<sup>26</sup> In 349 subjects randomly selected from 39,345 American women in the Women's Health Study (WHS), mean

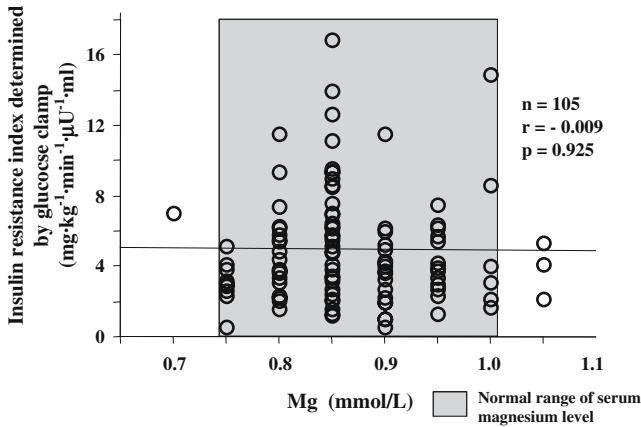


fasting insulin level was significantly decreased in the highest quartile group of total dietary magnesium intake.<sup>21</sup> Huerta and colleagues were the first to report, in a study of 48 children, that dietary magnesium intake (mg/kcal) was inversely correlated with HOMA-IR ( $r_s = -0.43$ ,  $p = 0.002$ ) and positively correlated with QUICKI ( $r_s = 0.43$ ,  $p = 0.002$ ).<sup>49</sup>

### **Clinical Evidence Obtained Using Glucose Clamp Technique, Minimal Model Analysis, and Steady-State Plasma Insulin and Plasma Glucose (SSPG)**

In human studies, the euglycemic hyperinsulinemic glucose clamp is the gold standard for assessing whole-body insulin sensitivity and separately estimating rates of oxidative and nonoxidative glucose metabolism in combination with indirect calorimetry. Using this technique, Paolisso and coworkers successfully demonstrated the effect of chronic magnesium supplementation on insulin sensitivity in a double-blind crossover study of elderly type 2 diabetic patients<sup>34</sup> and healthy elderly subjects.<sup>35</sup> Chronic supplementation of 2 g magnesium per day for 4 weeks increased total body glucose disposal by 128% and glucose oxidation rate during hyperinsulinemia by 152%, compared to the placebo period.<sup>35</sup> These improvements in insulin sensitivity were positively correlated with net change in erythrocyte magnesium level (Pearson's  $r = 0.67$ ,  $p < 0.01$ ). On the other hand, neither basal hepatic glucose production nor rate of nonoxidative glucose metabolism during hyperinsulinemia was significantly altered compared with the placebo period. Alzaid and coworkers subsequently reported that physiological hyperinsulinemia and hyperglycemia induced by the glucose clamp technique decreased plasma magnesium level in both type 2 diabetic and control subjects, and that this decrease was correlated with the whole-body rate of disappearance of glucose during euglycemic clamp ( $r = 0.55$ ,  $p < 0.01$ ). This finding indicates that magnesium uptake to insulin-sensitive tissue by insulin is also impaired, as well as glucose uptake in type 2 diabetes.<sup>50</sup> In 179 young adults who had been followed longitudinally, total dietary magnesium intake in milligrams/kilograms of fat-free mass was found to be weakly but significantly correlated with whole-body rate of glucose disposal during euglycemic hyperinsulinemic clamp ( $r = 0.15$ ,  $p < 0.05$  overall; in men,  $r = 0.25$ ,  $p < 0.02$ ) and with sum of plasma insulin levels during the oral glucose tolerance test.<sup>51</sup>

We have examined insulin resistance in Japanese individuals with type 2 diabetes in detail in various clinical conditions by means of euglycemic hyperinsulinemic glucose clamp with an artificial pancreas (STG-22, Nikkiso Co. Ltd, Tokyo). To the best of our knowledge, no previous studies have examined the association of magnesium with insulin resistance determined using glucose clamp technique in Japanese type 2 diabetic patients. Total serum magnesium level and insulin resistance assessed by euglycemic hyperinsulinemic clamp were retrospectively investigated in a cross-sectional study of 104 type 2 diabetic patients attending the Diabetes Center of Osaka City University Hospital,



**FIGURE 16.1.** The relationship between total serum magnesium level and insulin resistance index assessed by euglycemic hyperinsulinemic clamp in 104 type 2 diabetic patients at the Diabetes Center of Osaka City University Hospital. Most of our Japanese type 2 diabetic patients had normal levels of serum magnesium, with a mean of  $0.9 \pm 0.1$  (SD) mmol/L, as indicated by the dotted area. No significant correlation was found between serum magnesium level and insulin resistance index ( $r = -0.009$ ,  $p = 0.925$ ). Possible explanations for this finding are provided in the text.

as previously described elsewhere.<sup>46,48</sup> As shown in Figure 16.1, we failed to find any significant association of serum magnesium level with insulin resistance in our diabetic patients, who regularly visited our hospital and were treated by diabetologists (unpublished data). This discrepancy between our findings and those of previous studies is probably due to our evaluation of total serum magnesium level, rather than ionic serum or intracellular level of magnesium, in well-controlled diabetic patients. Total serum magnesium levels were within normal range in most of our subjects. As described above, total serum magnesium level does not precisely reflect the state of intracellular magnesium deficiency. Furthermore, it is likely that the insulin resistance in our subjects was more strongly affected by confounding factors, such as obesity, fat deposition, and various drugs, than by magnesium per se.

Another technique for assessing insulin sensitivity is the intravenous glucose tolerance test with minimal model analysis and steady-state plasma insulin and plasma glucose (SSPG) technique. Using minimal model analysis, Nadler and colleagues found a significant decrease by 75% in insulin sensitivity after a magnesium-deficient diet, with reduction of both serum and intracellular free magnesium levels in six subjects.<sup>52</sup> In 98 healthy subjects, Rosolova and colleagues found the highest fasting insulin and SSPG levels during SSPG in the group with the lowest tertile of plasma magnesium levels and an inverse correlation of plasma magnesium level with SSPG ( $r = -0.27$ ,  $p < 0.01$ ).<sup>53,54</sup>

## Magnesium and Development of Diabetes

### *Magnesium Intake and Development of Type 2 Diabetes*

There is increasing clinical and epidemiological evidence that low magnesium intake is associated with development of type 2 diabetes (Table 16.1). In 1992, the Nurse Healthy Study, which included 84,360 women with a 6-year follow-up period, first demonstrated that high dietary magnesium intake was associated with reduction of risk of development of diabetes, with a relative risk of 0.68 compared with the group with the lowest quintile of magnesium intake.<sup>55</sup> Various prospective epidemiological studies have subsequently been performed, as shown in Table 16.1. The Iowa Women's Healthy Study, a prospective cohort study including 35,988 older women with 6-year follow-up, found a strong inverse association of total grain, whole grain, total dietary fiber, cereal fiber, and dietary magnesium intakes with incidence of diabetes after adjustment for potential nondietary confounding variables.<sup>17</sup> The lowest multivariate-adjusted relative risk of diabetes was 0.67 compared to the highest quintile of dietary magnesium intake. Similarly, the Health Professionals Follow-up Study, which included 42,898 men with 12 years follow up, found a relative risk of development of diabetes of 0.58 compared with the lowest quintile of whole-grain intake.<sup>56</sup> Subsequently, the Nurse Health Study, which had a 16-year follow-up period, investigated the beneficial effects of intake of nuts and peanut butter, principal food sources of magnesium, on the development of diabetes. The relative risk of development of diabetes in women consuming peanut butter five times or more per week was 0.79 compared with those never or almost never consuming peanut butter.<sup>57</sup> The ARIC study found that the incidence of type 2 diabetes during 6-year follow up increased approximately twofold across the highest-to-lowest serum magnesium level in Caucasian participants.<sup>58</sup> The relative risk of diabetes was 1.76 with a 95% confidence interval of 1.18–2.61, compared with the group with lowest serum magnesium levels. However, in that study, no significant association was found between dietary magnesium intake and the incidence of diabetes.

Cross-sectional studies have also supported the association of magnesium intake with metabolic disorders related to development of diabetes such as fasting insulin level, waist, and body mass index (BMI).<sup>18,26,51</sup> For example, in the Framingham Offspring Study, including 2941 subjects, intake of whole grains, which are major food sources of magnesium, was closely associated with fasting insulin level as well as body mass index and waist-to-hip ratio.<sup>18</sup> These associations were less strong after adjusting for dietary magnesium.

Recently, two prospective studies by Lopez-Ridaura and colleagues and Song and colleagues with large numbers of subjects and long follow-up periods have yielded definite findings regarding magnesium intake and development of diabetes.<sup>20,21</sup> In a study involving follow up of 85,060 women and 42,872 men, a food-frequency questionnaire was validated every 2 to 4 years over 18 years in women and 12 years in men. The relative risk (RR) of type 2 diabetes after

**TABLE 16.1.** Principal prospective epidemiological studies concerning associations of dietary magnesium (Mg) intake, serum magnesium level, and the development of type 2 diabetes.

Report	Study cohort	Subjects (N)	Follow-up period (years)	Relative risk (RR <sup>a</sup> ) of diabetes
Colditz, 1992 <sup>55</sup>	Nurse Healthy Study	84,360 American women	6 years	0.68 ( $p^a = 0.02$ ) for magnesium compared with in the lowest quintile of magnesium intake.
Kao, 1999 <sup>58</sup>	Atherosclerosis Risk in Communities Study	12,128 nondiabetic middle-aged adults	6 years	1.76 (95% CI, 1.18–2.61; $p^a = 0.01$ ) compared with lowest serum Mg
Meyer, 2002 <sup>17</sup>	The Iowa Women's Health Study	35,988 older women	6 years	No association with magnesium intake 0.67 ( $p^a = 0.0003$ ) across quintiles of magnesium intake
Fung <sup>56</sup>	Health Professionals Follow-up Study	42,898 men	12 years	0.58 (95% CI, 0.47–0.70; $p < 0.0001$ ) compared with the lowest quintile of whole-grain intake
Jiang, 2002 <sup>57</sup>	Nurse Healthy Study	83,818 women	16 years	0.79 (95% CI, 0.68–0.91; $p^a < 0.001$ ) for women consuming peanut butter 5 times or more per week <sup>b</sup>
Lopez-Ridaura, 2004 <sup>20</sup>	—	85,060 women and 42,872 men	18 years in women and 12 years in men	0.66 (95% CI, 0.60–0.73; $p < 0.001$ ) for women and 0.67 (95% CI 0.56–0.80; $p < 0.001$ ) for men.
Song, 2004 <sup>21</sup>	The Women's Health Study	39,345 American women	6 years	0.89 ( $p = 0.05$ ) for women and 0.78 ( $p = 0.02$ ) for overweight or obese women

Abbreviations: RR, multivariate-adjusted relative risk of diabetes by confounding factor or by comparison with the group with the lowest risk of diabetes; 95% CI, 95% confidence interval.  
<sup>a</sup>The details about the relative risk (RR) of diabetes are described in the text.  $p$  for trend across quintile groups.  
<sup>b</sup>Compared with those who never/almost never ate peanut butter.

adjustment for various confounding factors was found to be 0.66 [95% confidential interval (95% CI), 0.60–0.73;  $p < 0.001$ ] for women and 0.67 (95% CI, 0.56–0.80;  $p < 0.001$ ) for men, in a comparison of highest and lowest quintiles of total magnesium intake.<sup>20</sup> In the Women's Health Study, with a cohort of 39,345 American women and an average follow-up period of 6 years, the multivariate-adjusted RR of diabetes of the lowest quintile of magnesium intake was 0.89 compared to the highest ( $p = 0.05$ ), while that for overweight or obese women with BMI greater than 25 kg/m<sup>2</sup> was 0.78 ( $p = 0.02$ ).<sup>21</sup> These findings strongly suggest the usefulness of increased consumption of major food sources of magnesium, such as whole grains, nuts, and green leafy vegetables, in preventing type 2 diabetes.

### ***Interventional Trial of Magnesium Supplementation in Type 2 Diabetes***

Controversy exists concerning whether oral magnesium supplementation improves metabolic profile, and especially glycemic control, in type 2 diabetic patients (Table 16.2). Table 16.2 shows the findings of well-conducted clinical trials concerning this issue. Some studies of subjects with type 2 diabetes<sup>35,59–61</sup> have found decreases in fasting plasma glucose and insulin level as well as HbA1c with improvement of insulin sensitivity, as represented by index of homeostasis model assessment (HOMA-R) and euglycemic hyperinsulinemic clamp, while others have not.<sup>11,62–64</sup> These discrepancies in findings probably arise from the differences in the magnesium salts and doses used, the degree of magnesium deficiency, and the period of intervention.

In a recent randomized, double-blind, placebo-controlled trial conducted by Rodriguez-Moran and coworkers,<sup>60</sup> a notable effect of oral supplementation of MgCl<sub>2</sub> solution (2.5 g per day) for 16 weeks was found in a total of 63 type 2 diabetic patients treated with glibenclamide who had low serum magnesium levels (below 0.74 mmol/L). It is surprising that after 16-week treatment, the group with magnesium supplementation had lower HOMA-R ( $3.8 \pm 1.1$  vs.  $5.0 \pm 1.3$ ,  $p = 0.005$ ), fasting glucose level ( $8.0 \pm 2.4$  mmol/L vs.  $10.3 \pm 2.1$  mmol/L,  $p = 0.01$ ), and HbA1c ( $8.0\% \pm 2.4\%$  vs.  $10.1\% \pm 3.3\%$ ,  $p = 0.04$ ) with higher plasma magnesium ( $0.74 \pm 0.10$  mmol/L vs.  $0.65 \pm 0.07$  mmol/L,  $p = 0.02$ ) compared with the placebo group. Subsequently, they also demonstrated improvement of the HOMA-R index to 57% below baseline mean value after 3-month supplementation, with increase in serum magnesium in nondiabetic subjects with insulin resistance.<sup>65</sup> The magnesium chloride used in their studies is believed to have excellent bioavailability and thus to be useful for improving serum magnesium level within the first month of treatment.<sup>60,65</sup>

In addition to better glycemic control, other beneficial effects of oral magnesium supplementation can be expected in type 2 diabetic patients, such as improvement of lipid profile, decrease in triglyceride level, and improvement of hypertension.<sup>64</sup> The major side effects of oral magnesium supplementation are headache, nausea, hypotension, and mild abdominal and bone pain,

**TABLE 16.2.** Principal previous reports concerning the clinical benefits of oral magnesium supplementation on metabolic profile.

Reports	Subjects	N	Supplementation (g/day, period)	Increase in Mg	Results
Paolisso, 1992 <sup>35</sup>	T2DM	12	Mg pidolate 4.5 g, 4 weeks	Erythrocytes	Increase in insulin secretion and [activity?] Decreases in FPG and PPG
Paolisso, 1994 <sup>39</sup>	T2DM	9	Mg pidolate, 3 weeks	Plasma Erythrocytes	Increases in insulin sensitivity and glucose oxidation No change in glucose level No changes in HbA1c
Eibl, 1995 <sup>63</sup>	T2DM	40	Mg citrate 30 mmol, 3 months	Plasma	No changes in FPG, HbA1c, lipid profile, or blood pressure
De Valk, 1998 <sup>64</sup>	T2DM	50	Mg aspartate 15 mmol, 3 months	Plasma Urinary [excretion?]	No changes of FPG, Fructosamine, HbA1
Lima Mde, 1998 <sup>11</sup>	T2DM	128	Mg oxide 20.7 mmol, 30 days	No changes in plasma or monocytes Serum	Decrease of HOMA-R Decrease of FPG, HbA1c Decrease of FRI, BP, TG, HOMA-R Decrease HOMA-R index
Rodriguez-Moran, 2003 <sup>60</sup>	T2DM	63	Mg chloride 2.5 g, 16 weeks	—	—
Yokota, 2004 <sup>61</sup>	T2DM	9	Natural Mg 0.3 g, 30 days	—	—
Guerrero-Romero, 2004 <sup>65</sup>	Insulin-resistant	60	Mg chloride 2.5 g, 3 months	Serum	—

Abbreviations: FPG or PPG, fasting or postprandial plasma glucose; HOMA-R, insulin resistance index of homeostasis model assessment; Mg, magnesium; T2DM, type 2 diabetes mellitus.

which usually require neither specific treatment nor cessation of magnesium supplementation.

## Perspectives on Research in Magnesium and Diabetes

Magnesium, the second most abundant cation in the body, acts as an important cofactor for many enzymes and is a natural calcium blocker, and consequently is involved in many biological reactions in various cells and tissues. This makes the physiological and pathological functions and critical roles played by magnesium difficult to clearly determine. Many problems remain to be solved, as stated above. The following issues are of great importance to the clinical treatment of diabetes: (1) the intracellular mechanisms of insulin resistance in skeletal muscle, fat, and liver, and insulin secretion by pancreatic  $\beta$  cells in magnesium deficiency; (2) the clinical effects of oral supplementation of magnesium on metabolic profiles, especially in individuals with glucose intolerance, diabetes, and related disorders such as hypertension and hyperlipidemia; and (3) the beneficial effects of consumption of major food sources, such as whole grains, nuts, and green leafy vegetables, in preventing the development of diabetes and related disorders in high-risk populations.<sup>66</sup> Further randomized prospective clinical trials can be anticipated to address the third of these issues.

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# 17

## **Magnesium Metabolism in Insulin Resistance, Metabolic Syndrome, and Type 2 Diabetes Mellitus**

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Magnesium plays a key role in regulating insulin action, insulin-mediated glucose uptake, and vascular tone. Intracellular magnesium depletion may result in a defective tyrosine-kinase activity at the insulin receptor level, in a postreceptorial impairment in insulin action, and clinically in a worsening of insulin resistance. Intra- and extracellular alterations of magnesium metabolism have been identified in clinical states characterized by insulin resistance, such as metabolic syndrome, hypertension, altered glucose tolerance, type 2 diabetes, and aging. Several studies, from our and other's groups, have confirmed the clinical relevance of alterations of magnesium homeostasis in these conditions and have highlighted the importance of an accurate definition of the magnesium status. While measurements of total serum magnesium levels have been proven inadequate for this purpose because important magnesium depletions are required before total serum level decreases, two technologies,  $^{31}\text{P}$  nuclear magnetic resonance ( $^{31}\text{P}$ -NMR) spectroscopy and magnesium-specific ion-selective electrodes, that, respectively, measure intracellular and extracellular free levels of magnesium, have a higher sensitivity in detecting magnesium deficits. A number of evidences have confirmed that magnesium supplementation is indicated in conditions associated with magnesium deficit, although well-designed therapeutic trials with oral magnesium supplements to study the beneficial effects in metabolic syndrome and in type 2 diabetes are needed.

An increasing number of evidences have suggested a clinical relevance for the altered magnesium (Mg) metabolism present in states of increased peripheral insulin resistance. As discussed below, we have suggested a role for Mg deficit as a possible unifying mechanism of the insulin resistance of hypertension and conditions associated with altered glucose tolerance, including metabolic syndrome and type 2 diabetes mellitus.<sup>1</sup> Our group has used a ion-based approach to demonstrate: (1) the critical importance of Mg metabolism in regulating insulin sensitivity, as well as vascular tone and blood pressure homeostasis; (2) that Mg deficiency, defined on the basis of intracellular free magnesium levels (Mgi), and or serum ionized magnesium (MgI) is a common feature of insulin resistant-states, as well as various cardiovascular and meta-

bolic processes and aging; (3) the ability of environmental factors such as dietary nutrient sugar and mineral content to alter the set point of steady-state cell ion activity.<sup>2,3</sup>

## Magnesium and Glucose Metabolism

Magnesium is the second most abundant intracellular cation (after potassium) present in living cells and its plasma concentration is remarkably constant in healthy subjects. Ninety-nine percent of Mg is distributed in the intracellular fluid, and 1% is distributed in the extracellular fluid. The levels of Mg in the plasma of healthy people are remarkably constant, being on average 0.85 mmol/L and varying less than 15% from this value. Circulating Mg exists in three forms: a protein-bound fraction (25% bound to albumin and 8% bound to globulins), a chelated fraction (12%), and the physiologically active ionized fraction (MgI, 55%).<sup>4</sup> Because Mg is predominantly an intracellular ion, and in the serum only the ionized active form is metabolically available, its total serum concentrations (MgT) may not reflect the Mg status or intracellular pool, and intracellular Mg depletion can be seen with normal MgT concentrations.<sup>5-8</sup>

Magnesium is directly involved in numerous important biochemical reactions, and particularly is a necessary cofactor in over 300 enzymatic reactions and specifically in all those processes that involve the utilization and transfer of adenosine triphosphate (ATP). Thus, intracellular Mg is a critical cofactor for several enzymes in carbohydrate metabolism, and because of its role as part of the activated Mg-ATP complex required for all of the rate-limiting enzymes of glycolysis, regulates the activity of all enzymes involved in phosphorylation reactions. Intracellular free magnesium concentration is critical in the phosphorylation of the tyrosine-kinase of the insulin receptor, as well as all other protein kinases, and all ATP and phosphate transfer-associated enzymes, such as the CaATPases in plasma membrane and endoplasmic reticulum. Magnesium deficiency may result in disorders of tyrosine-kinase activity on the insulin receptor, an event related to the development of a of postreceptorial insulin resistance and decreased cellular glucose utilization<sup>9</sup>; that is, the lower the basal MgI, the greater the amount of insulin required to metabolize the same glucose load, indicating decreased insulin sensitivity. Specifically, in skeletal muscle and fat tissue, insulin resistance would be the expected outcome in the presence of suppressed cellular Mg. Significant decrements in these enzyme activities can already be observed at the range of Mg values seen in disease states such as type 2 diabetes and hypertension.<sup>10</sup>

## Diagnostic Tools Available to Define Magnesium Status of Magnesium-Deficient Subjects

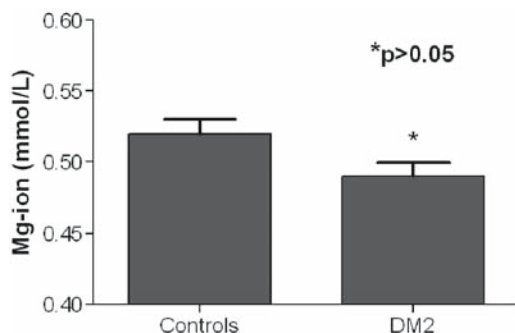
One of the principle reasons Mg metabolism has not become more the focus of routine attention in clinical practice has been the absence of an easily available, accurate, and reproducible measure of Mg status. Measurements of MgT

levels, which includes the protein-bound, chelated, and ionized fractions, that are the clinical measurement routinely used for assessing circulating Mg are not useful, and have been proven inadequate for this purpose because important Mg depletion is required before its total serum levels decrease.<sup>5,11</sup> Two technologies, <sup>31</sup>P-NMR spectroscopy and Mg-specific ion-selective electrodes (ISE), that measure Mgi and extracellular MgI, respectively, are a major advance in this regard, and have a higher sensitivity in detecting Mg deficits. This is because MgT levels are a late marker of a depletion of Mg stores (the complexed serum MgT is the last store to be depleted when an already important Mg depletion has occurred), while the declines in ionized unbound MgI may occur significantly earlier. While <sup>31</sup>P-NMR techniques are a research-based test because of the expenses in setting up and maintaining the NMR equipment, the development of a Mg-selective electrode apparatus may be particularly useful in routine clinical use. Using these techniques, a deficiency of intra- and ionized extracellular Mg levels have been consistently demonstrated in diabetes, often when MgT levels were within normal limits,<sup>5</sup> and significant relationship have been found with blood pressure, and cardiovascular and metabolic parameters.<sup>3</sup> From the intracellular point of view, these relations appear to be continuous, and do not display a threshold value within the range of clinically observable cellular free Mg levels, that is, the lower the free Mgi, the stiffer the blood vessels, the higher the blood pressure, the greater the insulin resistance, etc.<sup>1,3,12</sup>

## Magnesium Deficiency in Type 2 Diabetes Mellitus and Metabolic Syndrome

The presence of a Mg deficit in diabetic patients has long been recognized.<sup>13,14</sup> Epidemiological studies have found a high prevalence of hypomagnesaemia in subjects with diabetes, especially in those with poor glycemic control.<sup>15,16</sup> Because of the lack of sensitivity of serum total MgT, a suppressed level of intracellular Mgi and serum ionized MgI can be found in many subjects with total serum MgT still in the normal range (see above). Using the Nova-8 Mg ISE to measure serum ionized Mg in a preliminary sample of 50 subjects with type 2 diabetes, we have recently found significantly lower MgI levels compared to normal controls ( $0.49 \pm 0.01$  mmol/L vs.  $0.52 \pm 0.01$  mmol/L,  $p < 0.05$ ), without significant changes in MgT (Figure 17.1). When MgI and Mgi levels were measured concurrently in the same subjects using <sup>31</sup>P-NMR and the ISE Mg-selective electrode, Resnick and colleagues found that both Mgi and MgI (but not serum total MgT) were significantly reduced in type 2 diabetes subjects, and that a close direct relationship was present between the ionized extra- and the intracellular Mg measurement.<sup>5</sup>

Low dietary Mg intake and increased Mg urinary losses<sup>17-19</sup> are the main causes of the Mg deficit in diabetic subjects. The use of loop and thiazide



**FIGURE 17.1.** Ionized Mg levels (MgI, mmol/L) in type 2 diabetic subjects versus normal controls.

diuretics, which promote Mg wasting, may worsen Mg depletions. A Mg-deficient diet has been found to be associated with a significant impairment of insulin-mediated glucose uptake.<sup>20</sup> Hyperglycemia and hyperinsulinemia may have a role in the increased urinary Mg excretion, thus contributing to Mg depletion, and the reduced sensitivity to insulin may affect Mg transport.

Magnesium deficiency, which may take the form of a chronic latent Mg deficit rather than clinical hypomagnesemia, may have clinical importance because of the crucial role of Mg as a cofactor in many enzymatic reactions regulating glucose metabolism. A deficient Mg status may not just be a secondary consequence of diabetes, but experimental and epidemiological data suggest that it may precede and cause insulin resistance and altered glucose tolerance, and even type 2 diabetes.<sup>21</sup> However, independent to the cause of poor plasma and intracellular Mg content, a depletion of Mg seems to be a cofactor for a further derangement of insulin resistance.<sup>1,22</sup>

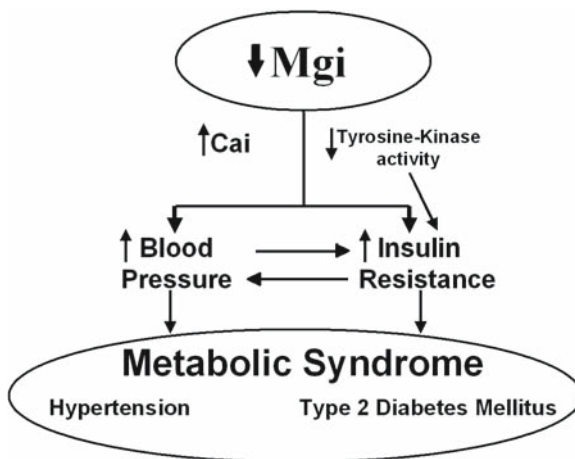
At the cellular level, using gold-standard NMR techniques, our group have shown lower steady-state Mgi and reciprocally increased Cai levels in subjects with type 2 diabetes mellitus, compared with young nondiabetic subjects.<sup>23–25</sup> We have recently extended our experience with NMR technique to study cytosolic free Mg directly in situ, in vivo, and in human living tissues, such as muscle and brain, and have shown that in living tissues Mgi values are quantitatively and inversely related to both systolic and diastolic blood pressures.<sup>26</sup>

Intracellular free magnesium levels quantitatively predict the fasting and postglucose levels of hyperinsulinemia, as well as peripheral insulin sensitivity. Specifically, (1) fasting insulin levels<sup>27</sup>; (2) the integrated insulinemic response to a standard oral glucose tolerance test<sup>28</sup>; (3) the steady-state plasma glucose response to insulin infusion; and (4) indices for peripheral insulin sensitivity derived from euglycemic hyperinsulinemic clamps, are all inversely related to Mg levels, whether measured as Mgi in situ in brain, free or total Mg in peripheral red cells,<sup>21,29,30</sup> or even as circulating Mg.<sup>31,32</sup> Furthermore, direct and inverse relations, respectively, are observed between Mgi and Cai levels and HbA1c, fasting blood glucose, and the glycemic response to oral glucose loading in normal, hypertensive, and diabetic subjects.<sup>33</sup>

## Aging, Insulin Resistance, and Magnesium

Old age is frequently associated with insulin resistance and glucose intolerance. We have specifically studied the behavior of ion content with age and have shown a continuous age-dependent fall of Mgi levels in peripheral blood cells of healthy elderly subjects,<sup>25</sup> these alterations being indistinguishable from those occurring, independently of age, in essential hypertension or diabetes.<sup>25</sup> In other terms, essential hypertension and/or type 2 diabetes appear to determine an acceleration of natural age-dependent Mg depletion, suggesting that these ionic changes may be clinically significant, underlying the predisposition in elderly subjects to cardiovascular and metabolic diseases, and might therefore help to explain the age-related increased incidence of these diseases. At the same time, the naive concept of both hypertension and type 2 diabetes as diseases of accelerated vascular aging may be more literally true than previously thought, because both of these diseases display these same ionic changes at all ages. Thus, having these conditions is ionically the same as getting older, for example, a 48-year-old diabetic having cellular ionic alterations indistinguishable from a healthy 84-year-old, suggesting a role for Mg deficit in the increased incidence of hypertension and glucose intolerance with age.<sup>2,25</sup>

Altogether, this accumulating evidence of the relevance of altered cellular Mg metabolism to tissutal insulin sensitivity suggests a critical role of Mg metabolism in contributing to the clinical coincidence of Mg depletion to states of insulin resistance, such as hypertension, metabolic syndrome, type 2 diabetes, as well as the increased incidence of each of these conditions with age, a condition itself characterized by a tendency to intracellular Mg depletion. Thus, pathophysiologically, Mgi depletion can directly promote tissutal insulin resistance and altered vascular tone, thus helping to understand the mechanisms underlying the clinical association among these apparently different conditions (Figure 17.2).



**FIGURE 17.2.** Overall hypothesis in which intracellular Mg deficiency may mediate the relationship between insulin resistance, hypertension, and type 2 diabetes mellitus.



## Insulin and Glucose Acute Effects on Dynamic Changes of Cellular Magnesium

Although these data suggest that cellular free ion content may determine clinically relevant biologic outcomes such as blood pressure, cardiac mass, and cellular insulin responsiveness,<sup>2</sup> it is also concomitantly true that metabolic and dietary factors have a role in contributing to insulin sensitivity and in regulating intracellular ion metabolism. Thus, dietary salt<sup>34</sup> or sugar loading allowed us to assess the role of different dietary circumstances in regulating blood pressure and cellular ion metabolism. Specifically, the transient hyperglycemia of oral glucose loading reproduces in normal subjects the same altered ionic profile of depleted Mgi/increased Cai levels that occurs chronically in diabetic subjects,<sup>27</sup> which dynamically appears to be equally and inversely true for serum ionized Mg. The contribution of hyperglycemia and hyperinsulinemia to the intracellular Mg depletion of diabetes has been confirmed by in vitro studies from our group showing that both glucose and insulin may, in turn, alter Mgi levels. Thus, glucose in a specific, concentration- and time-dependent manner, at concentrations achieved clinically, and independent of insulin lowered Mgi and reciprocally elevated Cai.<sup>35</sup> Barbagallo and colleagues reported that hyperglycemia also alter ionic content in cultured vascular smooth muscle cells, suggesting an ionic mechanism for the increased vasoconstriction present in chronic diabetic states.<sup>36</sup> This glucose-mediated effects are independent of insulin action, because hyperglycemia induces these changes both in vascular smooth muscle cells, and in erythrocytes, where glucose transport is unaffected by insulin.<sup>35,37,38</sup> Among its many actions, insulin has specific ionic effects to stimulate the transport of Mg from the extracellular to the intracellular compartment, thus increasing Mgi content.<sup>39</sup> Using NMR techniques to measure Mgi, we have shown that the ionic action of insulin is specific, dose-related, and independent of cellular glucose uptake. Insulin in the incubation medium was able to induce an accumulation of Mgi which shifted from a basal values of  $177 \pm 11$  mmol/L to  $209 \pm 19$  mmol/L.<sup>39</sup> The ionic effects of insulin were time and dose dependent. In the dose-response study, the dose of insulin at which we observed these ionic effects started at  $10 \mu\text{U/mL}$  and peaked at  $200 \mu\text{U/mL}$ , this dose corresponding roughly to the maximal physiological response in humans, adding to the physiological relevance of this effect. Such results further suggest that insulin is an important modulator of intracellular Mg content; furthermore, there are indications that, as in other energy producing systems, an ATPase-dependent pump is involved in the mechanism by which insulin regulates the erythrocyte Mgi content.

The overall hypothesis that Mgi content is a crucial determinant of cellular responsiveness is supported by other data from our group showing that the ability of insulin at physiologically maximal concentrations to stimulate Mgi is impaired in cells from hypertensive individuals, in which the basal Mgi

content is reduced,<sup>21</sup> confirming that insulin action is strictly dependent from the cellular Mgi content; for all subjects, independently of their designation as normotensive or hypertensive, cellular Mgi responsiveness to insulin was closely and directly related to basal cellular Mgi levels; that is, the lower the basal Mgi, the less responsive was the cell to insulin. Furthermore, a blunting of Mgi responses to insulin could be reproduced in normal cells that were Mg depleted by prior treatment either with A23187 in a calcium-free medium or with high glucose concentrations (15 mmol/L).<sup>40</sup> Once again, insulin responsiveness followed basal Mgi levels ( $r = 0.637$ ). Altogether, these data demonstrating ionic aspects of cellular insulin resistance has led us to hypothesize that the insulin resistance, currently defined by measurements of tissue glucose uptake, might equally well be defined on the basis of altered cellular ion responsiveness. Similar cellular behavior was found in cell responsiveness to glucose action. As was the case for insulin the lower the Mgi, the less responsive is the cell to glucose.<sup>37</sup> Thus, this ionic insulin resistance is probably not specific for insulin, but may rather be one tissue manifestation of a general property of cells in which steady-state Mgi and/or Cai level may determine cell responsiveness to insulin, glucose, or to other external stimuli. It is possible to hypothesize therefore, that the insulin resistance of hypertension and diabetes, currently defined by measurements of tissue glucose uptake, might equally well be defined on the basis of altered cellular ion responsiveness (Figure 17.2).

The link between Mg deficiency and the development of insulin resistance and type 2 diabetes is strengthened by the observation that several treatments for diabetes appear to increase Mg levels. Metformin, for example, raises Mg levels in the liver. Pioglitazone, a thiazolidinedione anti-diabetic agent that increases insulin sensitivity, increases free Mg concentration in adipocytes.<sup>41</sup> Other research of our group have demonstrated that the action of the insulin mimeting substances vanadate and IGF-1 are both associated to a direct effect to stimulate intracellular free Mg levels,<sup>42</sup> and that the effects of antioxidants glutathione and vitamin E to improve glucose and insulin metabolism may derive at least in part from their action on Mg metabolism. Barbagallo and coworkers demonstrated that the action of glutathione and vitamin E to increase insulin sensitivity in hypertensive subjects is associated with a concurrent increase in Mgi, and that a significant direct relationship between glucose disposal increase and Mgi levels was present.<sup>29,30</sup>

## **Is Providing Oral Magnesium Supplementation Effective in Reversing the Magnesium and Clinical Abnormalities?**

The effects of Mg supplements on the metabolic profile of type 2 diabetic subjects are still controversial,<sup>43</sup> benefits having been found in some,<sup>44-46</sup> but not all, clinical studies.<sup>47-49</sup> Differences in baseline Mg status and metabolic control may explain the differences among these studies. Thus, a recent clinical trial

specifically conducted among diabetic subjects with low total serum Mg levels (index of an already advanced Mg deficit) found a beneficial effect of oral Mg supplementation on fasting and postprandial glucose levels and insulin sensitivity<sup>43</sup> and we have shown an improvement in insulin-mediated glucose uptake measured by euglycemic insulin clamp in diabetic subjects after oral Mg supplementations.<sup>21</sup> A significant relationship was found between the increase in plasma and erythrocyte Mg concentration and the parallel progressive increase in the insulin sensitivity in diabetic patients that were supplemented with increased dosage of Mg supplements (5.1–11.5 mmol of elemental Mg).<sup>21</sup> Among nondiabetic, apparently healthy subjects, there are also some evidences of a relatively small but significant beneficial effects of Mg supplements on insulin sensitivity.<sup>32,50</sup> Recent epidemiological data have showed a significant inverse association between Mg intake and diabetes risk supporting the priority of Mg deficit in the development of glucose intolerance and diabetes.<sup>51</sup> Thus, taking into account that both dietary Mg and serum plasma Mg content have been associated with an increased risk to develop glucose intolerance and diabetes,<sup>13</sup> the use of Mg supplements could be an alternative tool for the prevention of type 2 diabetes,<sup>52</sup> a hypothesis that needs to be confirmed by specific and well-designed trials with Mg that are needed in the near future.<sup>21,53</sup>

Altogether, with the recent advances in the accurate measurement of intracellular and extracellular Mg levels, we have now the tools to translate what is known of the critical importance of Mg in glucose and insulin metabolism into clinical practice, both in routinely monitoring Mg status and in the therapeutic use of Mg supplementation in those conditions and those subjects in whom Mg deficiency can be demonstrated.

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# Cardiovascular Disease

# 18

## Cardiovascular Disease and Magnesium

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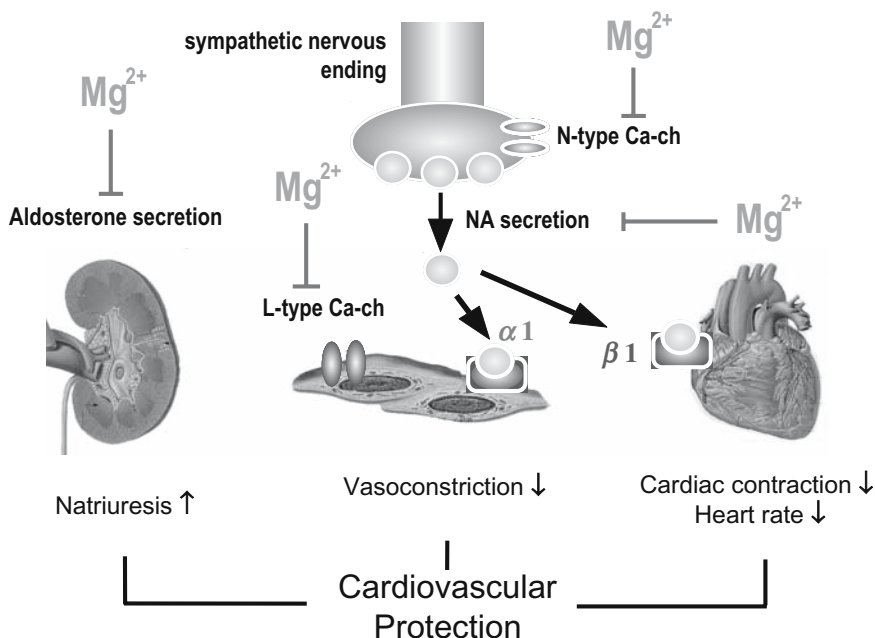
The effects of magnesium on cardiovascular function and disease have received increasing attention during the last decade.  $Mg^{2+}$  is the second most abundant intracellular cation after potassium ( $K^+$ ), and a critical cofactor in more than 300 enzymatic reactions critically involving in energy metabolism, glucose utilization, protein synthesis, fatty acid synthesis and breakdown, adenosine triphosphatase (ATPase) functions, and virtually all hormonal reactions in the cardiovascular system. The mechanisms of cardiovascular protective effects of magnesium associated with depression embrace calcium antagonistic action on L-type and N-type calcium (Ca) channels, suppression of catecholamine release from sympathetic nervous terminal, and suppression of aldosterone secretion from adrenal gland (Figure 18.1).

### Blood Pressure Control and Hypertension

It is now clear that the magnesium ion, although not directly involved in the biochemical process of contraction, modulates vascular smooth muscle tone and contractility by affecting calcium ion concentrations and its availability at critical sites.<sup>1</sup> Magnesium ions actively promote relaxation, offset calcium-related excitation-contraction coupling, and decrease cellular responsiveness to depolarizing stimuli, by stimulating  $Ca^{2+}$ -dependent  $K^+$  channels, which serve to offset the potential depolarizing influence of cellular calcium accumulation by activating the membrane sodium (Na)-K-ATPase pump, which is critical for the maintenance of the resting cellular membrane potential, by competitively inhibiting Ca binding to calmodulin, and by stimulating both plasma membrane and sarcoplasmic reticulum Ca ATPases.

Magnesium status has a direct effect upon the relaxation capability of vascular smooth muscle cells and the regulation of the cellular cations important to blood pressure—cellular sodium:potassium (Na:K) ratio and intracellular calcium ( $iCa^{2+}$ ).  $Mg^{2+}$  (intra- and extracellular) exists in three states: (1) free, ionized fraction (the physiologically active form); (2) complexed to anions (citrate, phosphate, bicarbonate); and (3) protein bound. In extracellular fluid,





**FIGURE 18.1.** Cardiovascular protective effects of magnesium. Ca-ch, calcium channel; NA, noradrenaline.

free  $Mg^{2+}$  composes 61% of total  $Mg^{2+}$ , 6% is complexed, and 33% is protein bound.<sup>2</sup> Both cellular and whole body Na:K ratios are crucial to the maintenance of normal blood pressure; when sodium becomes too high and potassium too low, high blood pressure is one result. At the cellular level, proper function of the sodium–potassium pump maintains potassium at a high intracellular concentration and sodium at a high extracellular concentration. Magnesium is required for the proper function of the sodium–potassium pump, which requires Mg-ATP as a source of energy and is responsible for maintaining the separation of sodium and potassium across cellular membranes. Physiologically, when magnesium status is low enough to cause hypomagnesemia, serum potassium level drops even when potassium intake is adequate or above adequate. Thus, when magnesium status becomes low or deficient, the Mg-ATP-driven sodium–potassium pump can be hindered, proper potassium metabolism is disturbed, potassium flows out of the cell, sodium flows into the cell, and even if adequate potassium is nutritionally maintained under these circumstances, an effective potassium deficit and abnormal Na:K ratio is expressed until the magnesium status is repaired. Hypertension, in such cases, can only be cured by adequate nutritional magnesium intake but is often treated with anti-hypertensive medications, some of which deplete the body of K and/or Mg.

As a result, nutritional magnesium has both direct and indirect impacts on the regulation of blood pressure and therefore on the occurrence of hypertension. Hypertension occurs when cellular Na:K ratios become too high, a consequence of a high-sodium, low-potassium diet or, indirectly, through a magnesium-deficient state that causes a pseudopotassium deficit.

Likewise, magnesium deficiency impairs proper calcium metabolism, affecting blood pressure. When cells' natural calcium channel blocker magnesium becomes deficient, calcium will rush abnormally into cells, creating high  $iCa^{2+}$ , low serum calcium, and low urinary calcium states even when calcium intake is adequate. Many aspects of the rise in intracellular calcium ion concentration and its impact on hypertensive elements have been elucidated at the cellular level.<sup>3,4</sup> What has not been studied nor considered in many of these studies is magnesium's impact on calcium ion metabolism; when magnesium is low, there is a concomitant rise in intracellular calcium ion due, primarily, to a magnesium deficiency, creating a magnesium:calcium (Mg:Ca) imbalance. In the case of vascular smooth muscle cells, such an imbalance instills a perpetually high  $iCa^{2+}$ , causing a perpetual vasoconstriction state and the condition we know as hypertension.<sup>5</sup> In the case of neurons, healthy intracellular magnesium ion concentrations block N-type  $Ca^{2+}$  channels at nerve endings, thus inhibiting norepinephrine release and maintaining normal blood pressure.<sup>6</sup> Many other hypertensive effects of raised  $iCa^{2+}$  can be set in motion by low intracellular magnesium. Such effects become added to the loss of magnesium ion's direct vasodilator effect. Thus, we see that long-term normal blood pressure requires maintenance of healthy  $iCa^{2+}$  levels which, in turn, require adequate cellular magnesium. High  $iCa^{2+}$  and high cellular Na:K ratio both occur when cellular magnesium becomes too low and the Mg-ATP-driven sodium-potassium pump and calcium pump become functionally impaired. High  $iCa^{2+}$  has several vasoconstrictive effects which lead to hypertension, an indirect result of low magnesium status. Dietary calcium is directly proportional to dietary magnesium. Serum magnesium does not reflect true magnesium status as do intracellular magnesium measurements.

At the whole body level, when magnesium status is low enough to exhibit hypomagnesemia, serum calcium drops below normal regardless of the intake of calcium, and only the ingestion of magnesium in adequate amounts can dispel the hypocalcemia. Low magnesium status causing a low serum calcium also causes a low urinary calcium. Resnick and coworkers showed intracellular magnesium-to-calcium ion ratio to be the crucial factor in the etiology of hypertension and other aspects of metabolic syndrome X.<sup>7</sup> Resnick's work has shown that Mg:Ca cellular ratios below a certain level result in hyperinsulinemia, insulin resistance, platelet aggregation, cardiac hypertrophy, and hypertension. Confirming this view, Kisters and colleagues reported aortic smooth muscle cells of the spontaneously hypertensive rat to have a Ca:Mg ratio of 3.5 compared with normal rats' ratio of only 2.2.<sup>8</sup>

Another mechanistic consideration is the linkage of  $Mg^{2+}$  deficiency with formation of reactive oxygen species (ROS) and potential effects on

redox-sensitive growth factor signaling pathways. The knowledge in the field of ROS and vascular function and pathology is currently growing at an exponential rate, and it is becoming clear that these highly reactive molecules are probably not only involved in vascular pathology, but also in normal cell signaling.<sup>9</sup> Anti-oxidants are compounds that hinder the oxidative processes and thereby delay or prevent oxidative stress. In this sense, magnesium is one of the physiological, efficient anti-oxidant.  $Mg^{2+}$  deficiency leads to an increase in thiobarbituric acid-reactive substances (TBARS), which indicates an increase in ROS and which is reversed by tempol, a superoxide dismutase mimetic. A broader question that begs answering is how  $Mg^{2+}$  deficiency might induce these effects. The traditional belief is that  $Mg^{2+}$  exerts many of its cellular actions by serving as a  $Ca^{2+}$  antagonist and there is no doubt that  $Mg^{2+}$  can compete with  $Ca^{2+}$  for binding sites on a variety of cellular targets. It is also thought that  $Mg^{2+}$  deficiency may affect cell function by causing the concentration of free intracellular  $Mg^{2+}$  to fall below its KD for key enzymes and hence decrease their activity (i.e., the  $Mg^{2+}$ -dependent  $Na^+$  and  $K^+$  activated ATPase that establishes the  $Na^+$  and  $K^+$  gradients across the cell membrane and the  $Mg^{2+}$ -dependent myosin ATPase which is critical for muscle contraction). It is well known that platelet  $Ca^{2+}$  mobilization is elevated in humans and animals with elevated blood pressure. This finding has been interpreted by many to indicate that there is a basic defect  $Ca^{2+}$  metabolism in platelets that might extend to other reactive cells, including vascular smooth muscle. However, the increase in platelet  $Ca^{2+}$  might also result from an increase in shear stress. This basic question needs to be addressed for the effect of  $Mg^{2+}$  on blood pressure and generation of ROS. In view of the continued interest regarding the role of  $Mg^{2+}$  in blood pressure regulation and vascular pathology, particularly in light of the hugely successful Dietary Approaches to Stop Hypertension (DASH) trials, additional work needs to be conducted in this area. Focus should continue to be directed towards understanding mechanisms of action, with a concerted effort being made towards making the animal models more closely match the human condition, and with an increased focus on molecular mechanisms, linking between an essential dietary nutrient and the key molecular pathways involved in regulating vascular smooth muscle growth and structure.

## Acute Myocardial Infarction

Over the past decade, several reviews have focused on the relevance of magnesium in cardiac disease. A special interest was developed on the importance of magnesium as a pharmacological agent in the treatment of acute myocardial infarction (AMI). Evidence exists that magnesium is the biological element that could diminish or even prevent reperfusion injury in AMI. Coronary artery disease is characterized pathomorphologically by arteriosclerosis and is expressed clinically by reduced coronary blood flow with consecutive myocardial ischemia and hypoxemia. Magnesium depletion and tissue ischemia

accelerate myocardial hypoperfusion, myocardial Ca overload, coronary arteriosclerosis, and platelet aggregation, suggesting the role of magnesium in the pathogenesis of ischemic heart disease and sudden death (Figure 18.2).

Clinical application of magnesium is still controversial in acute myocardial infarction because of the conflicting results of previous clinical trials. Magnesium therapy has been reported to reduce mortality in acute myocardial infarction in Leicester Intravenous Magnesium Intervention Trial 2 (LIMIT-2).<sup>10</sup> In LIMIT-2, magnesium administration demonstrated the 24% reduction in the 28-day mortality rate of acute myocardial infarction. However, it could not demonstrate significant effect on mortality in International Study of Infarct Survival 4 (ISIS-4).<sup>11</sup> One of the reasons suggested was a lower mortality rate of the control group in ISIS-4. The ISIS-4 investigators enrolled 58,050 patients: 29,011 to magnesium and 29,030 to control. The control group mortality was 7.2% in ISIS-4 compared with 7.6% in the magnesium group. The Magnesium in Coronaries (MAGIC) trial investigated the benefits of early administration of intravenous magnesium to high-risk patients with acute ST-segment elevation myocardial infarction (STEMI).<sup>12</sup> At 30 days, the mortality rate was 15.3% in the magnesium group and 15.2% in the placebo group.

It is possible that the effects of magnesium are not sufficiently powerful to demonstrate significant reduction in mortality rate in mild-to-moderate acute myocardial infarction, which has already received interventional therapy and full medication with other effective agents.

Despite these conflicting results of clinical trials, several previous reports of experimental myocardial infarction have suggested that magnesium treat-

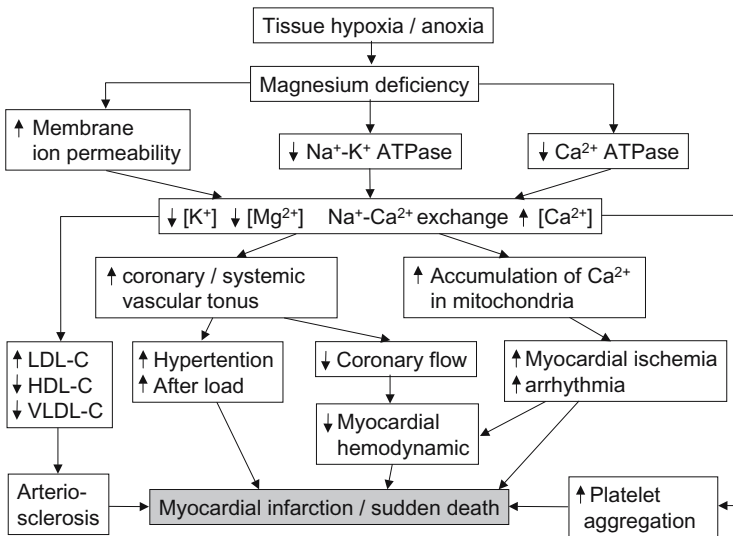


FIGURE 18.2. Myocardial ischemia and magnesium kinetics.

ment is potentially effective to reduce ventricular arrhythmias and reduce infarct size. However, the mechanism of its efficacy is still not fully understood. We have demonstrated that magnesium administration has an infarct-size-limiting effect independent of hemodynamic changes and incidence of arrhythmias in myocardial infarction in rabbits.<sup>13</sup> A high dose of magnesium significantly reduced the myocardial infarct size accompanied by a significant reduction of double products. The negative inotropic and chronotropic actions of a high dose of magnesium potentially produces energy sparing effects and reduces the myocardial infarct size. However, a lower dose of magnesium demonstrated a similar infarct-size-limiting effects independent of hemodynamic changes. We focused on the effects of magnesium on the reduction of myocardial infarct size, which is one of the major determinant of the mortality rate in acute myocardial infarction. The infarct-size-limiting effect of magnesium is attributable, at least in part, to attenuation of calcium overload and augmentation of adenosine mechanism (Figure 18.3).<sup>13</sup> Adenosine is a major cardioprotective substance in ischemia. Ischemic myocardium is salvaged by administration of adenosine or by inhibition of adenosine breakdown. The main pathway of adenosine synthesis in ischemic myocardium is decomposition of adenosine monophosphate by ecto 5'-nucleotidase (5'-ND), the main enzyme of producing adenosine in ischemic myocardium.<sup>14</sup> Magnesium is an important cofactor of 5'-ND. We confirmed that the activity of ecto 5'-ND extracted from rabbit myocardium was enhanced by magnesium in a dose-dependent fashion. It is conceivable that the infarct-size-limiting effect of magnesium is mediated, at least in part, by adenosine through the enhancement of activity of ecto 5'-ND (Figure 18.3).<sup>13</sup>

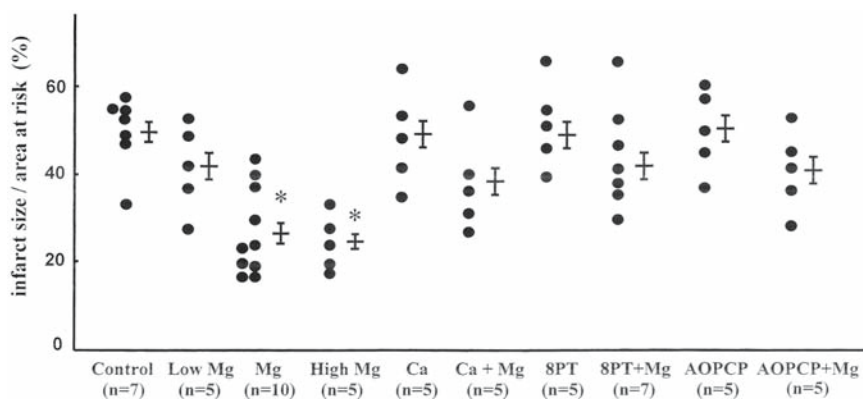


FIGURE 18.3. Myocardial infarct size limiting effects of magnesium. \* $p < 0.05$  versus control.<sup>13</sup>

Calcium overload in myocardium is one of the most important mechanisms of myocardial reperfusion injury.<sup>15</sup> It has been reported that the pretreatment with calcium channel antagonists has infarct-size-limiting effects. We have demonstrated that calcium administration abolished the infarct-size-limiting effect of magnesium, suggesting that there exists a competitive interaction between magnesium and calcium in ischemia-reperfusion myocardium (Figure 18.3).<sup>13</sup> Magnesium is known as a natural calcium antagonist. Its calcium antagonistic action is multifunctional, including inhibition of voltage-dependent L-type calcium channel, suppression of  $\text{Na}^+/\text{Ca}^{2+}$  exchanging system, inhibition of calcium release from sarcoplasmic reticulum (SR), potentiation of calcium sequestration into SR, and suppression of calcium binding to specific site of troponin C. Although it was not a study on the myocardial infarct size, Ferrari and coworkers reported that magnesium reduces mitochondrial calcium overload and protects myocardial injury in isolated rabbit hearts. Although calcium administration potentially augments calcium overload mechanism, the elevated extra- and intracellular calcium levels are not the sole determinant of the myocardial infarct size because calcium administration itself did not increase the infarct size. In either case, the infarct-size-limiting effect of magnesium was abolished by calcium administration. It is conceivable that magnesium reduces myocardial infarct size, at least in part, via the magnesium-calcium interaction mechanism in rabbits.

Magnesium depletion has been implicated in the pathogenesis of ischemic heart disease as a result of altering blood lipid composition and accelerating atherogenesis, and causing coronary artery spasm. However, the evidence supporting the role of magnesium in the pathogenesis of ischemic heart disease, at this time, is not very convincing. Magnesium deficiency has been shown to increase the infarct size.<sup>16</sup> Several beneficial effects of magnesium other than infarct-size-limiting effect have been proposed in myocardial infarction: coronary vasodilation increasing coronary blood flow, inhibition of catecholamine release,<sup>17</sup> and inhibition of platelet aggregation protecting against thrombosis. It is noteworthy that magnesium supplementation is inexpensive, easy to administer, and relatively free from side effects. In conclusion, magnesium administration has an infarct-size-limiting effect independent of hemodynamic changes and incidence of arrhythmias in myocardial infarction in rabbits. The infarct-size-limiting effect of magnesium is attributable, at least in part, to attenuation of calcium overload and augmentation of adenosine mechanism.

Ischemic-reperfusion injury of the microvasculature results in progressive diminution of perfusion to previously ischemic tissues despite restoration of flow in the conduit arteries supplying these tissues, that is, the no-reflow phenomenon.<sup>18</sup> The no-reflow phenomenon is a significant clinical problem. It occurs in coronary interventions and in patients receiving thrombolytic therapy during AMI. No reflow is associated with a higher incidence of early and prolonged congestive heart failure (CHF) compared with the absence of

no reflow. The primary insult in no reflow is probably reperfusion-induced (and oxygen free radical-mediated) injury to endothelium.<sup>19</sup> Circumstantial evidence suggests that  $Mg^{2+}$  reduces endothelial injury. First, deficiency of  $Mg^{2+}$  potentiates oxygen free radical-induced postischemic injury in working isolated rat hearts. Second, agents that attenuate the initial ischemic injury, namely  $Ca^{2+}$  antagonists administered before reperfusion, also reduce the severity of no reflow and preserve endothelial function. Finally, experimental areas of no reflow are decreased, and vascular endothelial and smooth muscle function are preserved after administration of  $Mg^{2+}$  cardioplegia (16 mM).<sup>20</sup>

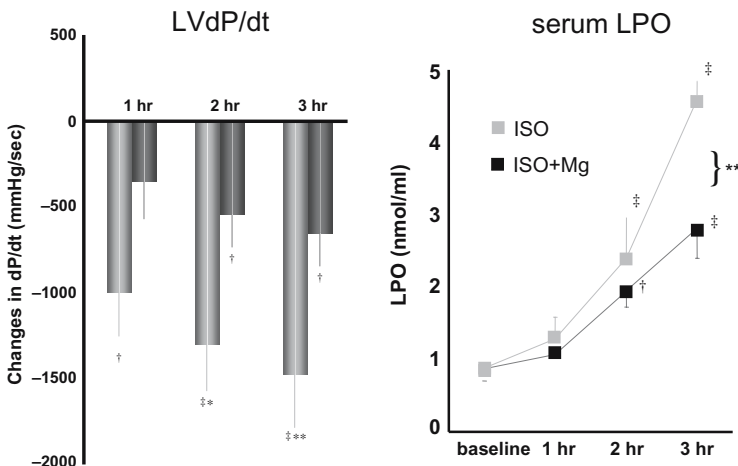
## Catecholamine

Myocardial dysfunction is accompanied by increased catecholamine concentrations in blood and urine. The serum catecholamine level is an indicator of the clinical severity of heart failure.<sup>21</sup> Although sympathetic activation is a crucial compensatory mechanism in the initial stage of heart failure, excess catecholamine itself induces cardiac cell injury, leading to cardiac dysfunction. Therefore,  $\beta$ -adrenergic receptor blockade improves prognosis as well as cardiac function in chronic heart failure. Excess isoproterenol (ISO), a selective  $\beta$ -adrenergic receptor agonist, induces myocardial necrosis and apoptosis, interstitial fibrosis, and left ventricular (LV) hypertrophy. The mechanism of cardiac dysfunction induced by excess ISO may be explained by calcium overload<sup>22</sup> and free radical generation.<sup>23</sup> Moreover, excess ISO induces  $\beta$ -adrenergic desensitization, one of the hallmarks of heart failure, which potentially exacerbates cardiac dysfunction. We have demonstrated that magnesium supplement prevents ISO-induced cardiac dysfunction and  $\beta$ -adrenergic desensitization in dogs.<sup>24</sup> The mechanism of cardiac dysfunction induced by excess ISO remains not fully understood. The most plausible explanation of ISO cardiotoxicity is intracellular calcium overload through  $\beta$ -adrenergic receptor stimulation. Cardiomyocytes are injured by excessive  $Ca^{2+}$  influx,<sup>25</sup> leading to irreversible cell injury, apoptosis, and necrosis. Therefore, ISO-induced cardiac dysfunction is effectively prevented by calcium channel blockades as well as  $\beta$ -adrenergic receptor blockades.  $Mg^{2+}$  inhibits extracellular  $Ca^{2+}$  influx via a voltage-sensitive  $Ca^{2+}$  channel (L-type  $Ca^{2+}$  channel),  $Ca^{2+}$  release from sarcoplasmic reticulum (SR), that is,  $Ca^{2+}$  induced  $Ca^{2+}$  release, and potentiate sequestration of released  $Ca^{2+}$  by SR in myocardial cells. These findings suggest that magnesium supplementation potentially suppresses  $Ca^{2+}$  overload in myocardium.

Prolonged exposure of hearts to  $\beta$ -adrenergic agonist results in decreased inotropic responsiveness to agonists, that is,  $\beta$ -adrenergic desensitization. It represents the mechanism that contributes to the progression of heart failure. The process of  $\beta$ -adrenergic desensitization involves decreases in  $\beta$ -adrenergic receptor density, which is triggered by phosphorylation of receptors, a rapid uncoupling of receptor from Gs, and decreases in the basal adenylate cyclase

activities and calcium channel density. Although the mechanism of ISO-induced  $\beta$ -adrenergic receptor desensitization has not been entirely clarified, the time-dependent decrease in inotropic response to ISO was significantly prevented by magnesium supplement (Figure 18.4).<sup>24</sup> Magnesium is known to modulate the receptor-G protein-catalytic interactions at several sites. Magnesium acts as a cofactor with ATP at the catalytic site, and is required for GTPase activity and Gs activation as well as GTP binding. Feldman reported that  $\beta$ -adrenergic desensitization is obscured in a high-magnesium condition by maintaining the activity of adenylate cyclase and cyclic adenosine monophosphate (cAMP)-dependent protein kinase.<sup>26</sup> These findings suggest that magnesium supplement may inhibit  $\beta$ -adrenergic desensitization by the regulation of the receptor-G protein-catalytic interactions.

Another possible effect of magnesium on cardiac dysfunction and  $\beta$ -adrenergic desensitization is an antioxidative action. Excess ISO can generate oxygen free radicals, suppress the activity of anti-oxidative enzyme, consequently augment oxidative stress. Excess catecholamine itself can be oxidized and generate oxidative products. An oxidation product of catecholamines may be responsible for impaired inotropic responses to adrenergic stimulation, as well as myocardial necrosis and contractile failure. The time-dependent increase in lipid peroxides, radical products of polyunsaturated fatty acid, was significantly diminished by magnesium supplement (Figure 18.4).<sup>24</sup> A chain reaction of lipid peroxidation by ROS impairs myocardial cell membrane, and, consequently, causes cardiac dysfunction. Magnesium has been reported to reduce radical production in ischemia reperfusion of myocardium. The anti-oxidative effects of magnesium may contribute to prevention of cardiac dysfunction and  $\beta$ -adrenergic desensitization induced by excess ISO.



**FIGURE 18.4.** Magnesium suppresses isoproterenol-induced cardiac dysfunction and oxidative stress. ISO, isoproterenol; LPO, lipid peroxides. †  $p < 0.05$  versus baseline; ‡  $p < 0.01$  versus baseline; \*  $p < 0.05$  versus ISO; \*\*  $p < 0.01$  versus ISO.<sup>24</sup>



## Arrhythmia

Extracellular and/or intracellular magnesium depletion has been implicated in a variety of cardiovascular disturbances, including causation of ventricular arrhythmias, predisposing to digitalis intoxication, modulating vascular tone, and promoting atherogenesis. Administration of  $\text{MgSO}_4$  during electrophysiological evaluation of patients has demonstrated two effects of  $\text{Mg}^{2+}$  relevant to the treatment of supraventricular tachyarrhythmias: (1) prolongation of atrioventricular nodal conduction time (anterograde and retrograde) and refractory period<sup>27</sup>; and (2) suppression of conduction in accessory pathways with and without atrioventricular nodalike properties,<sup>27</sup> although conflicting results have been reported. Prolongation of atrioventricular nodal conduction by  $\text{Mg}^{2+}$  is most likely attributable to inhibition of  $\text{Ca}^{2+}$  current, the primary mode of impulse conduction through the atrioventricular node, but also may result from  $\text{Mg}^{2+}$ -induced attenuation of sympathetic activity at the atrioventricular node. Cardiac arrhythmia is an important complication of magnesium depletion that has been associated with a prolonged  $\text{QT}_c$  interval. In addition, magnesium supplementation has been shown to reduce the  $\text{QT}_c$  intervals even in patients with normal serum magnesium levels. Torsades de pointes is a repetitive polymorphous ventricular tachycardia that occurs in the presence of QT prolongation, and is usually induced by drugs that prolong the QT interval. Because of its effect on prolonging the QT interval, magnesium depletion has also been implicated in the pathogenesis of torsades de pointes, although such a relation has rarely been shown. Because of its ability to shorten the QT interval, magnesium supplementation has been used with some success in treating torsades de points. In regard to cardiac function, there is a close association between magnesium and potassium. Magnesium has been shown to attenuate the electrophysiological effects of hyperkalemia. Furthermore, in view of the relationship between magnesium and intracellular and extracellular potassium depletion, magnesium depletion has been implicated as a potential cause of digitalis intoxication. This is supported by the finding that an acute induction of hypomagnesemia in dogs with dialysis facilitates the development of digitalis intoxication and arrhythmias. Moreover, ventricular arrhythmias, including those induced by digitalis, are sensitive to magnesium therapy. Some evidence indicates that subclinical magnesium depletion might predispose patients with acute myocardial infarction to develop arrhythmias.

Other anti-arrhythmic effects of  $\text{Mg}^{2+}$  have been reported, although the underlying mechanisms have not been defined: restoration of sinus rhythm in critically ill medical and surgical patients with supraventricular tachycardias; suppression of intractable ventricular tachyarrhythmias; control of ventricular rate in new-onset atrial fibrillation (AF); prophylaxis of AF after coronary artery bypass grafting; slowing of digoxin-facilitated ventricular rate during AF in Wolff-Parkinson-White syndrome; abolition of pre-excitation ( $\delta$  wave) in patients with Wolff-Parkinson-White syndrome during normal sinus

rhythm; suppression of multifocal atrial tachycardia<sup>28</sup>; suppression of digoxin-induced ectopic tachyarrhythmias; prevention of bupivacaine-induced arrhythmias; and treatment of amitriptyline-induced ventricular fibrillation.

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# 19

## Magnesium: Forgotten Mineral in Cardiovascular Biology and Atherogenesis

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In this review, a rationale is presented for how hypercholesterolemia, hypertension, diabetes mellitus (DM), end-stage renal disease (ESRD), prolonged stress, and exposure to magnesium (Mg)-wasting drugs can lead to atherosclerosis, ischemic heart disease, and stroke. The data, accumulated so far, indicate that Mg deficiency caused either by a poor diet or errors in Mg metabolism may be a missing link between diverse cardiovascular risk factors and atherogenesis. Early data from our laboratory and others indicate that reduction in extracellular free Mg ions can induce an entire array of pathophysiological phenomena known to be important in atherogenesis, that is, vasospasm, hypoxia, increased vascular reactivity, and elevation in intracellular calcium (Ca). More recent data has demonstrated molecular events, pointing the way to atherogenesis: that is, formation of pro-inflammatory agents, generation of free radicals, platelet aggregation, modulation of macrophage and leukocyte mobility, and emigration across the endothelial wall. Finally, oxidation of lipoproteins, changes in membrane fatty acid saturation, changes in membrane plasmalogens, and *N*-phospholipids suggest alterations in intracellular lipid signals. It has been shown that Mg deficiency can modulate membrane sphingomyelinase, generate vasoactive and pathogenic sphingolipids, which could alter multiple intracellular signaling pathways, modulate transcription factors, and thus cause intimal plaque formation.

Hypercholesterolemia, hypertension, DM, immune injury, ESRD, renal dialysis, prolonged stress, obesity, and smoking are widely accepted as risk factors for atherosclerosis. No common link has been identified that forms a rational basis to these disorders and atherogenesis. Moreover, it is not clear how lipoproteins,  $\text{Ca}^{2+}$ , and macrophages gain access to the normally impermeable arterial walls or what allows pliable, physiological vascular smooth muscle (VSM) cells to change their state (phenotype) from a contractile to a noncontractile, synthetic cell,<sup>1,2</sup> and how these diverse risk factors lead to ischemic heart disease (IHD) and stroke.

Until relatively recently, it was not believed that Mg, a common essential dietary element and electrolyte, played any role in maintaining normal cardiovascular dynamics. This lack of understanding is most likely a reflection

of several factors: (1) scanty, uncontrolled experimental and clinical data regarding its precise actions on the heart and blood vessels; (2) poor appreciation of progressive shortfalls in Mg dietary intake since the turn of the century; (3) failure to recognize that patients with diets deficient in Mg are not necessarily hypomagnesemic; (4) an absence of the methodology needed to measure the free, ionized ( $\text{IMg}^{2+}$ ) rather than total Mg (TMg) in tissues, cells, and body fluids.

Over the past decade, experimental, epidemiological and clinical studies point to the active role of magnesium ions ( $\text{Mg}^{2+}$ ) in the maintenance of normal cardiovascular functions as well as in the etiology of cardiovascular pathology when problems arise in Mg intake and balance. Dietary Mg deficiency, as well as abnormalities in Mg metabolism, appear to play important roles in IHD, congestive heart failure, sudden death ischemic heart disease (SDIHD), atherosclerosis, a number of cardiac arrhythmias, hypertension, vascular complications in DM, immune injury, and ESRD. In this article, we attempt to review the cogent experimental, molecular, epidemiological, and clinical evidence that supports a role for Mg in atherogenesis and cardiovascular health and disease.

**Progressive Decline in Dietary Intake of Magnesium:  
Relationship to Cardiovascular Disease (CVD)**

At the turn of the century in the United States, ingestion of Mg was about 450 to 485 mg/day. This has been steadily decreasing each 5 to 10 years (Table 19.1). The most recent figures indicate values for men of ~185 to 260 mg/day and for women, ~172 to 235 mg/day. If we accept that the recommended daily allowance (RDA) = 350 mg/day, these values represent dietary shortfalls of 90 to 178 mg/day. Surveys in Europe and Canada reveal similar shortfalls in dietary intake of Mg (e.g., men ~189–262 mg/day; women ~143–283 mg/day); these values represent deficits of at least 67 to 161 mg/day. Many investigators and nutritionists believe that the current RDA recommended by the U.S. National

**TABLE 19.1.** Progressive decline in dietary intake of magnesium over the past 100 years.

Years	Mg intake/day
1900–1908	475–500
1909–1913	415–435
1915–1929	385–398
1935–1939	360–375
1947–1949	358–370
1957–1959	340–360
1965–1976	300–340
1978–1985	225–318
1987–1992	175–248

**TABLE 19.2.** Physiological functions of magnesium.

Enzyme functions	Structural functions
7 glycolytic enzymes	Proteins
T TCA cycle enzymes	Polyribosomes
Membrane-bound ATPases	Nuclei Acids
Kinases: creatinine kinase	Mitochondria
Alkaline P'Tase	Multienzyme Complexes, e.g., G-Proteins,
12 photosynthetic enzymes	N-MDA receptor complex
	Membrane channels
Membrane functions	Calcium antagonist
Hormone-receptor binding	Muscle contraction/relaxation
Gating of Ca <sup>2+</sup> channels	Neurotransmitter release
Transmembrane flux of ions	Action potential conduction in nodal tissue
Adenylate/GMP cyclase system	
Ca <sup>2+</sup> -Ca <sup>2+</sup> release	

Academy of Sciences is too low and it should be 450 to 500 mg/day; thus, the deficits become very pronounced, thereby raising the possibility for development of disease processes, particularly when numerous biochemical (Sidebar) and physiological functions (Table 19.2) could be compromised.

According to recent epidemiological studies, in which dietary variables were assessed in human volunteers by the 24-h recall method, Mg had the strongest association with blood pressure.<sup>3,4</sup> It is known that strict vegetarians exhibit significantly decreased incidences of atherosclerosis and IHD, sudden-death ischemic heart disease, and high blood pressure. Is this a consequence of the increased dietary intake of Mg? Legumes, beans, nuts, soybeans, green leafy vegetables, and unprocessed cereals, which form the basis of vegetarian diets, are rich in Mg. Greenlanders who have a low incidence of IHD, SDIHD, and high blood pressure consume diets rich in Mg (both foods and drinking water) whereas Danes, who consume diets rich in processed food and low in Mg, have high incidences of these cardiovascular disorders. However, when Greenlanders move to Denmark and reside there for some years their incidences of these CVD are approximately equal to the indigenous Danes.<sup>5</sup> Similar patterns have been noted for South African Bantu natives, Bedouins in the Arabian desert, and Aborigines in Australia upon moving into Western societies.<sup>6</sup>

Pivotal to magnesium's wide-sweeping range of physiological effects is its role as a prime gatekeeper of cellular activities, membrane functions, and its function in regulating a great number of critical cellular and subcellular processes (see Table 19.2 and Sidebar).

Although it was recognized by two French physicians, Hazard and Wurmser, in 1932, that systemic administration of magnesium ions can produce rapid reduction in arterial blood pressure and vasodilatation, very little else was known about the cardiovascular actions of Mg<sup>2+</sup> until the late 1960s. In 1961, one of us (BMA), while investigating the physiological responses of isolated blood vessels to vasoactive pressor and dilator substances, found that removal

### Roles of Magnesium in Cell Metabolism

Two major roles for Mg exist in biological systems: (1) It can form chelates with various intracellular anionic ligands, for example, ADP, ATP. It is this ability to form pyrophosphates which is necessary to drive numerous biological reactions, but also is key for most cellular uptake systems of ions and foodstuffs, as well as for control of muscle tension and the shape of cells. (2) It can compete with calcium ions ( $\text{Ca}^{2+}$ ) for binding sites on membranes (external and internal) and proteins.

$\text{Mg}^{2+}$  catalyzes or activates more than 500 enzymes in the body and is pivotal in the transfer, storage, and utilization of energy. It activates phosphate groups and reactions that involve ATP as well as other nucleotide complexes. The intracellular level of free  $\text{Mg}^{2+}$  ( $[\text{Mg}^{2+}]_i$ ) serves to regulate intermediary metabolism through activation of such rate-limiting enzymes as hexokinase, pyruvate dehydrogenase, enolase, and creatine phosphokinase. It regulates seven enzymes in glycolysis and four key enzymes in the tricarboxylic acid cycle.  $\text{Mg}^{2+}$  can cause a conformational change during catalytic processes (e.g.,  $\text{Na}^{2+}\text{-K}^{+}\text{ATPase}$ ), by promoting aggregation of multi-enzyme complexes (e.g., aldehyde dehydrogenase) or by a mixture of mechanisms (e.g.,  $\text{F}_1\text{-ATPase}$ ).

Within the cell nucleus,  $\text{Mg}^{2+}$  regulates DNA synthesis. Large numbers of  $\text{Mg}^{2+}$  ions are bound to the pentose-phosphate backbone of DNA. By regulating DNA and RNA (i.e., RNA synthetase, mRNA attachment to ribosomes, etc.) synthesis and structure,  $\text{Mg}^{2+}$  plays a vital role in regulating cell growth, reproduction, and membrane structure. Magnesium's role in regulating cell membrane permeability, transmembrane electrolyte flux, hormone and agonist-receptor binding, and cell adhesion are becoming widely accepted. Dietary deficiency of Mg (in different mammals) usually results in loss of intracellular  $\text{K}^{+}$  from organs and cells; intracellular  $\text{Ca}^{2+}$  and  $\text{Na}^{2+}$  rise under such conditions (e.g., Table 19.2). Magnesium's role in regulating membrane permeability has been long recognized.

of the  $\text{Mg}^{2+}$  from an artificial physiological salt solution resulted in production of vasospasm and enhanced responses to vasoactive pressors and decreased responses to dilator agents.<sup>3,7</sup> Since no one, up to that time, had reported such dramatic results of a  $\text{Mg}^{2+}$ -free medium on either VSM tone or reactivity, we did not at first believe the findings. Repetition of our findings thus was undertaken over the next several years, with several different types of blood vessels, until verification of our data remained unquestionable. Such early and exciting findings, as well as others, became compelling to us.

## Magnesium Depletion from the Body Is Common

A variety of commonly used drugs, such as alcohol, diuretics, chemotherapeutic agents (e.g., cisplatin, bleomycin), cyclosporine, certain antibiotics (amphotericin B), and fungicides, cardiac glycosides, and drugs of abuse (e.g., morphine, heroine, cocaine), among others,<sup>3,6-10</sup> produce a loss of body Mg; alcohol being the most well-known Mg waster. Diarrhea, malnutrition, vomiting, dehydration, high-salt diets, high intake of soft drinks, malabsorption syndromes, and certain renal disorders also result in considerable body loss of Mg.

Six to sixty-five percent of all hospitalized patients exhibit low serum Mg; patients with numerous cardiovascular disorders associated with atherogenesis demonstrate the most profound deficits in body Mg.<sup>3,10-21</sup> Did the disease(s) produce the Mg deficiency or was an existing Mg deficiency responsible, in part, for the etiology of the disease state?

## Magnesium Exists in Several Forms in Blood and Body Fluids: Importance of Free Magnesium Ions

Blood normally contains magnesium ions in three states: bound to plasma proteins; complexed to small anion ligands, such as bicarbonate, citrate, sulfate, phosphate, lactate, or to peptides; and free. Most clinical laboratories measure total magnesium levels by colorimetry or atomic absorption spectrophotometry. However, it is the free ionized form of magnesium that is physiologically active. Usual estimates of free  $Mg^{2+}$  have relied upon total Mg measurements in protein-free ultrafiltrates, which exclude the protein-bound Mg.<sup>22,23</sup>

Because the levels of anions can vary significantly in pathological states, and in view of the role apparently played by Mg in cellular homeostasis (Table 19.2), it is desirable to directly measure free  $Mg^{2+}$  in blood<sup>24</sup> and other body fluids. With this aim in mind,  $Mg^{2+}$ -sensitive electrodes have been designed to obtain these measurements in the presence of potential cationic interferences.<sup>25</sup>

Twenty-five years ago (1980), we demonstrated that diverse large and small mammalian coronary arteries subjected to reductions in extracellular  $Mg^{2+}$  concentrations underwent rapid spasm and potentiation of circulating vasoconstrictor hormones.<sup>26</sup> Similar observations have been made in human coronary arteries in vitro and during anginal attacks.<sup>12</sup>

We therefore initiated ion-sensitive electrode studies to determine whether patients with acute myocardial infarction (AMI) or coronary heart disease exhibited significantly lowered levels of free  $Mg^{2+}$ .<sup>27</sup> We found that free, but not total, Mg was usually lowered in such patients and that free  $Mg^{2+}$  as a percentage of total Mg was also significantly lowered (Table 19.3). These studies have provoked a number of cardiologists to examine the potential use of  $Mg^{2+}$  in the therapy of acute myocardial infarction or congestive heart failure. The



**TABLE 19.3.** Plasma ionized magnesium versus total magnesium in ischemic heart disease patients scheduled for coronary bypass surgery.

Group	N	Mg (mM/L)	
		IMg <sup>2+</sup>	TMg
Controls	42	0.60 ± 0.005	0.84 ± 0.008
IHD	35	0.51 ± 0.008 <sup>a</sup>	0.82 ± 0.006

Abbreviations: IHD, ischemic heart disease.  
IMg<sup>2+</sup> obtained with NOVA ISE; TMg obtained with AAS.  
<sup>a</sup>Significantly different from controls (*p* < 0.001).

results so far support the notion that Mg compounds might be of significant therapeutic value in these cardiac syndromes.

**Mg<sup>2+</sup> Controls Vascular Contractility, Basal Vascular Tone, and Produces Vasospasm**

As early as 1971, Mg<sup>2+</sup> ions have been shown to directly alter baseline tension or tone of blood vessels.<sup>28–32</sup> Decrements in [Mg<sup>2+</sup>]<sub>o</sub> result, in a concentration-dependent manner, in rapid elevations in contractile tension development in numerous mammalian and human arteries and arterioles. Elevation in [Mg<sup>2+</sup>]<sub>o</sub>, on the other hand, above the physiological level inhibits spontaneous mechanical activity and lowers baseline tension. The elevations in mechanical activity, produced, in response to lowering [Mg<sup>2+</sup>]<sub>o</sub>, are inhibited when external Ca<sup>2+</sup> ions are lowered or chelated. This demonstrates that influx of extracellular Ca<sup>2+</sup> ions are necessary for these contractile responses. In addition, a variety of neurohumoral agents that induce a contraction in VSM exhibit heightened contractile responses as external Mg<sup>2+</sup> is lowered, and attenuated contractile activity is observed as [Mg<sup>2+</sup>]<sub>o</sub> is elevated. How are these actions of [Mg<sup>2+</sup>]<sub>o</sub> brought about?

We first demonstrated in 1971, that Mg<sup>2+</sup> regulates Ca<sup>2+</sup> flux across VSM cell membranes,<sup>28</sup> as well as its release from intracellular storage sites.<sup>33,34</sup> Because a change in cytosolic free Ca<sup>2+</sup> concentration is necessary for contraction or relaxation, it may be physiologically relevant that Mg<sup>2+</sup> regulates the activity of VSM cells by competing with and modulating the levels of free intracellular Ca<sup>2+</sup>. Precise signaling pathways appear to be activated in these Ca<sup>2+</sup>-related changes (see below).

The vasodilator effect has been attributed, at least in part, to its Ca<sup>2+</sup> channel-blocking property as well as to modulation of intracellular free Ca<sup>2+</sup> in VSM cells. Recently, we have found that the precise concentration of [Mg<sup>2+</sup>]<sub>o</sub> also clearly controls the subcellular distribution of both [Ca<sup>2+</sup>]<sub>i</sub> and [Mg<sup>2+</sup>]<sub>i</sub>.<sup>33,35</sup>

The end result of lowered serum Mg<sup>2+</sup> and intracellular VSM cell Mg<sup>2+</sup> ([Mg<sup>2+</sup>]<sub>i</sub>) would be vasospasm, increased vascular tone, increased reactivity, reduction in peripheral blood flow (hypoxia), and, thus, local vascular injury,

a stimulus for hypertension and atherogenesis.<sup>1</sup> Using a combination of radio-labeled  $^{45}\text{Ca}$ , digital image analysis with fluorescent probes, and confocal laser-scanning microscopy with fluorescent probes, we found that  $\text{Mg}^{2+}$  gates special membrane  $\text{Ca}^{2+}$  channels in both VSM and endothelial cells and controls the release of intracellular free cytosolic  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) from these cells.<sup>3,7,33–35</sup>

The entry and release of  $[\text{Ca}^{2+}]_i$  and loss of membrane- $\text{Mg}^{2+}$  most likely initiates a number of intracellular signaling pathways (i.e., growth factors, cell adhesion factors, cytokines, nuclear-factor  $\kappa\text{B}$ , c-jun, c-fos and phospholipids, among others; see below) that could result in VSM cell proliferation (cell transformation), alteration in endothelial cell wall permeability, macrophage attraction, leukocyte adhesion, and  $\text{Ca}^{2+}$  cell entry. Low  $[\text{Mg}^{2+}]_o$ -induced vasospasm could accelerate uptake of oxidized lipids and/or stimulate generation of oxygen- and nitrosoradicals (see below).

## Low Magnesium Levels Can Lead to Formation of Pro-Inflammatory Agents: Possible Importance in Atherogenesis

Inflammation is such an everyday occurrence that most people do not even notice it. It does not just occur in the skin, but internally as well, particularly when viruses or bacteria invade the body. Inflammation is actually one of the body's major defense mechanisms.

Over time, however, inflammation can cause great harm to bodily tissues it is supposed to heal. This destructive side of inflammation has long been known to be pivotal in diseases like rheumatoid arthritis and multiple sclerosis. Recently, evidence has accumulated to implicate inflammation in development of atherosclerosis, as well as in many other diseases. Entry of various types of inflammatory cells into the arterial wall characterizes formation of the fatty streak.<sup>1,36</sup> Droplets of fat in the blood stream are initially thought to be absorbed by arterial walls, inducing release of hormone-like mediators called cytokines. Various cytokines are then thought to cause the VSM cells of the arterial walls to become sticky, which then attracts inflammatory white cells called monocytes. These squeeze between the endothelial cells, and, once in the arterial wall, undergo transformation to scavenger cells called macrophages, which ingest the fat droplets. These cells, over time, become overloaded with fat and give rise to plaques. Eventually, the inflammatory process thins the fibrous cap of the plaques and rupture occurs, spilling the contents along with some cytokines into the blood, which induces clotting and blocking of an artery, causing a heart attack or a stroke, depending on its location.

Much speculation centers around what the initiation and sustaining factors are for the above process.

Evidence implicates matrix metalloproteases (MMP) in plaque rupture. These enzymes can act in the shoulder region of the plaques to degrade the extracellular matrix, thus weakening the fibrous cap.<sup>36–38</sup> Matrix metalloproteases are produced by VSM cells and macrophages. Inflammatory cytokines

released in atherogenesis, such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin-1 (IL-1), and T-cell-derived interferon  $\gamma$  can induce MMP expression. Thus, a balance of several forces predicts the state of the fibrous cap; matrix production and its degradation determining plaque stability. MMP is also regulated by production and release of platelet-derived growth factor (PDGF) secreted by both the platelets and VSM cells.<sup>1,2,36</sup>

Feeding rats and hamsters diets deficient in Mg, for periods of only 14 to 21 days, results in cardiomyopathic lesions, cytokine formation and release (IL-1, IL-2, IL-6; TNF $\alpha$ ), and endothelin formation.<sup>40-42</sup> Using human umbilical vein endothelial cells (HUVE), Maier and colleagues<sup>43</sup> have shown that low [Mg<sup>2+</sup>]<sub>o</sub> produces an upregulation of IL-1, as well as upregulation of vascular cell adhesion molecule-1 (VCAM-1) and plasminogen activator inhibitor-1 (PAI-1). Other workers have demonstrated that low [Mg<sup>2+</sup>]<sub>o</sub> environments resulted in an upregulation of MMPs in cultured rat aortic VSM cells under basal and PDGF-stimulated conditions. Rayssiguier and Mazur<sup>44</sup> have reported that Mg deficiency in rats leads to a clinical type of inflammatory syndrome characterized by increased production of leukocytes, release of several cytokines, macrophage activation, and production of acute phase inflammatory proteins. Although other growth factors (PDGF, transforming growth factor B, fibroblast growth factors) have not yet been identified in Mg-deficient states, it is likely that Mg deficiency will be found to cause production of these well-known growth factors implicated in atherogenesis.<sup>1,2,36</sup>

## **Importance of Leukocytes, Adhesion Molecules, and Chemokines in Atherogenesis: Possible Roles of Magnesium**

Although the major risk factors for atherogenesis have been identified above, the precise cellular and molecular mechanisms of an atherothrombotic lesion initiation and progression remain to be understood. It is now clear that recruitment of blood monocytes and lymphocytes to the arterial intimal surface is a key characteristic of atherothrombotic lesions.<sup>1,36</sup> Attached monocytes migrate into the endothelial intima, transform themselves into macrophages, and become foam cells. Subsequently, growth and expansion of early lesions occur, and then plaque destabilization, resulting in thrombotic complications.

A *sine qua non* of the atherosclerotic inflammatory disease is the recruitment of leukocytes to the site of inflammation. Leukocytes migrate along chemotactic gradients and induce tissue damage by releasing enzymes, chemical mediators, and toxic oxygen-free radicals. Diverse cytokines and chemokines produced locally, appear to regulate the type of leukocyte recruited. In this regard, IL-1 or TNF $\alpha$  induce endothelial cells to synthesize and express surface adhesion molecules, such as selectins, immunoglobulin gene superfamily proteins, integrins, and present chemokines (small heparin-binding proteins), which are bound to cell surface proteoglycans. The cell adhesion molecules and chemokines induce recruitment of leukocytes from the blood

to the arterial walls. Different adhesion molecules mediate each stage of leukocyte emigration. The chemokines direct the migration to sites of inflammation or injury.

In the settings of hypercholesterolemia, VCAM-1 and intercellular adhesion molecule 1 (ICAM-1) are upregulated and expressed in the endothelial cells.<sup>45,46</sup>

Dietary Mg deficiency in rats has been shown, in a few days, to be associated with a marked increase in total circulating leukocytes, mostly neutrophils.<sup>47,48</sup> This Mg deficiency-induced leukocytosis is also associated with infiltration of the spleen with polymorphonuclear leukocytes (PMN) and macrophages concomitant with a rise in IL-6 levels. Using rabbits fed diets of low Mg, we have reported an activation of splenic and Kupfer cell macrophages.<sup>49</sup> Weglicki and his colleagues have shown that Mg deficiency in rats results in increased levels of IL-1, IL-6, and TNF $\alpha$  in T-lymphocytes.<sup>50</sup> To our knowledge, there is no data as yet to indicate whether a Mg-deficient state will give rise to increased levels of selectins, and integrin adhesion molecules or chemokines. Some studies have indicated that a Mg-deficient state *in vitro*, can result in upregulation of one key member of the immunoglobulin gene superfamily—VCAM-1.<sup>43</sup> It will be important to determine whether Mg deficiency in intact animals will result in early upregulation and expression of selectins, integrin molecules, and chemokines in intimal endothelium and VSM cells.

## Low Levels of Magnesium Enhance Platelet Aggregation and Thrombotic Tendencies

A number of studies dating back to the late 1950s indicate that low  $[Mg^{2+}]_o$  promotes blood coagulation.<sup>6,51</sup> More recently, several investigators have demonstrated that platelet aggregation is enhanced in low  $[Mg^{2+}]_o$  environments<sup>52,53</sup> and that megakaryocyte numbers and white cell counts are increased concomitantly in Mg-deficient hamsters,<sup>54</sup> thus providing, potentially, an important link between early atherogenesis and subsequent thrombotic tendencies.

## Low Magnesium Levels Result in Lipid Peroxidation and Free Radicals

Until recently, it was not known how Mg deficiency could promote cardiovascular damage. During the past 10 years, reports from several laboratories, including ours, have suggested that Mg deficiency can lead to formation of several different types of oxygen-free radicals, lipid peroxidation, and formation of ferrylmyoglobin.

In the course of normal metabolism, oxygen is responsible for formation of some highly toxic and reactive byproducts, such as superoxide ( $O_2^{\cdot-}$ ), hydrogen

peroxide ( $\text{H}_2\text{O}_2$ ), hydroxyl radicals ( $\text{OH}^\cdot$ ), and singlet oxygen ( $^1\text{O}_2$ ). Oxidative stress causes extensive tissue damage, including lipid peroxidation of membranes, oxidation of proteins, and damage to DNA and RNA.  $\text{H}_2\text{O}_2$  and  $\text{OH}^\cdot$  radicals are particularly reactive,  $\text{H}_2\text{O}_2$  penetrating cell membranes easily. Other radicals that can be formed and are destructive are  $\cdot\text{NO}$ , peroxynitrite ( $\text{OONO}^-$ ), and hypochlorite ( $\text{HOCl}^-$ ). All of these molecules have been shown to promote vasoconstriction, spasm, and damage of small blood vessels.<sup>62–66</sup>

Because macrophages are the most potent producers of these reactive oxygen metabolites (ROMs), their invasion of blood vessel walls in the atherogenic process raises the possibility of ROMs in initiation of atherosclerosis. The major targets of ROMs in biological systems are lipids, proteins, and DNA. In the case of atherogenesis, one of the primary events is an attack on the low-density lipoproteins (LDL) in the blood.<sup>67,68</sup> Before LDL can be taken up by macrophages, it has to be oxidatively modified and subsequently gets endocytosed by macrophages to form foam cells. Although the two primary metabolites  $\text{O}_2^{\cdot-}$  and  $\text{H}_2\text{O}_2$  each are not very reactive in this regard, the combination are thought to form the highly reactive hydroxyl radical (the Haber–Weiss reaction). Endothelial cells (EC) are known to generate the superoxide radical anion<sup>69</sup> and thus become a prime candidate for in situ modification of LDL. Activated macrophages and neutrophils, which appear in large numbers in atherogenesis, contain an enzyme myeloperoxidase (MPO) that converts  $\text{H}_2\text{O}_2$  and chloride ions to  $\text{HOCl}^-$ ,  $^1\text{O}_2$  and  $\text{OH}^\cdot$ . All these can initiate lipid peroxidation and it is, thus, not surprising to find that MPO is involved in LDL oxidation.<sup>70,71</sup> Myeloperoxidase has been found in atherosclerotic plaques,<sup>71</sup> and when it combines with  $\text{HOCl}^-$  and  $\text{O}_2^{\cdot-}$ , it will produce  $\text{OH}^\cdot$  (the Long–Bielski reaction), thus causing potentially significant damage in the vascular wall. Most of these reactions are catalyzed by metal ions ( $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ); surprisingly many of these ROMs, once formed, in turn release free Fe ions from ferritin. Such free Fe could combine in muscle tissues (heart and skeletal muscle) with myoglobin to form the reactive ferrylmyoglobin (see below).

With respect to Mg deficiency in rats, several reports have appeared that demonstrate that macrophages and PMNs exhibit release of superoxide anions and some free radical species in very early Mg deficiency<sup>72–75</sup> concomitant with increased numbers of PMNs and increased lipid peroxidation. Other studies performed by Rayssiguier's group in intact rats indicate that these phenomena are accompanied by changes in the physical status of cell membranes. Using rats, rabbits, and monkeys, others have demonstrated experimentally that Mg deficiency-induced hyperlipidemias are accompanied by vascular wall lipid infiltration and thick atheromatous plaques.<sup>49,72</sup> In some studies, correlations have been seen between the degree of Mg deficiency-induced lipid peroxidation (TBARS levels) and lipoprotein oxidizability.<sup>72</sup> Other investigators employing rats and hamsters also reported lipid peroxidation after 7 to 21 days.<sup>42</sup> Using bovine EC in culture in low  $[\text{Mg}^{2+}]_o$  (0.4 mM), these workers demonstrated induced formation of oxygen radicals.<sup>76</sup> Using primary rat aortic VSM cells and canine VSM cells, our group has demonstrated that exposure of these cells to low  $[\text{Mg}^{2+}]_o$  (0.15–0.48 mM) for 3 h resulted in concentration dependent

risers in malondialdehyde (MDA) levels, with higher levels of MDA after 18 to 24 h of low  $[Mg^{2+}]_0$  exposure.<sup>77</sup> Using stroke-prone rats, Touyz and coworkers<sup>78</sup> have shown that low dietary Mg intake resulted in formation of ROMs concomitant with an exacerbation of elevated systolic blood pressure along with structural changes in the vascular wall. In this context, it should be pointed out that Mg deficient diet-induced thickening in arterial walls has been seen to occur prior to rises in arterial blood pressure,<sup>79,80</sup> suggesting that cell hyperplasia and proliferation of endothelial and VSM cells occurs earlier than thought in Mg-deficient states. To this, should be added the early findings of Gunther and coworkers, who reported, using human and rat erythrocytes, that oxygen-free radicals, like  $H_2O_2$ , by destroying the  $Na^+/Mg^{2+}$  anti-port in cells can result in efflux (and loss) of free  $Mg^{2+}$ .<sup>81</sup> Such a situation, if found in vivo, would exacerbate Mg deficiency-induced pro-oxidant states.

Lastly, we should mention another potential avenue for Mg deficiency-induced cardiovascular damage and atherogenesis via formation of the highly reactive ferrylmyoglobin.<sup>82</sup> The generation of free Fe mentioned above would be expected to combine with myoglobin found in cardiac and skeletal muscle to form the latter radical (see below). Experimentally, it has been shown that Mg deficiency-induced formation of ferrylmyoglobin in intact, perfused rat hearts will lead to cardiac failure.<sup>82</sup> This work also showed the link between  $Ca^{2+}$  overload induced by Mg deficiency and cardiac myocyte membrane damage.

All the above seem to point to a true link between Mg deficiency, lipid peroxidation, generation of ROMs, and atherogenesis, but definitive studies remain to be done.

## Elevated Magnesium Levels Ameliorate Atherogenesis and Homocysteine-Induced Magnesium Depletion

Hypercholesterolemia has been widely accepted as a high risk factor for development of atherosclerosis. Evidence from both animals and humans suggests that the dietary and blood level of  $Mg^{2+}$  may modulate the serum levels of lipids and lipoproteins. Ever since the early experimental and clinical studies by Bejal and Czech (1956) and Gruneis (1953), respectively, there have been reports that either dietary or systemic administration of Mg salts might ameliorate atherogenesis.<sup>49,79,90</sup> Magnesium is necessary for the activity of lecithin cholesterol acyltransferase (LCAT) and lipoprotein lipase (LPL), which lower triglyceride levels and elevate high-density lipoprotein (HDL) cholesterol levels.<sup>67,68</sup> Our laboratory, using rabbits, has reported that the dietary level of Mg modulates the serum level of cholesterol and triglycerides in normal animals; the lower the intake of Mg, the higher the serum lipid levels.<sup>49</sup>

Dietary deficiency of Mg (compatible with reduced dietary intake of Mg seen in the adult population of the western world) in rabbits fed a high-cholesterol diet exacerbates atherogenesis and lipid deposition in arterial muscle, and stimulates (or activates) macrophages of the reticuloendothelial system. We

have demonstrated, in this study, that pretreatment of animals with orally administered Mg aspartate HCL: (1) attenuated the atherosclerotic process markedly, and (2) lowered serum cholesterol and triglycerides in the atherosclerotic animals. These studies indicate that the extent of the atherogenic lesions were poorly correlated with the level of serum cholesterol and highly dependent on the level of dietary Mg and the Ca/Mg ratio. We noted that due to the high blood levels of lipids and lipoproteins, a true Mg-deficient state can often be masked or hidden.

A similar masked or hidden Mg deficiency has been observed, by our group, in renal transplant patients as well as in ESRD patients<sup>17,18</sup>; patients who are characterized by extensive, unexplained atherogenesis. Patient studies in our medical center with subjects having undergone renal transplantation or that have end-stage kidney failure, who are at high risk to develop atherosclerosis, demonstrate a concomitant marked, lowering of ionized Mg (but not TMg), decreased urinary output of Mg, hyperinsulinemia, elevated total serum cholesterol and/or triglycerides.  $\text{ICa}^{2+}/\text{IMg}^{2+}$  ratios, signs of elevated vascular tone, increased vascular reactivity, and risk of atherogenesis, were also significantly elevated in our stable renal transplant recipients and subjects with ESRD. The TMg in these patients is usually normal or elevated, respectively, suggesting that in advanced atherogenesis and states of hyperlipidemia, a normal TMg would often be seen, despite the fact that the body and vascular walls would be deficient in  $\text{IMg}^{2+}$ .

An increased plasma homocysteine (HC) concentration is common in patients with stroke, coronary heart disease, and peripheral vascular disease, and confers an independent risk for atherogenesis. Several studies have shown that HC causes vascular endothelial injury, proliferation of VSM cells, and altered blood coagulation.<sup>55,56</sup> Recent investigations have reported that elevated plasma HC result in production of phospholipids, activation of protein kinase C, and induction of c-fos and c-myc in VSM cells.<sup>57</sup> These findings suggest that hyperhomocysteinemia may contribute to progression of peripheral and cerebral atherosclerosis and infarction. However, the mechanism(s) whereby hyperhomocysteinemia induces heart attacks and stroke remain to be determined.

Existence of an interrelationship between vitamin status and plasma HC was noted by an inverse relationship between HC and plasma folate or vitamin B<sub>12</sub> concentrations.<sup>58</sup> These studies were a direct result of early suggestions of McCully.<sup>59</sup> Because the major enzymes involved in HC metabolism are  $\text{Mg}^{2+}$  dependent, it is distinctly possible that  $\text{Mg}^{2+}$  plays an important role in HC-related atherogenesis,  $[\text{Ca}^{2+}]_i$  overload, and coronary and cerebral vasospasm and stroke, particularly as  $\text{Mg}^{2+}$  depletion has been shown to initiate atherogenesis and vasospasm (see above). Using primary cerebral VSM cells, our laboratory demonstrated that HC itself induces Mg depletion.<sup>60</sup> However, concomitant addition of vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, or folic acid, either alone or together, failed to prevent the HC-induced cellular loss of  $[\text{Mg}^{2+}]_i$ . These recent findings are thus compatible with the hypothesis that an increased serum HC concentration



causes abnormal metabolism of  $Mg^{2+}$  in cerebral VSM cells, thus priming these cells for HC-induced atherogenesis, cerebrovasospasm, and stroke.

Although hyperhomocysteinemia (HHC) is an independent factor for coronary artery disease, some studies have indicated that patients with hyperhomocysteinemia are not susceptible to atherosclerosis. There may be an important link between HHC, Mg, and extracellular matrix metalloproteinase-2. In this context, it has been shown recently by Guo and coworkers,<sup>61</sup> using cultured rat aortic VSM cells, that HHC (50–1000  $\mu M$ ) increased the production of MMP-2 in a concentration-dependent manner, and folic acid plus Mg was found to reduce this increased MMP-2 production. These preliminary observations are suggestive of the possibility that therapeutic administration of folic acid with Mg may exert ameliorative actions on atherogenesis.

### **Deficits in Magnesium Can Induce Formation of Ferrylmyoglobin Radicals in Cardiac Muscle: New Possible Link to Coronary Arterial Atherogenesis**

Cardiac tissue contains a large amount of myoglobin, which is involved in the intracellular transport and storage of oxygen. These functions depend on its ability to undergo reversible oxygenation by the ferrous heme group. The heme iron of deoxymyoglobin can be oxidized by hydrogen peroxide, which is currently thought to yield an oxy ferryl complex ( $Fe^{IV} = O$ ), similar to compound II of peroxidases, and a transient protein radical.<sup>83</sup> This high oxidation state of myoglobin is known as ferrylmyoglobin. Because there is a lack of significant amounts of catalase in cardiac tissue, the heart has a characteristic susceptibility to hydroperoxide toxicity. The subsequent formation of this ferrylmyoglobin radical can further degrade deoxyribose after releasing iron from the porphyrin ring of the myoglobin, and thus lipid peroxidation can occur.<sup>84–86</sup> It has been suggested that oxidation of myoglobin may be involved in the initiation and propagation of free radical damage in some tissues, and such reactive species may cause cellular injury in muscle.<sup>87–89</sup> We hypothesize that such reactions might underlie initiation of myocardial dysfunction in ischemia and  $Mg^{2+}$  deficiency-induced cardiac injury and may play a key role in coronary artery atherogenesis.

Using reflectance spectrophotometry in isolated perfused working rat hearts, subjected to an acutely Mg-deficient environment (0.3 mM) for 30 min, we found that 80% of the myoglobin converted to its deoxygenated form; reduced cytochrome oxidase aa<sub>3</sub> also increased about 80% in low  $[Mg^{2+}]$ .<sup>82</sup> This process, set into motion by acute Mg deficiency, resulted from a direct accessibility of the exogenous peroxide to the cytosolic protein. Ferrylmyoglobin formation could be prevented or dissipated by one-electron reduction of this hypervalent form of myoglobin using ascorbate. Interestingly, the



ferrylmyoglobin radical was formed before appearance of either creatinine phosphokinase or LDH, suggesting a cause and effect for cardiac myocyte membrane damage and, potentially, CAD.

We suggest the link between Mg deficiency-induced  $\text{Ca}^{2+}$  overload and cardiac myocyte membrane damage is formation and action of the ferrylmyoglobin radical; the latter myocardial injury should be prevented by use of one-electron reductants like ascorbate.

## **Multiple Cellular Signaling Pathways Are Activated in VSM by Alterations in Extracellular Magnesium**

Although low levels of  $[\text{Mg}^{2+}]_o$  lead to vasoconstriction, vasospasm, and decreased blood flow, forerunners of atherogenesis, whilst elevated levels of  $[\text{Mg}^{2+}]_o$  lead to relaxation, vasodilatation, and increased peripheral blood flows, up until very recently, the signaling pathways (except for  $\text{Ca}^{2+}$ ) for these diverse responses in vascular smooth muscle cells remained largely unknown. Using small and large peripheral and cerebral blood vessels, we and other workers have now identified a number of signaling pathways whereby low  $[\text{Mg}^{2+}]_o$  leads to vasoconstriction.<sup>8,33,34,77,78,91-96</sup> These include activation of several protein kinase C isozymes, nonreceptor tyrosine kinases, P-I-3 kinases, mitogen-activated protein kinases (MAPK), and MAPK kinases. With respect to the relaxant effects of elevated  $[\text{Mg}^{2+}]_o$ , our laboratory found that high concentrations of  $[\text{Mg}^{2+}]_o$  appear to eventuate in fresh rat aortic endothelial cell production and release of NO followed by activation of guanylate cyclase, leading to cGMP and vessel relaxation<sup>34</sup>; the smaller the blood vessel, the greater the dependence on mediation by NO. This work should be contrasted with recent work of others, using a passaged cell line of murine microvascular EC, who exposed the EC for 3 days to low  $[\text{Mg}^{2+}]_o$ .<sup>97</sup> These workers found that 0.1 mM  $[\text{Mg}^{2+}]_o$ , a very low concentration (incompatible with life), resulted in an elevation of NO synthesis. Such experiments should be repeated with fresh (or primary) EC and extracellular levels of  $[\text{Mg}^{2+}]_o$  ranging from 0.3 to 0.48 mM. Whether or not the activation/deactivation of NO synthase is dependent on concentration and type of EC, and species, and its role in atherogenesis will have to await further study.

## **Magnesium-Dependent Changes in Fatty Acid Saturation, Synthesis and Release of Sphingolipids and Plasmalogen Content of VSM: Relation to Intracellular Signaling Pathways and Atherogenesis**

In atherogenesis and vascular disease, it has been demonstrated that membrane phospholipids are altered. The failure to focus on the latter may be one of the chief reasons that neither the frequency of restenosis after percutaneous

transluminal coronary angioplasty has improved nor have varied recommended treatments met with more success. Approximately 20 years ago, our laboratory reported that dietary Mg deficiency in rats resulted in alterations of membrane phospholipids and fatty acid saturation in intact cardiac tissue.<sup>98</sup> A few years later, it was shown that phosphoinositide-derived second messengers are important in regulation of  $\text{Ca}^{2+}$  in VSM.<sup>99</sup> Over the past decade these initial findings have given rise to numerous studies on this theme.

We investigated whether  $\text{Mg}^{2+}$  modulates fatty acid saturation and plasmalogen ( $\alpha,\text{B}$ -unsaturated ether) content in rat aortic and canine cerebral VSM membranes.<sup>100</sup> In our studies, using ion exchange chromatography to separate lipids and proton nuclear magnetic resonance (NMR) spectroscopy, we noted that exposure of primary cultured aortic and cerebral arterial VSM cells to low  $[\text{Mg}^{2+}]_o$  (0.3–0.48 mM) for 18 h increased the fatty acid content of these cells about threefold. Fatty acid saturation was increased about twofold when these cells were exposed to a medium containing around 0.2 mM  $[\text{Mg}^{2+}]_o$ , compared to one containing 1.2 mM  $[\text{Mg}^{2+}]_o$ . These data show that the level of fatty acid saturation in VSM cells is sensitive to  $[\text{Mg}^{2+}]_o$  concentrations over the human pathophysiological range (0.3–0.6 mM) found by our ion-selective electrode work in patients with hypertension, IHD, AMI, DM, ESRD, and stroke, all subjects known to have increased levels of atherosclerosis.

In our studies, we found that the decrease in fatty acid unsaturation observed in VSM cells exposed to low  $[\text{Mg}^{2+}]_o$  was accompanied by a two to three carbon decrease in average chain length.<sup>100</sup> In contrast to the  $\text{Mg}^{2+}$ -dependent increase in double bonds, the vinyl ether content decreased with increasing  $[\text{Mg}^{2+}]_o$ . In addition, we found that the ratio of fatty acid double bonds to vinyl ether double bonds in VSM cells increased about fivefold as  $[\text{Mg}^{2+}]_o$  was increased from the pathophysiological to the physiological range (0.3–0.8 mM). We believe such data suggest that different desaturases must be involved in the synthesis of plasmalogens and unsaturated fatty acids, although one cannot rule out the possibility that  $\text{Mg}^{2+}$  has different effects on the same enzyme acting on different substrates. Because plasmalogens are high in excitable tissues like contractile tissues,<sup>101</sup> the increased contractility of VSM in low  $[\text{Mg}^{2+}]_o$  in vivo (reviewed above), may be facilitated by increased plasmalogen content in Mg-deficient states, thus potentially playing an important role in the early atherogenic process.

The observed low  $\text{Mg}^{2+}$ -induced decrease in fatty acid chain length and in the number of double bonds most likely reflects free radical oxidation of the double bonds and chain shortening. It is possible that this oxidative modification of the polyunsaturated fatty acid residues of membrane phospholipids could generate platelet-activating factor (PAF)-like lipids,<sup>102</sup> believed to be associated with leukocyte emigration and diapedesis.<sup>102</sup> About 10 years ago, it was found that exposure of smooth muscle cells to oxidized LDL, which produces PAF-like lipids, was mitogenic; this is significant because atherogenesis requires mitosis of intimal VSM cells. Interestingly, the reported mitogenic activity was blocked by specific PAF-receptor antagonists.<sup>103</sup> We believe that the  $\text{Mg}^{2+}$ -dependent changes in membrane lipids reported by our group may

link various CVD risk factors (hypercholesterolemia, hypertension, DM, ESRD, IHD, smoking, immune injury, obesity, prolonged stress) and atherogenesis. Such attributes of  $Mg^{2+}$  could be pivotal in generating and controlling the diverse intracellular signaling pathways.

In view of the above potential relationships between Mg regulation of membrane phospholipids, fatty acid content, and makeup, it seems logical to believe that  $Mg^{2+}$  might modulate atherogenesis through additional lipid second messengers like sphingolipids. Sphingomyelin (SM) is one of the major lipids in cell membranes and lipoproteins. Although direct links between atherogenesis and SM have not been established, experimental in vitro data suggest that SM and/or its metabolites may have proatherogenic potential. For example, SM-rich lipoproteins can be transformed to foam cell substrates by sphingomyelinase (SMase).<sup>104</sup> Second, the SM content of lipoprotein particles could influence lipid metabolism by influencing binding or activity of lecithin-cholesterol acyltransferase<sup>105</sup> and lipoprotein lipase.<sup>106</sup> Third, ceramide and/or other metabolic products of SM breakdown and synthesis are known to be potent regulators of cell proliferation, activation, and apoptosis,<sup>107</sup> possess vasoconstrictor and spasmogenic properties,<sup>108–111</sup> and thus may be initiators of plaque growth and stability. We have found that incorporation of [3H]-palmitic acid into phosphatidyl-choline (PC) and SM, in primary rat aortic VSM cells and canine cerebral arterial VSM cells, was altered within 15 to 30 min after modifying the extracellular Mg level.<sup>112</sup> In these studies, decreased  $[Mg^{2+}]_o$  produced a fall in [3H]-SM and [3H]-PC over the first 2 h. After 18-h incubation, the [3H]-PC/[3H]-SM ratio changed from 20:1 to 50:1. There was a reciprocal relationship between ([3H]-ceramide formation and [3H]-1,2-diacylglycerol (1,2-DAG) levels. Collectively, these results indicate that a fall in  $[Mg^{2+}]_o$  produces a rapid and sustained decrease in membrane SM and a rise in intracellular ceramide. Thus, a major effect of lowering  $[Mg^{2+}]_o$  appears to be a downregulation of SM synthase. Ceramide and its downstream products are known to activate different serine/threonine protein kinase cascades and protein phosphatases that regulate the function of several transcription factors, such as nuclear factor- $\kappa$ B (NF- $\kappa$ B), shown, recently, to be regulated by  $[Mg^{2+}]_o$ ,<sup>77</sup> which coordinately controls genes encoding inflammatory cytokines and cell adhesion molecules. If low  $[Mg^{2+}]_o$  is a trigger for promotion of transcription of inflammatory proteins and cell adhesion molecules, via a breakdown of SM to form ceramides (and other products), it could be a major contributing factor in atherogenesis, which as, reviewed above, has both inflammatory and leukocyte components. This should be a fruitful avenue for further investigation.

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# **Skeletal Diseases and Calcium Metabolism**

# 20

## Overview of Skeletal Diseases and Calcium Metabolism in Relation to Magnesium

Hirotoshi Morii

Whereas 69.3% of magnesium (Mg) exists in bone tissues and is incorporated in bone crystal as a constituent, Mg also regulates functions of osteoblasts and osteoclasts, as well as osteocytes. Calcium-sensing receptor (CaR) is a key regulator of parathyroid hormone (PTH) secretion and CaR requires magnesium for the action. Another important aspect of Mg is the role of this element as a nutrient. Mg is supplied as a nutrient from food.

### Magnesium and Osteoporosis

Rude and colleagues dedicated a study to bone metabolism and magnesium in the present monograph. After publishing a review article entitled “Magnesium Deficiency: A Possible Risk Factor for Osteoporosis,”<sup>1</sup> the group has concentrated on this issue from many standpoints. After that one of the problems was the correlation between magnesium deficiency and cytokines related to bone turnover.<sup>2</sup> This study group demonstrated OPG decrease and RANKL increase in magnesium deficiency by the immunocytochemistry, thus reflecting the increased osteoclastogenesis and decrease in bone mass in magnesium deficiency. Ryder studied the effect of dietary magnesium intake and bone mineral density (BMD).<sup>3</sup> This group showed that in white, but not black, men and women, magnesium intake was positively associated with BMD of the whole body after adjustment of age, self-report of osteoporosis or fracture in adulthood, caloric intake, calcium and vitamin D intake, body mass index (BMI), smoking status, alcohol intake, physical activity, thiazide diuretic use, and estrogen use in women ( $p = 0.05$  for men and  $p = 0.005$  for women). Bone mineral density was  $0.04 \text{ g/cm}^2$  higher in white women and  $0.02 \text{ g/cm}^2$  higher in white men in the highest than in the lowest percentile of magnesium intake.<sup>3</sup>

However, there have been many discussions of the possibility of magnesium deficiency as a risk factor of osteoporosis. Morii cited a study from WHI-OS (Women’s Health Initiative Observational Study), reported in 2002, in which the study group demonstrated that high magnesium intake does not confer a protective effect against fracture and in fact may increase the risk of wrist/lower arm fracture in the cohort.

Morii showed in the present review that Mg restored bone toughness partially compared to calcium in Ca-Mg deficient animals but plays a role in maintaining bone strength.

## **Magnesium and Bone Cells**

Intracellular magnesium is important in the function of cells, including osteoblasts, osteoclasts, and osteocytes. Schwartz observed the effect of magnesium deficiency on bone-cell differentiation and bone formation using in vivo matrix-induced endochondral ossification. Demineralized bone matrix was implanted subcutaneously in young male rats fed a semisynthetic Mg-deficient diet (50 ppm) for 7 days. Control diet was supplemented to contain 1000 ppm Mg. The implants were harvested 7, 9, 11, 15, and 20 days after implantation. Implants removed from Mg-deficient animals showed retardation in cartilage and bone differentiation and matrix calcification. Magnesium content was markedly reduced compared to the controls. Bone marrow development was also retarded with Mg deficiency.<sup>4</sup>

Gruber induced dietary Mg deficiency in the mouse, which showed hypercalcemia. Animals received osteoprotegerin (OPG) injections for 12 days. Serum calcium was similar in Mg-deficient mice treated with OPG and control mice receiving OPG. Both groups had significantly higher serum calcium than controls or Mg-deficient animals receiving vehicle alone. Mg-depleted mice that received OPG in doses that inhibit osteoclastic bone resorption remained hypercalcemic. Thus OPG–osteoclast relation may have differed in Mg deficiency.

## **Calcium Sensing Receptor (CaR) and Magnesium**

This issue was discussed in detail by Schlingman in this monograph (Chapter 22). CaR plays important roles in the regulation of PTH and re-absorption mechanisms of divalent cations from tubules. Magnesium plays important roles in these processes. Morii also reviewed some aspects of CaR in relation to magnesium. Kawata showed that CaR is activated by  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Ga}^{3+}$ , and neomycin dose in a dependent manner. It was also demonstrated that calcimimetic administration induced decrease in serum Mg levels.<sup>5</sup> It was postulated that the inhibition of PTH secretion or some mechanism related to the actions on renal tubules may have participated.<sup>5</sup>

## **Magnesium and Vitamin K in Relation to Bone Metabolism**

This issue is fully discussed by Amizuka in the present monograph. Kobayashi and colleagues also concentrated on this issue in recent years. It is very interesting that bone abnormalities are reversed by vitamin K. Further studies are needed from this standpoint.

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# 21

## Magnesium and Osteoporosis

Hirotoishi Morii, Takehisa Kawata, Nobuo Nagano, Takashi Shimada, Chie Motonaga, Mariko Okamori, Takao Nohmi, Takami Miki, Masatoshi Kobayashi, K. Hara, and Y. Akiyama

Magnesium is the second most abundant cation in bone and has been recognized as one of essential elements of bone. It is estimated that 1300 g of calcium, 14 g of magnesium, and 600 g of phosphorus exist in bones of a 70-kg human adult.<sup>1</sup> Basic structure of bone is composed of hydroxyapatite  $[\text{Ca}_5(\text{OH})(\text{PO}_4)_3]$ , but Newman proposed that magnesium is incorporated in bone crystal in the following formula:  $[\text{Ca}_9^{++}(\text{H}_2\text{O}^+)_2(\text{PO}_4^{\equiv})_6(\text{OH}^-)_2][\text{Ca}^{++} \cdot \text{Mg}_{0.3}^{++} \cdot \text{Na}_{0.3}^{+} \cdot \text{CO}_3^{-} \cdot \text{Cit}_{0.3}]$ .<sup>2</sup>

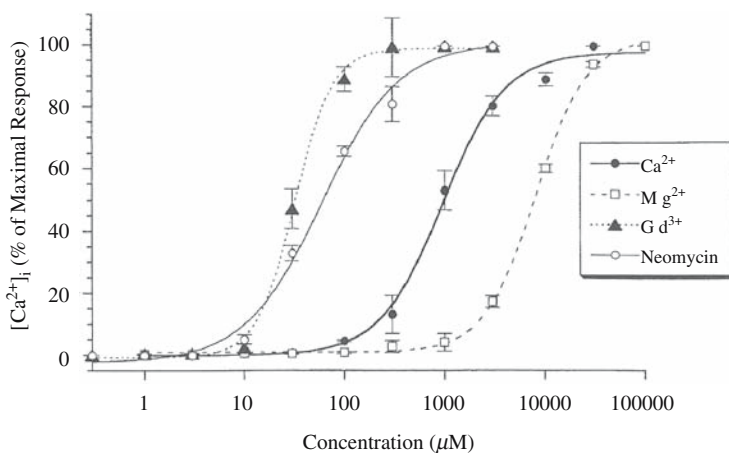
Thus, magnesium may be involved in bone metabolism as a component of bone mineral, as well as by regulating functions of osteoblast and osteoclast. Magnesium has been known to regulate parathyroid hormone (PTH) secretion and function directly or indirectly via the regulation of calcium-sensing receptor (CaR).

### Calcium-Sensing Receptor and Magnesium

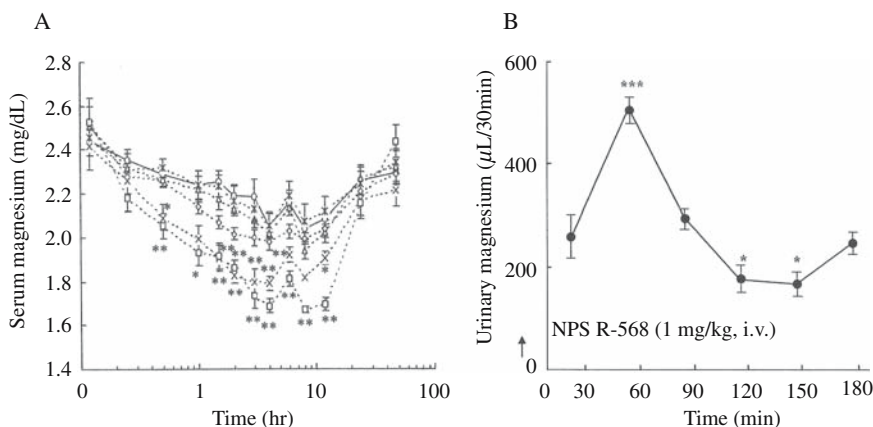
Calcium-sensing receptor was cloned from bovine parathyroid cells by Brown and colleagues in 1993<sup>3</sup> and was shown to respond to changes in extracellular concentration of calcium and influence on PTH secretion. Magnesium has an action of suppressing PTH secretion as a ligand of CaR, just as calcium. However, the affinity of magnesium to CaR is much less compared with calcium and Murakami showed that  $\text{EC}_{50}$  in raising intracellular level was 0.9 mM for calcium and 7.9 mM for magnesium (Figure 21.1).<sup>4</sup>

Calcimimetics were developed to increase the sensitivity of CaR to calcium so that hyperparathyroidism may be controlled, especially in secondary hyperparathyroidism in chronic renal failure (Figure 21.2).<sup>5</sup>

Thus, magnesium plays a role in the mechanism of suppressive effect of calcium in controlling the secretion of PTH and in the action of calcimimetics.



**FIGURE 21.1.** Effect of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Gd}^{2+}$ , and neomycin on  $[\text{Ca}^{2+}]_i$  in HuCaR-HEK293. Hu CaR-HEK 293 cells loaded with fura2 were suspended in buffer containing 1 mM CaV1.2. The cells were treated with indicated concentrations of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Gd}^{2+}$ , and neomycin. The percentage of maximal peak increase in  $[\text{Ca}^{2+}]_i$  was plotted. Each point is the mean  $\pm$  standard error (SE) of three experiments.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Gd}^{2+}$ , and neomycin increased  $[\text{Ca}^{2+}]_i$ . The  $\text{EC}_{50}$  values for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Gd}^{2+}$ , and neomycin were 0.9, 7.9, 0.032, and 0.06 mM, respectively.



**FIGURE 21.2.** Effect of NRS-568 on serum total magnesium and urinary total excretion. Single oral administration of NRS-568 (1, 3, 10, 30, or 100 mg/kg) decreased serum total magnesium (A) ( $\circ$ , vehicle; \*, 1 mg/kg;  $\triangle$ , 3 mg/kg;  $\diamond$ , 10 mg/kg;  $\times$ , 30 mg/kg;  $\square$ , 100 mg/kg). Single injection of NPS-568 (1 mg/kg) increased urinary magnesium excretion (B).

## Magnesium Intake and Osteoporosis

### *Effect of Magnesium Deficiency on Bone in Ovariectomized Rats*

Sprague-Dawley rats 9 weeks of age were ovariectomized and fed a calcium- and magnesium-free diet (AIM-93M) for 10 weeks, which included 3 weeks before ovariectomy. For the calcium group, whey calcium (at 1.09%, as this compound contains 28% as elemental calcium in this solution) was mixed, and for the magnesium group, magnesium sulfate at 0.64% (0.1% as magnesium) mixed in addition to calcium carbonate group ( $\text{CaCO}_3$ ) to which 0.79% calcium carbonate was administered. Each group consisted of 6 animals. Body weight, food consumption, and ingested water were estimated. Strength and toughness of femur were tested after sacrifice at the end of 10 weeks. Strength was tested by braking femur by pressing at three points of the femur. Toughness was tested by twisting femur.

Body weight showed steady increase until 10 weeks in the four groups of animals. There were no significant differences among the groups (Figure 21.3a). Significant increase was demonstrated in the strength between the control ovariectomized group (OVX) and two kinds of calcium groups (Whey and  $\text{CaCO}_3$ ) or the magnesium group (Figure 21.3b), although the increase was not so pronounced in magnesium group compared with the calcium groups. There were significant differences in the toughness of femur between OVX and two calcium groups, respectively. The difference in toughness between OVX and Mg, was not significant, although there was a slight increase in the value of power of toughness in the Mg group.

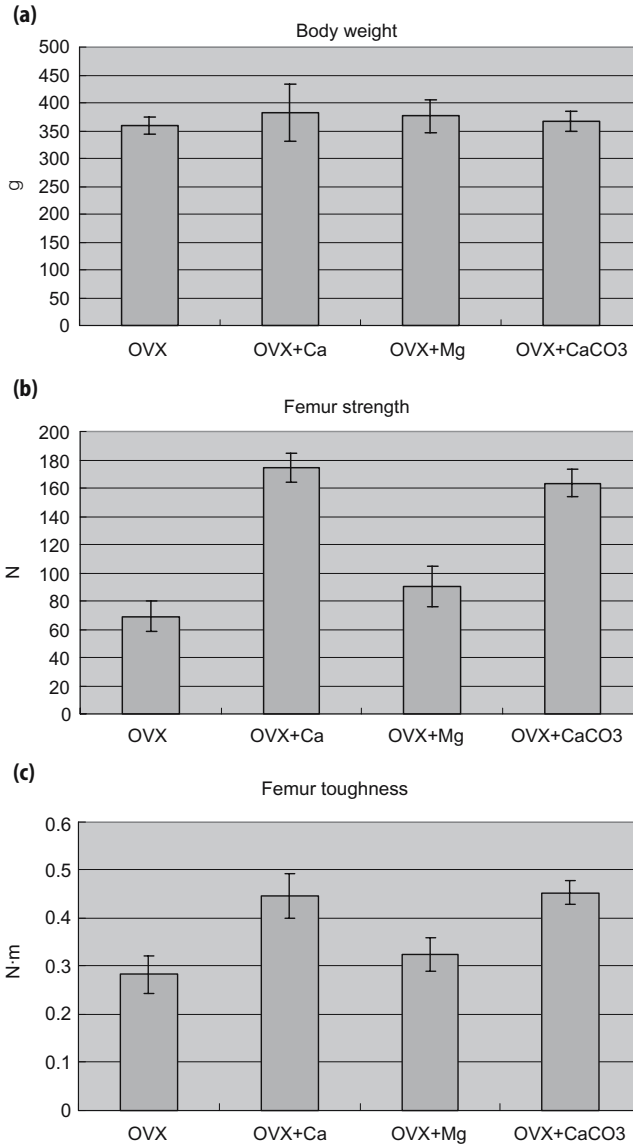
It may be concluded from this small study that calcium is effective in promoting bone strength and toughness in any form of the compound and that magnesium has similar effect as calcium, but the effect is rather permissive compared with the effect of calcium.

### *Magnesium Nutrition and Osteoporosis*

Rude published an interesting review article on magnesium deficiency as a risk factor for osteoporosis, collecting papers until 2000.<sup>6</sup> In his review article, Rude cited a paper in which magnesium intake was positively related to quantitative ultrasound properties of bone. Regarding the effect of magnesium on bone turnover markers, Rude stated that magnesium supplementation did not have effect on serum osteocalcin, bone-alkaline phosphatase, or urinary pyridinoline and deoxypyridinoline excretion. As to magnesium therapy in osteoporosis, Rude reported a significant increase in bone density of the proximal femur and lumbar spine in celiac sprue patients who received approximately 575 mg of magnesium per day.<sup>7</sup>

In experimental study, Rude showed in 2003 that in all magnesium-depleted mice hypomagnesemia developed and skeletal magnesium content fell significantly. Serum calcium increased but PTH was normal and osteoprotegerin did





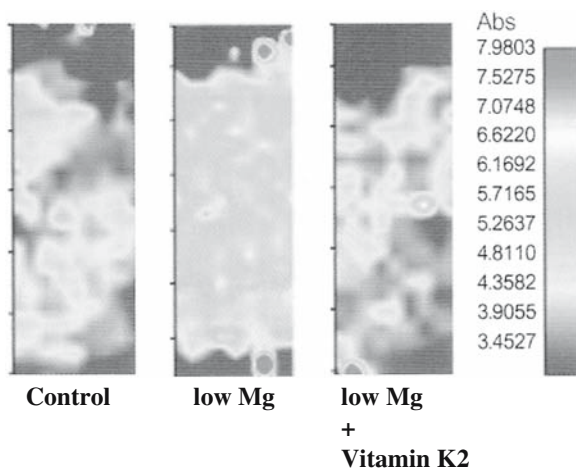
**FIGURE 21.3.** (a) Body weight in ovariectomized rats fed calcium- and magnesium-free diet for 10 weeks (OVX) after ovariectomy, added whey calcium (OVX+Ca), added magnesium sulfate (OVX+Mg), and added calcium carbonate (OVX+CaCO<sub>3</sub>). (b) Femur strength in OVX, OVX+Ca, OVX+Mg, and OVX+CaCO<sub>3</sub>. OVX versus OVX+Ca,  $p < 0.001$ ; OVX versus OVX+Mg,  $p < 0.05$ ; OVX versus OVX+CaCO<sub>3</sub>,  $p < 0.001$ . (c) Femur toughness in OVX, OVX+Ca, OVX+Mg, and OVX+CaCO<sub>3</sub>. OVX versus OVX+Ca,  $p < 0.001$ ; OVX versus OVX+CaCO<sub>3</sub>,  $p < 0.001$ .

not improve hypercalcemia. Serum calcium did not change after dietary calcium was reduced to 50% and the cause of hypercalcemia was supposed to be compensatory increase of intestinal absorption of calcium in magnesium deficiency. Growth plate was decreased, trabecular bone volume decreased, and osteoclast number increased. Substance P increased in megakaryocyte and lymphocyte, interleukin 1 (IL-1), and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) increased in osteoclasts.<sup>8</sup>

Jackson investigated the relationship between magnesium intake and hip, wrist/lower arm, and other clinical fractures in 89,717 postmenopausal women aged 50 to 79 years enrolled in WHI-OS (Women's Health Initiative Observational Study). It was shown that high magnesium intake does not confer a protective effect against fracture and, in fact, may increase the risk of wrist/lower arm fracture in the cohort.<sup>9</sup>

## Magnesium Deficiency and Vitamin K

There appeared interesting studies on the relationship between magnesium deficiency and vitamin K. It was demonstrated that the maximum load decreased and elasticity decreased in magnesium-deficient rats, in spite that cortical thickness and mineral contents of femur were preserved.<sup>10</sup> Kobayashi also showed mineral/matrix was higher in magnesium deficiency compared with controls using Fourier transform infrared microscopy (FTIRM), but that the ratio was reversed to normal by administration of menatetrenone (Figure 21.4).<sup>11</sup> In a further study Kobayashi measured amino acid composition in the



**FIGURE 21.4.** Mineral/matrix analyzed by FTIRM. Magnesium deficiency causes increased mineral/matrix but is reversed to the normal level by administration of menatetrenone.

femoral diaphysis. The total amino acid level in the magnesium-deficient rats was significantly lower than that in the normal rats. It was speculated that an increase in the mineral/matrix in the magnesium-deficient rat was associated with a decrease in the collagen area, but not with an increase in the mineral area.

## Summary and Conclusion

Magnesium plays an important role in the action of CaR, which regulates PTH secretion. Epidemiological studies showed inconclusive effect of magnesium deficiency in the pathogenesis and supplementation in the treatment of osteoporosis. Experimental studies demonstrated that magnesium deficiency causes osteoporosis and magnesium supplementation results in the amelioration of the disease. However, the effect of magnesium seems permissive compared with calcium in restoring bone fragility in calcium- and magnesium-deficient states. It was discovered that bone abnormalities due to magnesium deficiency would be compensated by menatetrenone in experimental animals.

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## Calcium-Sensing Receptor and Magnesium

Karl Peter Schlingmann

Divalent cation metabolism is a complex process involving the coordinated function of several organ systems and endocrine glands. The calcium-sensing receptor (CaSR) plays a central role for the homeostasis of both calcium and magnesium as it mediates not only the level of parathyroid hormone (PTH) secretion from the parathyroid, but also directly regulates the rate of divalent cation re-absorption in the kidney. Moreover, its expression in intestinal and bone cells suggests a role in all of the organs involved in maintaining systemic calcium and magnesium homeostasis. Several deactivating as well as activating CaSR mutations have been identified leading to hereditary hyperparathyroidism and autosomal-dominant hypocalcemia (ADH), respectively. Furthermore, the Bartter-like phenotype observed in patients with activating CaSR mutations demonstrates a functional link between renal calcium and magnesium handling and urine concentrating mechanism. This review focuses on the role of the CaSR for body magnesium homeostasis with special respect to renal magnesium handling and summarizes the diverse biological functions of the CaSR in regulating divalent mineral metabolism.

Magnesium is the dominant divalent intracellular cation and is essential for a variety of cellular processes such as enzyme function, DNA and protein synthesis, or the regulation of ion channels. Under physiological conditions, serum magnesium levels are maintained at almost constant values. Magnesium homeostasis primarily depends on the balance between intestinal absorption and renal excretion. Magnesium deficiency can result from reduced dietary intake, intestinal malabsorption, or renal loss. The control of body magnesium homeostasis primarily resides in the kidney. Of the magnesium filtered in the glomerulus, the vast majority is re-absorbed along the tubule. Whereas in the thick ascending limb of the loop of Henle (TALH), magnesium is reclaimed passively via the paracellular route, magnesium re-absorption in the distal convolute (DCT) involves active transcellular transport. Magnesium transport processes in both the TALH and the DCT sensitively respond to changes in body magnesium in order to maintain magnesium balance.

In contrast to calcium, there are no hormones specifically regulating magnesium homeostasis. Rather, magnesium metabolism is influenced by a number

of hormones primarily regulating calcium metabolism, renal salt and water handling, and glucose homeostasis, such as PTH, vitamin D<sub>3</sub> metabolites, and calcitonin, as well as ADH and aldosterone, and insulin and glucagone.<sup>1</sup> In addition to these hormonal stimuli, epithelial magnesium transport in gastrointestinal tract and kidney is directly influenced by extracellular magnesium and calcium levels. Furthermore, PTH secretion is regulated by extracellular calcium and magnesium in such a way that levels exceeding a certain threshold inhibit PTH secretion.

The molecular identity of the cellular sensor transducing the information of extracellular divalent cation levels to the interior of target cells was elucidated by Brown and coworkers, who cloned and characterized the CaSR.<sup>2</sup> The CaSR belongs to the family of G-protein-coupled receptors and bears several low-affinity cation binding sites in the extracellular domain, allowing the cooperative interaction with multiple cations at physiological, millimolar concentrations.<sup>3</sup> Mutations affecting the CaSR making it either less or more sensitive to calcium and magnesium cause various clinical disorders: Individuals heterozygous for inactivating mutations, that is, rendering the CaSR to be less sensitive to extracellular divalent cations, show familial hypocalciuric hypercalcemia (FHH), while carriers of inactivating mutations in the homozygous state exhibit severe neonatal hyperparathyroidism (NSHPT). Mutations causing increased sensitivity of the CaSR to extracellular divalent cations produce a hereditary form of hypoparathyroidism called ADH. In addition to the pathognomonic changes in calcium metabolism, patients also exhibit concomitant changes in magnesium homeostasis.

The aim of this review is to summarize the data from molecular genetic, biochemical, and electrophysiological studies on the diverse biological roles of the CaSR in regulating divalent mineral metabolism.

## Ca<sup>2+</sup>/Mg<sup>2+</sup>-Sensing Receptor

Modulation of PTH secretion from the parathyroid gland by extracellular calcium and magnesium involves the interaction with a specific cell-surface receptor. Experimental data had indicated the existence of such an divalent cation-sensing mechanism since raising extracellular calcium lead to the activation of phospholipase C, which in turn resulted in the accumulation of inositol trisphosphate and the release of calcium from intracellular stores.<sup>4</sup> Finally, Brown and coworkers used a functional cloning approach in *Xenopus* oocytes to clone the CaSR-cDNA from bovine parathyroid gland.<sup>2</sup> Subsequently, CaSR expression was demonstrated in different human tissues, including parathyroid, kidney, gastrointestinal tract, thyroidal C-cells, brain, and bone cells.<sup>5,6</sup>

The CaSR belongs to family C of the G-protein-coupled superfamily, which further includes eight metabotropic glutamate receptors, two GABA receptors, three taste receptors, and six orphan receptors.<sup>7</sup> Structural homologies and conservation of specific domains in some family members suggest an

evolutionary link between inorganic cation and amino acid sensing.<sup>8</sup> The deduced amino acid sequence of the CaSR protein shows the characteristic signature of all G-protein-coupled receptors with seven membrane-spanning domains, a very large extracellular N-terminal domain, and an intracellular carboxy terminus. Analysis of the amino acid sequence reveals potential sites for N-linked glycosylation, as well as phosphorylation by protein kinase C.<sup>5,9</sup> Biochemical analyses indicate that the CaSR mainly exists as a dimer on the cell surface with the two molecules covalently linked by disulfide bonds.<sup>10</sup> The extracellular domain of the CaSR does not contain any of the known high-affinity calcium binding motifs but instead bears several regions rich in negatively charged amino acids that allow the cooperative interaction with multiple cations in the physiological, millimolar concentration range.<sup>3</sup>

After ligand binding, CaSR signal transduction involves coupling through G proteins to at least three phospholipases, providing for coordinate, receptor-mediated regulation of multiple signal transduction pathways. Activation of phospholipase C (PLC) and consecutive mobilization of intracellular calcium appear to be the key mechanism by which the CaSR exerts its biological actions. Activations of phospholipases A<sub>2</sub> and D seem to be rather secondary events largely depending on prior PLC activation.<sup>11</sup>

Functional characteristics of wild-type CaSR, as well as various naturally occurring and genetically engineered mutants, have been studied extensively in heterologous expression systems including HEK293 cells and *Xenopus* oocytes.<sup>12</sup> CaSR activation is usually quantified via monitoring oscillations of intracellular calcium or via measurement of inositol trisphosphate production. Using this approach, increasing extracellular calcium yields a sigmoidal dose-response curve consistent with functional studies of native parathyroid CaSR in vivo and in vitro.<sup>5,9,13</sup>

Although calcium is regarded as the primary physiological ligand, other divalent and trivalent cations can also activate the receptor. In addition, CaSR activity is allosterically modulated by amino acids with a preference for aromatic and small aliphatic L-amino acids, as well as by structurally related phenylalkylamines called calcimimetics and calcilytics.<sup>14,15</sup>

Does magnesium serve as a physiologically relevant CaSR agonist in vivo? Studies on CaSR activation by extracellular magnesium reveal several interesting CaSR characteristics. Generally, magnesium is considered a partial agonist exerting an intrinsic activity of half to two thirds compared to that of calcium at equimolar concentrations.<sup>16,17</sup> However, it is still unclear whether this lesser potency is due to the cations capacity to interact with the receptor binding site(s) or due to other contributing factors. Due to the lack of direct binding assays, many studies used rather indirect techniques to study CaSR activation and measured downstream signals like inositol trisphosphate production, intracellular calcium release, or suppression of cyclic adenosine monophosphate (AMP) levels. It is well imaginable that addition of extracellular magnesium possibly exerts a number of additional effects on CaSR-expressing cells that interfere with CaSR-mediated intracellular events like blocking calcium

influx pathways.<sup>6</sup> Furthermore, cationic CaSR agonists ability to potentiate each other's action on the CaSR and CaSR sensitivity to magnesium greatly depends on extracellular calcium levels. In general, the activation of PLC and A<sub>2</sub> by the CaSR requires a certain extracellular calcium level with a threshold of around 1.5 mM. Accordingly, magnesium is substantially more potent in activating the CaSR in the presence of 1.5 mM than 0.5 mM calcium.<sup>16</sup> On the other hand, magnesium could potentially activate the CaSR simply by rendering it more sensitive to extracellular calcium.

Three serine residues (Ser147, Ser169, and Ser170) as well as one proline (P823) in the seven transmembrane region have been identified as pivotal for full responsiveness to extracellular calcium.<sup>17,18</sup> It remains to be clarified if CaSR activation by various cations (and especially magnesium) results in a transduction of distinctive intracellular signals, that is, by recognition via distinctive binding sites and/or activation of differing second messenger profiles. Yet, no binding sites specific for magnesium have been reported. However, Ruat and colleagues demonstrated that the relative degree of PLC and PLA<sub>2</sub> activation significantly differed depending on the cation activating the CaSR.<sup>16</sup>

A strong argument for the CaSR being a physiologically relevant magnesium sensor *in vivo* are the marked changes in magnesium metabolism observed in individuals with inactivating or activating CaSR mutations. These will be discussed below.

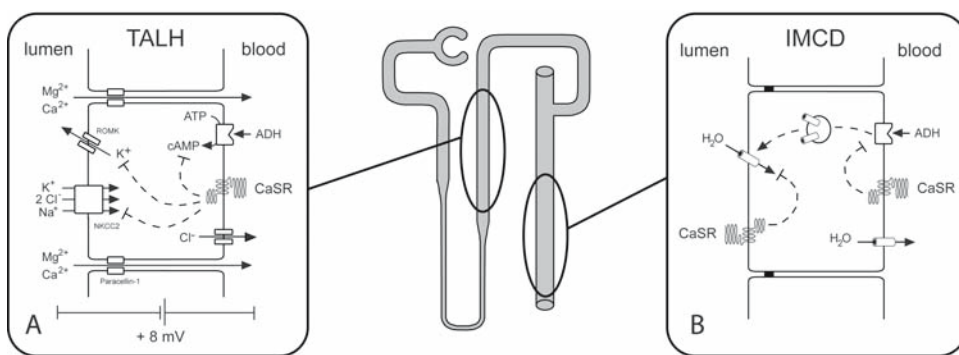
## Physiology of Renal Magnesium Handling

The kidney plays a central role in maintaining body magnesium homeostasis. Renal magnesium handling is a filtration–re-absorption process as there is little evidence for tubular magnesium secretion. Approximately 80% of total serum magnesium (the ultrafiltrable fraction) is filtered at the glomeruli, of which more than 95% is re-absorbed along the nephron. Magnesium re-absorption differs in quantity and kinetics depending on the different nephron segments. Five to 15% are re-absorbed by the convoluted and straight portions of the proximal tubule. The majority of filtered magnesium (70%–80%) is re-absorbed in the loop of Henle, especially in the cortical thick ascending limb (cTALH). Of the 10% to 15% of the filtered magnesium delivered to the DCT from the loop of Henle, 70% to 80% is re-absorbed so that approximately 3% of the filtered load normally appears in the urine. The re-absorption rate in the DCT defines the final urinary magnesium excretion rate as there is no significant re-absorption of magnesium beyond this nephron segment. However, magnesium transport processes in both the TALH and the DCT respond to changes in magnesium to effect sensitive control of magnesium balance.

Magnesium transport processes differ depending on the different nephron segments.<sup>19,20</sup> Magnesium re-absorption in the TALH is passive, occurring

through the paracellular pathway. Magnesium movement is influenced by electrostatic charges of tight junction proteins that comprise this route and specifically confer ion selectivity of the pathway to divalent cations. A paracellular protein, paracellin-1 or claudin-16, has been identified in the TAL that is involved in controlling magnesium and calcium permeability of the paracellular pathway. Mutations in *CLDN16* encoding paracellin-1 lead to a combined form of renal magnesium and calcium wasting, nephrocalcinosis, and progressive renal failure.<sup>21</sup> Paracellular divalent cation transport is driven by the lumen-positive transepithelial voltage, which is generated by transcellular salt re-absorption and potassium recycling across the apical membrane (Figure 22.1). Therefore, any influence that alters transepithelial voltage or the permeability of the paracellular pathway will alter magnesium re-absorption in the TALH.

In contrast, magnesium transport in the DCT is active and transcellular in nature. Physiological studies indicate that apical entry into the DCT cell is mediated by a specific and regulated magnesium channel driven by favorable transmembrane voltage.<sup>1</sup> Magnesium entry into the DCT cell is the rate-limiting step of transcellular magnesium re-absorption. The mechanism of basolateral transport into the interstitium is unknown. Magnesium has to be extruded against an unfavorable electrochemical gradient. Most physiological studies favor a sodium-dependent exchange mechanism. Driving force for magnesium entry into DCT cells and transcellular magnesium transport is the lumen-negative transepithelial potential in the DCT generated by transcellular sodium chloride re-absorption. A large number of hormones influence magnesium re-absorption within the DCT among others, including the



**FIGURE 22.1.** Effects of CaSR on electrolyte transport in TALH and IMCD. (A) TALH. Basolaterally expressed CaSR inhibits transcellular salt re-absorption upon activation by extracellular calcium and magnesium. Diminished active salt transport leads to a decrease in transepithelial lumen-positive potential, which represents the driving force for passive paracellular re-absorption of calcium and magnesium. (B) IMCD. Activation of basolateral CaSR by peritubular calcium and magnesium inhibits ADH-induced incorporation of AQP-2 water channels into the apical IMCD membrane. In addition, apically expressed CaSR specifically reduces ADH-elicited osmotic water permeability.



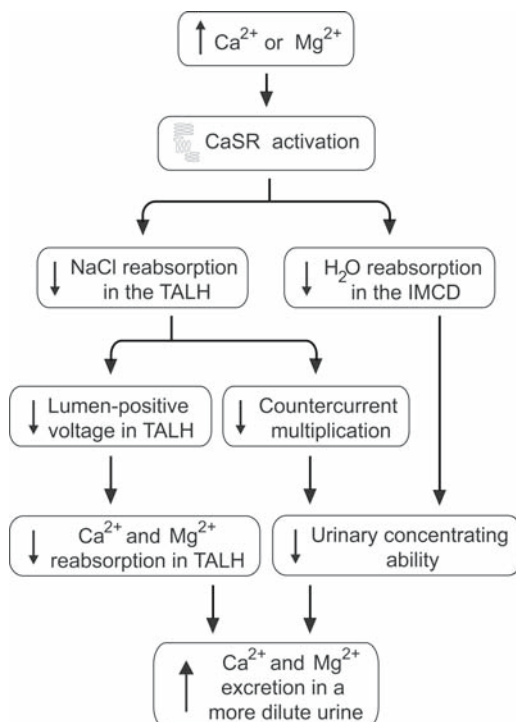
calcitropic hormones PTH, vitamin D<sub>3</sub>, and calcitonin, as well as ADH and aldosterone.<sup>1</sup> Interestingly, all of the hormones regulating transcellular magnesium transport in the DCT also influence paracellular magnesium transport in the TALH. Hormonal actions on magnesium transport in both nephron segments are mediated by changes in transepithelial voltage and paracellular permeability.<sup>19</sup>

## CaSR in the Kidney

Besides hormonal controls, the distal nephron is able to regulate magnesium uptake in direct response to diminished extracellular magnesium. The CaSR plays a crucial role in this regulatory process. The CaSR is expressed nearly along the entire nephron. Its highest expression level is at the basolateral membrane of the cTALH.<sup>22</sup> The CaSR's basolateral expression in this nephron segment is consistent with a model that it senses interstitial or serum calcium and magnesium levels and mediates a negative feedback control of divalent cation re-absorption via the paracellular pathway (Figure 22.1). The cellular events following an activation of the CaSR by extracellular magnesium and/or calcium are complex. Two effects have been attributed to account for the inhibitory effect of CaSR activation on paracellular calcium and magnesium transport in the TALH: (1) a decrease in divalent selective paracellular permeability is suspected; (2) a decrease in transepithelial lumen-positive voltage secondary to inhibition of transcellular salt transport.<sup>6</sup> Inhibition of salt transport is thought to occur indirectly via inhibition of the sodium-potassium-chloride cotransporter WKCC2 at the apical membrane (Figure 22.2). Intracellular signaling events following CaSR activation probably include a reduction in cAMP formation, activation of PLC, and/or stimulation of arachidonic acid production via PLA<sub>2</sub>.<sup>6</sup> Metabolites of arachidonic acid probably inhibit the apical potassium channel ROMK.<sup>23</sup> This functional coupling of inhibited salt transport and impaired divalent cation re-absorption in the TALH is also seen with furosemide administration and, furthermore, explains renal calcium and magnesium losses in patients with antenatal Bartter syndrome.<sup>20</sup>

Less is known about the functional consequences of CaSR activation in the DCT. CaSR activation is thought to interfere with hormone-stimulated calcium and magnesium uptake into DCT cells via inhibition of cAMP formation.<sup>24</sup> If there are also functional consequences of CaSR activation on transcellular salt re-absorption in the DCT remains to be clarified.

A further important role for renal salt and water handling is attributed to the CaSR in IMCD, where it is expressed both at the apical and basolateral membrane (Figure 22.1). Activation of CaSR results in a reduction of ADH-mediated aquaporin-2 (AQP-2) incorporation into the apical membrane and consecutively in reduced water permeability of the inner medullary collecting duct (IMCD).<sup>25</sup> The inhibitory



**FIGURE 22.2.** Integrative role of the CaSR in renal divalent mineral and water handling. In the presence of increased serum calcium and/or magnesium, CaSR activation leads to a reduction of divalent cation re-absorption in the TALH and to decreased water re-absorption in the IMCD. The net effect is an increased excretion of divalent minerals in a more dilute urine, preventing renal stone formation.

effect of CaSR on ADH-stimulated AQP-2 membrane trafficking is probably mediated by PLC activation and/or reduction in cAMP levels.<sup>3,25</sup>

It is proposed that the coordinated action of the CaSR in TALH and IMCD allows for a coordinate control of divalent mineral and body water metabolism and minimizes the risk of urinary tract stone formation in the face of an increase in calcium and magnesium excretion (Figure 22.2).<sup>26</sup>

Divalent cation-sensing mechanisms have been demonstrated in a number of other cell types in the body of which not all are directly involved in divalent cation metabolism. Control of osteoblast as well as osteoclast function by extracellular divalent cations should especially be mentioned. Elevations of extracellular divalents are able to directly inhibit osteoclast function<sup>27</sup> and stimulate osteoblast proliferation in cell cultures,<sup>28</sup> pointing to a role not only as substrate but also regulator of bone resorption and formation. However, the pharmacological profile of some of the cation-sensing mechanisms demonstrated *in vivo* and *in vitro* substantially differs from CaSR characteristics. In addition, CaSR knockout mice display an essentially normal skeletal

phenotype when preventing the deleterious effects of PTH.<sup>29</sup> These findings together point to the existence of additional molecules involved in the sensing of extracellular divalent cations, at least in bone. Indeed, additional molecules have been identified, including a ryanodine receptor, RyR-2, in osteoclasts as well as a novel G-protein-coupled receptor, GPRC6a, in osteoblasts as potential divalent cation sensors besides the CaSR.<sup>7,30</sup>

## Ca<sup>2+</sup>/Mg<sup>2+</sup>-Sensing Receptor–Associated Disorders

Several diseases associated with both activating and inactivating mutations in the CaSR gene have been described (Table 22.1). These genetic alterations of CaSR activity are presented here with a special focus on disturbances in magnesium metabolism.

Activating mutations of the CaSR result in ADH.<sup>31</sup> Patients typically manifest during childhood with seizures or carpopedal spasms. Laboratory evaluation reveals the typical combination of hypocalcemia and low PTH levels, but the majority patients also exhibit moderate hypomagnesemia with serum levels around 0.5 to 0.6 mmol/L.<sup>32</sup> Affected individuals are often given the diagnosis of primary hypoparathyroidism on the basis of inadequately low PTH levels despite their hypocalcemia. Serum calcium levels are typically in a range of 6 to 7 mg/dL. The differentiation from primary hypoparathyroidism is of particular importance because treatment with vitamin D can result in a dramatic increase in hypercalciuria and the occurrence of nephrocalcinosis and impairment of renal function in ADH patients. Therefore, therapy with vitamin D or calcium supplementation should be reserved for symptomatic patients with the aim of maintaining serum calcium levels just sufficient for the relief of symptoms.<sup>32</sup>

Activating CaSR mutations lead to a lower setpoint of the receptor or an increased affinity for extracellular divalent cations. This inadequate activation by physiological extracellular calcium and magnesium levels then results in

**TABLE 22.1.** CaSR-associated disorders.

CaSR-associated disorders	OMIM no.	Inheritance	Age at onset	Serum Mg <sup>2+</sup>	Serum Ca <sup>2+</sup>	Urine Mg <sup>2+</sup>	Urine Ca <sup>2+</sup>	Nephrocalcinosis	Renal stones
Autosomal-dominant Hypoparathyroidism (ADH)	601198	AD	Infancy	↓	↓	↑	↑– ↑↑	Yes <sup>a</sup>	Yes <sup>a</sup>
Familial hypocalciuric hypercalcemia (FHH)	145980	AD	Often asymptomatic	N to ↑	↑	↓	↓	No	?
Neonatal severe hyperparathyroidism (NSHPT)	239200	AR	Infancy	N to ↑	↑↑↑	↓	↓	No	?

<sup>a</sup>Frequent complication under therapy with calcium and vitamin D.

diminished PTH secretion and decreased re-absorption of both divalent minerals, mainly in the cTAL. For magnesium, the inhibition of PTH-stimulated re-absorption in the DCT may significantly contribute to an increased renal loss in addition to the effects observed in the TAL.<sup>33</sup>

Recently, a Bartter-like phenotype in patients with activating CaSR mutations has been described.<sup>33,34</sup> In addition to hypocalcemia and deficient PTH secretion, these patients developed renal salt and water loss associated with hypokalemic alkalosis. Furthermore, all patients were found to be profoundly hypomagnesemic. Functional expression of the underlying mutations revealed a complete CaSR activation under physiologic serum calcium concentrations (threshold for CaSR activation at  $<0.5$  mmol/L calcium).<sup>33,34</sup> As described above, this probably leads to an inhibition of salt re-absorption in the cTAL and a decrease in water permeability of the IMCD, resulting in renal salt and water losses with secondary hyperaldosteronism and hypokalemia.

Recently, mice with an activating CaSR mutation have been reported that display features of the human disease phenotype, including hypocalcemia, inappropriately low PTH levels, and ectopic calcifications.<sup>35</sup> However, urine calcium and magnesium levels were not significantly reduced compared to control animals and serum magnesium levels were found to be normal. Consistently, functional expression of the mutant CaSR in HEK293 cells displayed only a mild activation with a slight reduction of the  $EC_{50}$  for calcium (1.95 mmol/L compared to 2.25 mmol/L in wild-type mice).

Familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT) result from inactivating mutations present in either the heterozygous or homozygous (or compound heterozygous) state, respectively.<sup>36,37</sup>

Familial hypocalciuric hypercalcemia patients typically present with mild-to-moderate hypercalcemia, accompanied by few if any symptoms and often do not require treatment. Urinary excretion rates for calcium and magnesium are markedly reduced and serum PTH levels are inappropriately high. In addition, affected individuals also show mild hypermagnesemia, which has been attributed to an increase in tubular magnesium re-absorption in the distal nephron.<sup>38</sup>

In contrast, NSHPT patients with homozygous CaSR mutations usually present in early infancy with polyuria and dehydration due to severe symptomatic hypercalcemia. Unrecognized and untreated, hyperparathyroidism and hypercalcemia result in skeletal deformities, extraosseous calcifications, muscle wasting, and a devastating neurodevelopmental deficit. Early treatment with partial to total parathyroidectomy seems to be essential for outcome.<sup>39</sup> Data on magnesium metabolism in NSHPT patients is sparse. However, significant elevations of serum magnesium have been reported.<sup>38,40</sup> A Japanese patient has been reported with an inactivating CaSR mutation (P39A) in homozygous state that exhibits a mild phenotype without typical symptoms of primary hyperparathyroidism.<sup>41</sup> Next to moderate hypercalcemia, the patient shows pronounced hypermagnesemia ( $S_{Mg}$  1.36 mmol/L).

These findings concerning magnesium handling in patients with CaSR inactivation are also supported by observations in knockout mice, which carry a heterozygous or homozygous deletion of the *CaSR* gene. These mice also show significant hypermagnesemia therefore providing additional biological evidence for the CaSRs role in magnesium homeostasis.<sup>42</sup>

## CaSR Polymorphisms

In addition to numerous disease causing mutations in the *CaSR* gene, the NCBI database lists six non-synonymous single nucleotide polymorphisms. Three clustered polymorphisms in the C terminus of the CaSR, A986S, G990R, and Q1011E, are of special interest as they are common in the Caucasian population and have been linked to serum calcium levels in healthy probands.<sup>43,44</sup> The CaSR A986S polymorphism with an allele frequency of around 15% was especially considered a likely candidate locus for a genetic predisposition to various bone and mineral disorders.<sup>45</sup> However, subsequent studies in populations at risk for osteoporosis could not confirm this suspicion.<sup>46,47</sup> Unfortunately, there is no data on serum magnesium in these genotyped cohorts.

## Pharmacological CaSR Modulation

The discovery of the CaSR also led to the development of drugs that specifically alter its sensitivity to extracellular divalent cations that have now entered routine practice. Calcimimetics that amplify the sensitivity of the CaSR to extracellular calcium can suppress PTH levels with a resultant fall in serum calcium levels. Their therapeutic potential has been evaluated in patients with primary hyperparathyroidism as well as secondary hyperparathyroidism linked to renal disease. In contrast, calcilytics inhibit the effects of extracellular cations on CaSR, thereby increasing PTH secretion. Intermittent administration mimicking the cyclical pattern of endogenous PTH potentially promotes anabolic effects on bone that could be used for prevention and treatment of osteoporosis. Unfortunately, data on the effects of a pharmacological manipulation of the CaSR on magnesium metabolism is poor. However, changes in body magnesium paralleling those of calcium are expected. Although surely outweighed by the positive effects of a reduction of calcium phosphorus product on clinical outcome,<sup>48</sup> especially magnesium deficiency following calcimimetic administration, might be of clinical relevance in patients on hemodialysis. If calcilytics might exert beneficial effects on calcium and magnesium metabolism besides their anabolic effects on bone remains to be studied. Besides targeted pharmacological intervention, other polyvalent cationic drugs are able to activate the CaSR. For example, the renal wasting of calcium and magnesium, which is a common side effect of aminoglycosides like gentamicin, is attributed to an activation of the CaSR.<sup>49</sup>

## Conclusions

The discovery of the CaSR as a cellular sensor for extracellular divalent cations has greatly improved our understanding of divalent mineral metabolism. By CaSR activation, extracellular calcium as well as magnesium can directly serve as first messengers in and between a variety of tissues which, in turn, can directly respond to changes in ambient divalent cations. Extracellular calcium and magnesium are not only able to sensitively adapt the level of PTH secretion in the parathyroid but directly adjust the level of their excretion in the kidney by influencing tubular re-absorption processes. Moreover, the role of the CaSR along the kidney tubule provides insight into the close relationship between divalent mineral handling and renal concentrating mechanism. The phenotype of patients with inactivating and activating CaSR mutations that show profound changes in magnesium metabolism clearly indicates the importance of the CaSR as a magnesium sensor in vivo.

The role of the CaSR in tissues besides parathyroid and kidney is much less understood so far. Yet, the discovery of additional molecules sensing extracellular divalents demonstrates the complexity and differentiation of signaling pathways in response to extracellular divalents. Finally, drugs specifically altering CaSR sensitivity offer new therapeutic options for the treatment of primary and secondary forms of hyperparathyroidism, osteoporosis, and potentially further disorders involving disturbances in divalent cation metabolism.

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# Kidney

## 23

# Magnesium and the Kidney: Overview

Linda K. Massey

Normal human serum magnesium concentration is about 0.7 to 0.85 mmol/L. About 20% to 30% of serum magnesium is protein bound; this leaves 70% to 80% that is ultrafiltrable, which in turn is made up of 55% ionized magnesium and 15% magnesium complexed with anions such as phosphate and citrate. About 100 to 120 mmol per day is filtered, and about 4 to 6 mmol is excreted, so that about 95% of the glomerular filtrate is re-absorbed.<sup>1</sup>

The renal handling of magnesium in humans is a filtration–resorption process; there is no tubular secretion of magnesium. About 15% to 20% of the filtered magnesium is re-absorbed in the proximal convoluted tubule. Then approximately 65% to 75% of filtered magnesium is re-absorbed in the thick cortical ascending loop of Henle. Another 5% to 10% is re-absorbed in the distal convoluted tubule. Finally the collecting duct is only a minor site for magnesium re-absorption.<sup>2,3</sup>

Magnesium re-absorption in the proximal tubule appears to be passive. It follows changes in salt and water re-absorption and is associated with the rate of fluid flow. In the loop of Henle, there appears to be an additional active transport system; a decrease in magnesium re-absorption in this segment is independent of sodium chloride transport in either hypermagnesemia or hypercalcemia.<sup>2</sup> Recently, a protein, claudin 16, has been identified in the thick ascending loop of Henle (TALH) that forms a tight junction of the paracellular pathway.<sup>3,4</sup> Magnesium is re-absorbed in the distal convoluted tubule (DCT) through a transcellular, active transport process. In the DCT, magnesium transport into the cytosol is through selective channels and extrusion into the interstitium and is apparently mediated by a sodium/magnesium cotransporter. In vivo studies in animals and humans have demonstrated a tubular maximum for magnesium that reflects a composite of these tubular re-absorptive processes.

The role of the kidney in conserving magnesium during chronic mild magnesium deficiency is controversial. During acute experimental magnesium depletion in humans, urinary magnesium decreases to very low levels, less than 1 mmol/day within 3 to 4 days. However, the effects of long-term low dietary magnesium intake on renal conservation have been little studied. The few studies available are reviewed in the Dietary Reference Intakes.<sup>5</sup>

Despite the tight regulation of magnesium by the kidney, no one has described a dominant hormone or factor that is responsible for renal magnesium homeostasis. Because patients with either primary hyper- or hypoparathyroidism usually have normal serum magnesium concentrations and a normal tubular maximum for magnesium, it is unlikely that parathyroid hormone (PTH) is an important regulator of magnesium homeostasis. Although PTH increases magnesium re-absorption, the PTH-induced hypercalcemia opposes this increase in re-absorption. Glucagon, calcitonin, vasopressin, aldosterone, prostaglandins, insulin, and vitamin D also affect magnesium transport in the loop of Henle, but their physiological relevance is uncertain.<sup>2</sup>

The calcium-sensing receptor is also sensitive to magnesium, so an elevated serum magnesium concentration will decrease potassium movement into the lumen of the TALH, leading to a decreased lumen-positive voltage and a decrease in magnesium re-absorption.<sup>2</sup>

## **Diet and Drugs Affect Urinary Magnesium Excretion**

Any changes in the cotransport of sodium, chloride, and potassium, as well as active sodium re-absorption, results in changes in the transepithelial voltages in the loop of Henle, which will affect magnesium re-absorption. Although acutely increased dietary sodium chloride increases magnesium excretion, the chronic effects are largely unstudied.

Caffeine acutely increases urinary magnesium excretion relative to creatinine excretion for 9 h after its consumption.<sup>6</sup> Night time compensatory conservation was insufficient to offset these losses, resulting in a net 24-h urinary increase of 0.16 mm of magnesium. Adaptation of magnesium homeostasis to chronic consumption of caffeine is unstudied.

Diuretic therapy affects magnesium renal handling. Loop diuretics, such as furosemide, decrease magnesium absorption in the TALH by inhibiting sodium-potassium-chlorine movement into the cell, resulting in a diminished transepithelial voltage that leads to a decrease in both calcium and magnesium re-absorption. Whereas amiloride and tramterene increase magnesium transport in the DCT, chronic chlorothiazide use may result in renal magnesium wasting. These diuretics, which are used commonly in the treatment of hypertension, heart failure, and other edematous states, may cause hypermagnesuria, leading to possible hypomagnesemia and tissue magnesium deficiency.<sup>7</sup>

## **Diseases Impacted by Abnormal Urinary Magnesium**

Magnesium depletion is a common feature of diabetes mellitus, apparently related to glycemic control. Djurhuss and colleagues<sup>8</sup> directly showed the hypermagnesuric effect of elevated blood glucose by infusing 200% glucose into 10 patients with type 1 diabetes. Blood glucose increased from 5.3 to

12.3 mmol/L, but urinary glucose was only slightly increased. Hyperglycemia increased renal magnesium excretion and clearance 2.4-fold over a euglycemic day. Plasma magnesium decreased 3% during hyperglycemia. This study clearly separated the influence of plasma insulin from that of blood glucose, which proved to be the major effector of renal magnesium loss. This finding does not negate the additional role of insulin in magnesium metabolism, including the distribution of magnesium in cells versus plasma.

Low urinary magnesium is a risk factor for calcium oxalate nephrolithiasis. Magnesium acts as a calcium antagonist in oxalate binding. Magnesium oxalate is far more soluble than calcium oxalate, so reduces the saturation limit of calcium oxalate in the blood. Daily urines with a magnesium output of less than 2.0 mmol/day are the most likely to result in a risk index above the saturation limit for calcium oxalate.<sup>9</sup> The cause of low urinary magnesium in otherwise healthy stoneformers is likely to be dietary magnesium deficiency.

The extensive use of non-potassium-sparing diuretics predisposes many patients with chronic heart failure to magnesium deficiency. With reduced renal function, re-absorption of filtered magnesium is reduced in chronic renal failure, which may lead to magnesium deficiency.<sup>7</sup>

There are a number of primary inherited disorders of magnesium re-absorption, which are generally associated with disorders in calcium transport.<sup>4,10,11</sup> Cole and Quamme<sup>12</sup> have described extensively the five most characterized. First there is hypomagnesemia with secondary hypocalcemia, an autosomal-recessive disease segregating with chromosome 9q12–22.2. Second, an autosomal-dominant hypomagnesemia caused by isolated renal magnesium wasting that maps to chromosome 11q23. Third, a recessive hypomagnesemia with hypercalciuria and nephrocalcinosis caused by a mutation of the claudin 16 gene (3q27) that codes for a tight junction protein that regulates paracellular  $Mg^{2+}$  transport in the loop of Henle. Fourth, an autosomal-dominant hypoparathyroidism, a variably hypomagnesemic disorder caused by mutations in the extracellular  $Ca^{2+}/Mg^{2+}$ -sensing receptor, the *Casr* gene at 3q13.3–21. Finally, Gitelman's syndrome presents with renal magnesium wasting and is associated with mutations in the chlorothiazide sensitive NaCl cotransporter expressed in the distal convoluted tubule (SLC12A3 at 16q13).

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# 24

## Cellular Basis of Magnesium Transport

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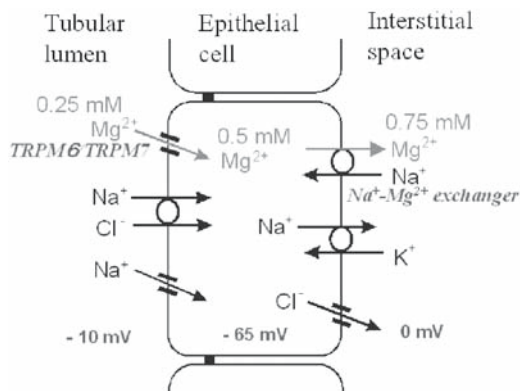
About 40% of magnesium that is contained in food and drinking water is absorbed from the gastrointestinal tract. On the other hand, the kidneys are the most important for control of body magnesium balance through its urinary excretion, which is primarily regulated by tubular re-absorption. The amount of magnesium excretion in the urine is regulated by hormones and other factors, and can vary widely.

Total serum magnesium levels lie in the range of 0.7 to 1.1 mM (i.e., 1.4–2.2 mEq/L), about 30% of which is bound to proteins (mainly albumin) and the rest (70%) is in the ionized form ( $\text{Mg}^{2+}$ ). Because magnesium molecules bound to macromolecules (such as proteins) do not permeate glomerular membranes, about 80% of serum magnesium is ultrafilterable. While the glomerular filtrate flows through the renal tubules, more than 95% of filtered magnesium is re-absorbed, and the rest is excreted in the urine; 15% to 20% of magnesium in the glomerular filtrate is re-absorbed in the proximal tubules, 65% to 75% is re-absorbed in the loop of Henle, and 5% to 10% is re-absorbed in the distal tubules.<sup>1</sup> Little absorption occurs in the collecting ducts. Fractions of magnesium re-absorbed in various portions of renal tubules are different from those for other ions; for example, about two thirds of sodium is re-absorbed in proximal tubules.

Epithelial cells of renal tubular walls are connected to each other by tight junctions, through which some solutes and water can permeate (the paracellular pathway). Transport through the paracellular pathway is passive; that is, it is driven by the difference of electrochemical potential between the tubular lumen and interstitial fluid. The other pathway is transcellular and active: movement of substances across luminal and basolateral membranes through epithelial cells. A large fraction of filtered magnesium is re-absorbed in the loop of Henle and the distal tubules, and re-absorption in these portions is thought to play important roles in regulation of urinary magnesium excretion. For example, it is known that re-absorption in the loop of Henle and the distal tubules increases in magnesium deficiency, and decreases when the serum magnesium level is elevated.

In the loop of Henle, the thick ascending limb (TAL) plays a key role in magnesium re-absorption. In the TAL, about 65% to 75% of ultrafiltered magnesium is re-absorbed passively through paracellular pathway.<sup>2</sup> The  $Mg^{2+}$  flux is thought to be driven by the potential difference because potential of the luminal side is slightly more positive than that of the basolateral side. Various hormones, such as parathormone, calcitonin, glucagons, and vasopressin, stimulate magnesium re-absorption in the TAL by an increase in the potential difference across the tubular walls or an increase in  $Mg^{2+}$  permeability of tight junctions.<sup>3</sup> The regulatory mechanisms of paracellular permeability are not fully understood, but paracellin-1, a tight junction protein, seems to provide an important clue. Familial renal hypomagnesemia is a hereditary disease that causes low serum magnesium levels through an abnormally increased urinary excretion of magnesium. By analyzing genes of families with this disease, Simon and colleagues<sup>4</sup> found various mutations of a gene, *CLDN16*, which encodes a protein localized in renal tight junctions. This protein, paracellin-1 or claudin-16, consists of 305 amino acids and has four putative transmembrane domains. The function of paracellin-1 is not yet clear, but it is speculated that the protein somehow contributes to a selective paracellular conductance by building a pore permitting fluxes of magnesium (and calcium). Lowering the  $Mg^{2+}$  permeability of tight junctions by mutations of paracellin-1 would cause a massive magnesium loss into the urine.<sup>4</sup>

In the distal convoluted tubules (DCT), 5% to 10% of filtered magnesium is re-absorbed.<sup>2</sup> In contrast to the case of the TAL described above, re-absorption in the DCT is thought to be transcellular and active.<sup>3</sup> Magnesium ion passively enters the cell through the luminal membrane (driven by a negative intracellular potential) and actively extruded across the basolateral membrane (Figure 24.1). For passive entry in the luminal side,  $Mg^{2+}$ -permeable channels have



**FIGURE 24.1.** A scheme of transcellular magnesium re-absorption in the distal convoluted tubules. Magnesium passively enters into tubular cells through TRPM6 and/or TRPM7 channels localized in the luminal membrane, and is actively extruded from the cells by the  $Na^+/Mg^{2+}$  exchanger localized in the basolateral membrane.



been postulated, and recent studies highlight two members of the transient receptor potential (TRP) channel superfamily as candidates for a physiological  $\text{Mg}^{2+}$  entry pathway<sup>5</sup>: TRPM6 and TRPM7 channels that permeate divalent cations, including  $\text{Mg}^{2+}$ . Schlingmann and colleagues<sup>6</sup> and Walder and colleagues<sup>7</sup> showed that primary hypomagnesemia with secondary hypocalcemia, a rare familial disease in which excess urinary excretion of magnesium leads to low serum magnesium levels, is caused by mutations of the *TRPM6* gene that encodes the TRPM6 channel. It was subsequently shown that the TRPM6 channel is localized in the DCT of kidneys, as well as the small intestine and colon, sites of transcellular re-absorption of magnesium.<sup>8</sup> TRPM7, the closest homologue of TRPM6, with 52% homology of the amino acid sequence, is ubiquitously distributed, and appears to have a central role in  $\text{Mg}^{2+}$  uptake in cells.<sup>9,10</sup> Both TRPM6 and TRPM7 channels permeate  $\text{Mg}^{2+}$  and are regulated by low intracellular  $\text{Mg}^{2+}$  concentration ( $[\text{Mg}^{2+}]_i$ ).<sup>8,11</sup> It is not clear, however, if TRPM6 alone can form functional channels. Some investigators have suggested the possibility of the assembly of functional complexes of TRPM6 and TRPM7.<sup>2,2</sup>

On the basolateral side, on the other hand, extracellular  $\text{Na}^+$ -dependent  $\text{Mg}^{2+}$  efflux (the putative  $\text{Na}^+/\text{Mg}^{2+}$  exchange) has been postulated as an active  $\text{Mg}^{2+}$  extrusion mechanism.<sup>13,14</sup> Although the transport has been characterized functionally in renal tubular cells and other types of cells, the transporter molecules are not yet identified.

In this chapter, we will focus on the properties of the putative  $\text{Na}^+/\text{Mg}^{2+}$  exchange of the basolateral membrane, based on our recent works on renal tubular cells and cardiac myocytes.

## **$\text{Na}^+/\text{Mg}^{2+}$ Exchange in Renal Tubular Cells**

Although the  $\text{Na}^+/\text{Mg}^{2+}$  exchange has been postulated as an active  $\text{Mg}^{2+}$  transport in the basolateral membrane, the transporter molecules are not yet identified, hindering further studies on molecular mechanisms of the transport. As the first step towards molecular cloning of the  $\text{Na}^+/\text{Mg}^{2+}$  exchanger, we established a mutant cell line from mouse cortical renal tubular (MCT) cells that could grow in culture media with very high extracellular  $\text{Mg}^{2+}$  concentration ( $[\text{Mg}^{2+}]_o$ ) through stepwise increases of  $\text{Mg}^{2+}$  concentration in the culture media and selection of high  $\text{Mg}^{2+}$ -tolerant cells.<sup>15</sup>

The standard culture medium containing 1 mM  $\text{Mg}^{2+}$  for MCT cell culture was replaced by media containing various concentrations of  $\text{Mg}^{2+}$  and incubated replacing the medium with fresh media twice a week. In a medium containing 41 mM or a lower concentration of  $\text{Mg}^{2+}$ , cells showed no apparent changes in their shape or growth rate. However, approximately 80% of the cells in the 61 mM  $\text{Mg}^{2+}$ -containing medium and all the cells in 81 mM or higher  $\text{Mg}^{2+}$ -containing medium died within 7 days. The surviving cells in 61 mM  $\text{Mg}^{2+}$ -containing medium started dividing thereafter, and the cells reached confluence 21 days after increasing the  $\text{Mg}^{2+}$  concentration. The cells were then

dissociated with trypsin and diluted 10-fold into fresh medium. After passages in medium containing 61 mM  $\text{Mg}^{2+}$  for 6 weeks, the culture medium was changed to medium containing 71 mM  $\text{Mg}^{2+}$ . Thereafter, the  $\text{Mg}^{2+}$  concentration of the medium was further increased every 10 to 12 weeks in increments of 10 mM. Massive cell death was observed when  $\text{Mg}^{2+}$  concentration was elevated from 71 mM to 81 mM and from 81 mM to 91 mM. The cells that had adapted to 81 mM  $\text{Mg}^{2+}$  and 101 mM  $\text{Mg}^{2+}$  could be dislodged and stored in 10% dimethylsulfoxide and 90% fetal calf serum (FCS) in liquid nitrogen, with 20% to 50% of viability at retrieval. Mouse cortical renal tubular cells could be adapted in Delbecco's modified Eagle's medium (DMEM) containing as high as 121 mM  $\text{Mg}^{2+}$  without losing their growing capacity.

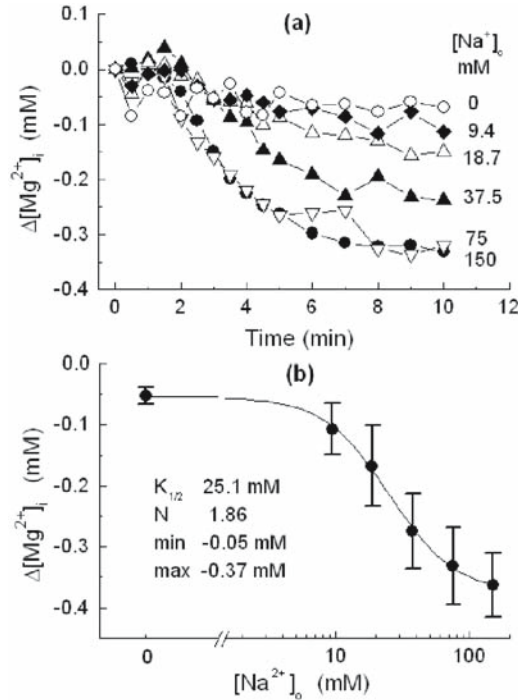
We measured  $[\text{Mg}^{2+}]_i$  with a fluorescent indicator fura-2 (Mag-fura-2) in clusters of wild-type cells and high  $\text{Mg}^{2+}$ -tolerant cells. In the presence of 150 mM extracellular  $\text{Na}^+$ ,  $[\text{Mg}^{2+}]_i$  in the highly  $\text{Mg}^{2+}$ -tolerant cells was significantly lower than that in the wild-type cells either at 1 mM or 51 mM  $[\text{Mg}^{2+}]_o$ . In the absence of extracellular  $\text{Na}^+$ , however, there was no significant difference in  $[\text{Mg}^{2+}]_i$  between the high  $\text{Mg}^{2+}$ -tolerant cells and wild-type cells.

To evaluate the rate of  $\text{Mg}^{2+}$  efflux in the high  $\text{Mg}^{2+}$ -tolerant and the wild-type cells, the rates of change in  $[\text{Mg}^{2+}]_i$  upon reduction of  $[\text{Mg}^{2+}]_o$  from 51 mM to 1  $\mu\text{M}$  were compared between the high  $\text{Mg}^{2+}$ -tolerant cells and wild-type cells. In the presence of 150 mM extracellular  $\text{Na}^+$ , reduction of  $[\text{Mg}^{2+}]_o$  to 1 mM caused a decrease in  $[\text{Mg}^{2+}]_i$  with the average rate much greater in the high  $\text{Mg}^{2+}$ -tolerant cells than in the wild-type cells. The decrease in  $[\text{Mg}^{2+}]_i$  was largely diminished in the absence of extracellular  $\text{Na}^+$  in both cell types, indicating the  $\text{Na}^+$ -dependent  $\text{Mg}^{2+}$  extrusion activity.

To analyze the  $\text{Na}^+$  dependence of the  $\text{Mg}^{2+}$  efflux, we measured the rate of decrease in  $[\text{Mg}^{2+}]_i$  in the high  $\text{Mg}^{2+}$ -tolerant cells at various extracellular  $\text{Na}^+$  concentration ( $[\text{Na}^+]_o$ ). Extracellular  $\text{Na}^+$  accelerated, in a concentration-dependent manner, the rate of decrease in  $[\text{Mg}^{2+}]_i$  induced by reduction of  $[\text{Mg}^{2+}]_o$  from 51 mM to 1 mM (Figure 24.2). The data were explained by a Hill-type curve with a Hill coefficient of about 2 and half activation at 25 mM  $[\text{Na}^+]_o$ .

We also examined the effects of imipramine, a known inhibitor of the  $\text{Na}^+/\text{Mg}^{2+}$  exchange in erythrocytes<sup>16,17</sup> and cardiac myocytes,<sup>18,19</sup> on the rate of decrease in  $[\text{Mg}^{2+}]_i$  at 150 mM  $[\text{Na}^+]_o$ . Imipramine slowed the decrease in  $[\text{Mg}^{2+}]_i$  in a concentration-dependent manner with a half-inhibitory concentration between 50 to 200  $\mu\text{M}$ , the range roughly comparable to reported  $\text{IC}_{50}$  values of the agent for putative  $\text{Na}^+/\text{Mg}^{2+}$  exchange in red blood cells and cardiac myocytes.<sup>16,17,19</sup>

The  $\text{Na}^+/\text{Mg}^{2+}$  exchange activity is observed in the wild-type MCT cells, and the transport probably serves as a physiological  $\text{Mg}^{2+}$  extrusion pathway. In the high  $\text{Mg}^{2+}$ -tolerant cells, the greatly enhanced activity of the active  $\text{Mg}^{2+}$  transport prevents increase in  $[\text{Mg}^{2+}]_i$  to high levels and is likely responsible for the  $\text{Mg}^{2+}$  tolerance. This mutant cell line may be useful to identify  $\text{Mg}^{2+}$



**FIGURE 24.2.** Effect of extracellular  $Na^+$  on the rate of changes in  $[Mg^{2+}]_i$  ( $\Delta[Mg^{2+}]_i$ ) in the high  $Mg^{2+}$ -tolerant cells. (a) Results from a series of experiments carried out on the same subculture. Extracellular  $Mg^{2+}$  was reduced from 51 mM to 1 mM at time zero in the presence of various  $Na^+$  concentrations: 150 mM (●), 75 mM (▽), 37.5 mM (▲), 18.8 mM (▼), 9.4 mM (◆), and 0 mM (○). Each symbol represents data obtained from a different cell cluster. (b)  $\Delta[Mg^{2+}]_i$  values measured in six series of experiments were plotted as a function of  $[Na^+]_o$ . Each symbol represents a mean  $\pm$  standard deviation (SD) of four cell clusters. A smooth line indicates the least squares fit by a Hill-type curve with parameters shown in the panel:  $\Delta[Mg^{2+}]_i = \text{min} + (\text{max} - \text{min}) \times [Na^+]_o^N / (K_{1/2}^N + [Na^+]_o^N)$ , where min and max denote, respectively,  $\Delta[Mg^{2+}]_i$  values at 0 and saturating  $[Na^+]_o$ ,  $N$  is the Hill coefficient, and  $K_{1/2}$  is  $[Na^+]_o$  that gives a midpoint value of  $\Delta[Mg^{2+}]_i$  between min and max. (Source: Reproduced from Watanabe et al.<sup>15</sup> by copyright permission of the American Physiological Society.)

transporter molecules and to understand molecular mechanisms of  $Na^+/Mg^{2+}$  exchange.<sup>15</sup>

## Characteristics of $Na^+/Mg^{2+}$ Exchange

Because significant leakage of the indicator from MCT cells hampered long-term measurements of  $[Mg^{2+}]_i$  in clusters of the small number of cells, we further studied detailed characteristics of the  $Na^+/Mg^{2+}$  exchange transport using cardiac ventricular myocytes as a model system of the transcellular

magnesium transport.<sup>20,21</sup> Cardiac myocytes are advantageous for accurate measurements of  $[Mg^{2+}]_i$  with optical signals, because the indicator fluorescence signals with good signal-to-noise ratio can be stably measured from single cells for a period of many hours, and fluorescence intensity ratios of the indicator can be converted to  $[Mg^{2+}]_i$  using the calibration curve constructed in the myocytes.<sup>22</sup> After the myocytes were loaded with  $Mg^{2+}$  by exposure to high  $[Mg^{2+}]_o$ , reduction of  $[Mg^{2+}]_o$  to 1 mM (in the presence of extracellular  $Na^+$ ) induced a decrease in  $[Mg^{2+}]_i$ . The rate of decrease in  $[Mg^{2+}]_i$  was higher at higher initial  $[Mg^{2+}]_i$ . We further calculated changes in total magnesium concentration to obtain quantitative information on  $Mg^{2+}$  flux across the cell membrane by utilizing concentration and dissociation constant values of known cytoplasmic  $Mg^{2+}$  buffers. The calculations indicated that, in the presence of extracellular  $Na^+$ ,  $Mg^{2+}$  efflux is markedly activated by cytoplasmic  $Mg^{2+}$  with a half-maximal activation of about 1.9 mM.<sup>20</sup> Thus, the  $Na^+/Mg^{2+}$  exchange activity is negligible at basal  $[Mg^{2+}]_i$ , and is strongly activated by small elevations of  $[Mg^{2+}]_i$ . On the other hand, raising  $[Mg^{2+}]_o$  slowed the decrease in  $[Mg^{2+}]_i$  with 50% reduction of the rate at about 10 mM  $[Mg^{2+}]_o$ .<sup>20</sup>

Dependence of the  $Na^+/Mg^{2+}$  exchange on intracellular and extracellular concentrations of  $Na^+$  was also studied in cardiac myocytes.<sup>21</sup> The myocytes were loaded with  $Mg^{2+}$  by exposure to high  $[Mg^{2+}]_o$  either in the presence of 106 mM  $Na^+$  plus 1 mM ouabain ( $Na^+$  loading) or in the presence of only 1.6 mM  $Na^+$  to deplete the cells of  $Na^+$  ( $Na^+$  depletion). The initial rate of decrease in  $[Mg^{2+}]_i$  from the  $Mg^{2+}$ -loaded myocytes were compared between the cells loaded with  $Na^+$  and those depleted with  $Na^+$ . Average  $[Na^+]_i$ , when estimated from fluorescence signals of the  $Na^+$  indicator SBFI, increased from 12 mM to 31 mM and 47 mM after  $Na^+$  loading for 1 and 3 h, respectively, and decreased to near 0 mM after 3 h of  $Na^+$  depletion. The intracellular  $Na^+$  loading significantly reduced the initial rate of decrease in  $[Mg^{2+}]_i$  on average, by 40% at 1 h and by 64% at 3 h, suggesting that the  $Mg^{2+}$  transport was inhibited by intracellular  $Na^+$  with 50% inhibition of the rate at about 40 mM. Extracellular  $Na^+$  concentration dependence of the rate of  $Mg^{2+}$  efflux revealed that the  $Mg^{2+}$  transport was activated by extracellular  $Na^+$  with half-maximal activation at 55 mM, a value somewhat higher than that obtained in the MCT cells.<sup>15</sup> Thus, the  $Mg^{2+}$  extrusion transport critically depends on extracellular  $Na^+$  for its activity, and elevated intracellular  $Na^+$  can strongly inhibit the transport.<sup>21</sup> These results are consistent with the putative  $Na^+/Mg^{2+}$  exchange as the primary transport mechanism for active extrusion of cellular  $Mg^{2+}$ .

Regarding the stoichiometry of the exchange, recent experiments estimated 1  $Na^+/1 Mg^{2+}$  (37°C)<sup>18,23</sup> or 1 to 2  $Na^+/1 Mg^{2+}$  (25°C).<sup>24</sup> However, more complex stoichiometries involving ions other than  $Na^+$  and  $Mg^{2+}$  have also been proposed. First, based on the  $Ca^{2+}$  dependence of noradrenaline-induced  $Mg^{2+}$  efflux observed in rat ventricular myocytes, Romani and colleagues<sup>25</sup> postulated that the  $Na^+/Ca^{2+}$  exchanger might play a role in the  $Mg^{2+}$  transport. Tashiro and colleagues<sup>26</sup> experimentally showed that  $Na^+$ -dependent  $Mg^{2+}$  transport activity developed after overexpression of the  $Na^+/Ca^{2+}$  exchanger

in CCL39 cells, suggesting that  $\text{Mg}^{2+}$  is transported by the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger at least under some experimental conditions. Later quantitative analysis, however, raised the question of the physiological significance of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger for cellular  $\text{Mg}^{2+}$  transport.<sup>27</sup> Furthermore, Rasgado-Flores and coworkers<sup>28</sup> found that  $\text{K}^+$  and  $\text{Cl}^-$  were involved in extracellular  $\text{Mg}^{2+}$ -dependent  $\text{Na}^+$  efflux in squid giant axons, and proposed the putative  $\text{Na}^+-\text{K}^+-\text{Cl}^-/\text{Mg}^{2+}$  exchanger that carries 1  $\text{Mg}^{2+}$  in exchange for 2  $\text{Na}^+$ , 2  $\text{K}^+$ , and 2  $\text{Cl}^-$ .<sup>29</sup>

To explore possible roles of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger and the putative  $\text{Na}^+-\text{K}^+-\text{Cl}^-/\text{Mg}^{2+}$  exchanger, we tested if the rate of  $\text{Mg}^{2+}$  efflux was modified by intracellular and extracellular concentrations of  $\text{Ca}^{2+}$  (expected for the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger)<sup>30</sup> and  $\text{K}^+$  and  $\text{Cl}^-$  (expected for the  $\text{Na}^+-\text{K}^+-\text{Cl}^-/\text{Mg}^{2+}$  exchanger).<sup>31</sup> We measured the  $[\text{Mg}^{2+}]_i$  of rat ventricular myocytes with the fluorescent indicator fura-2, and estimated the initial rate of  $\text{Mg}^{2+}$  efflux, as described above, with varied intracellular and extracellular concentrations of  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ . The initial rate of  $\text{Mg}^{2+}$  efflux was essentially unchanged by the addition of extracellular  $\text{Ca}^{2+}$  up to 2 mM.<sup>30</sup> Heavy intracellular loading of a  $\text{Ca}^{2+}$  chelator, either BAPTA or dimethyl BAPTA, by incubation with its acetoxy-methyl ester form (5  $\mu\text{M}$  for 3.5 h) did not significantly change the initial rate of  $\text{Mg}^{2+}$  efflux.<sup>30</sup>

Extracellular or intracellular, or both, concentrations of  $\text{K}^+$  and  $\text{Cl}^-$  were modified, while membrane potential was set at  $-13\text{ mV}$  with amphotericin B-perforated patch clamp technique.<sup>31</sup> None of the following conditions significantly changed the efflux rate: (1) changes in  $[\text{K}^+]_o$  between 0 mM and 75 mM; (2) intracellular perfusion with  $\text{K}^+$ -free ( $\text{Cs}^+$ -substituted) solution from the patch pipette in combination with removal of extracellular  $\text{K}^+$ ; or (3) extracellular and intracellular perfusion with  $\text{K}^+$ -free and  $\text{Cl}^-$ -free solutions.<sup>31</sup> It is thus likely that  $\text{Mg}^{2+}$  is transported in exchange with  $\text{Na}^+$ , but not with  $\text{Ca}^{2+}$ ,  $\text{K}^+$  or  $\text{Cl}^-$ , in mammalian cardiac myocytes.

The effects of intracellular and extracellular concentrations of  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$  and  $\text{Cl}^-$  have been studied (Table 24.1). Among these ion species, the

**TABLE 24.1.** Effects of extracellular and intracellular ions on  $\text{Na}^+/\text{Mg}^{2+}$  exchange (25°C).

Ion	Extracellular	Intracellular
$\text{Na}^+$	Required for activity <sup>15,21</sup> ( $K_a = 25\text{--}55\text{ mM}$ )	Inhibition <sup>21</sup> ( $K_i = 40\text{ mM}$ )
$\text{K}^+$	No effect <sup>31</sup> (0–75 mM)	No effect <sup>31</sup> ( $\text{Cs}^+$ replacement)
$\text{Mg}^{2+}$	Inhibition <sup>20</sup> ( $K_i = 10\text{ mM}$ )	Required for activity <sup>20</sup> ( $K_a = 1.9\text{ mM}$ )
$\text{Ca}^{2+}$	No effect <sup>30</sup> (0–2 mM)	No effect <sup>30</sup> (Inhibition by overload?)
$\text{Cl}^-$	No effect <sup>31</sup> (0–142 mM)	No effect <sup>31</sup> ( $\text{Ms}^-$ replacement)

Abbreviations:  $K_a$ , concentration for half-maximal activation;  $K_i$ , concentration for 50% inhibition;  $\text{Ms}$ , methanesulfonate.

Mg<sup>2+</sup> extrusion appears to require only intracellular Mg<sup>2+</sup> and extracellular Na<sup>+</sup> for its activity, while clear inhibition of the Mg<sup>2+</sup> transport is associated only with extracellular Mg<sup>2+</sup> and intracellular Na<sup>+</sup>. These results do not support complex exchange stoichiometries that involve ion species other than Na<sup>+</sup> and Mg<sup>2+</sup> in mammalian cardiac myocytes. The simple Na<sup>+</sup>/Mg<sup>2+</sup> exchanger appears to be functionally distinct from any other transporters so far identified in mammalian cells. Further studies are needed to determine the exchange stoichiometry of Na<sup>+</sup> and Mg<sup>2+</sup>.

## Conclusions

It has been shown that TRPM6 and TRPM7 channels open when [Mg<sup>2+</sup>]<sub>i</sub> decreases to low levels,<sup>8,11</sup> and that Na<sup>+</sup>/Mg<sup>2+</sup> exchange is activated when [Mg<sup>2+</sup>]<sub>i</sub> increases above its basal level.<sup>20</sup> It is thus tempting to speculate that [Mg<sup>2+</sup>]<sub>i</sub> plays a pivotal role as a regulator of Mg<sup>2+</sup> re-absorption in renal tubules; Mg<sup>2+</sup> influx through apical TRPM channels raises [Mg<sup>2+</sup>]<sub>i</sub>, which then activates the Na<sup>+</sup>/Mg<sup>2+</sup> exchanger to extrude Mg<sup>2+</sup> through the basolateral membrane. However, more quantitative information needs to be accumulated to describe how TRPM channels and the Na<sup>+</sup>/Mg<sup>2+</sup> exchanger constitute a physiological Mg<sup>2+</sup> re-absorption pathway.

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# 25

## Magnesium in Chronic Renal Failure

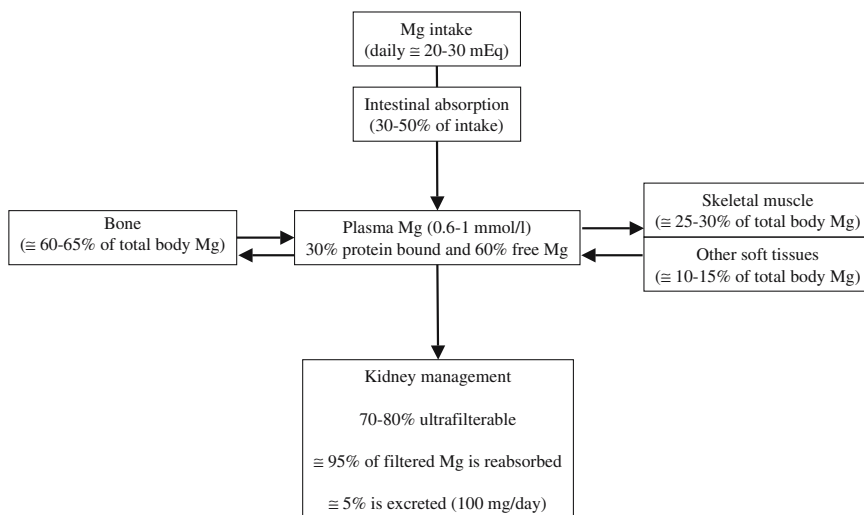
Juan F. Navarro and Carmen Mora-Fernández

Magnesium (Mg) is the fourth most abundant cation in the body. An average adult contains approximately 25 g (2000 mEq) of Mg.<sup>1,2</sup> Of the body's Mg, the vast majority of this ion is in the intracellular compartment (99%), and the remaining 1% is in the extracellular fluid.<sup>3</sup> The principal site of intracellular Mg is bone (60%–65%) where two distinct pools, cortical and trabecular, have been described. It is thought that Mg forms a surface constituent of the hydroxyapatite mineral component. Initially, much of this magnesium is readily exchangeable with serum and therefore represents a moderately accessible magnesium store, which can be drawn on in times of deficiency. Approximately 25% to 30% is localized within the skeletal muscle, and about 10% to 15% in other non-muscle soft tissues.<sup>4,5</sup>

The majority of the intracellular Mg is bound to several chelators, such as citrate, proteins, adenosine diphosphate (ADP) and triphosphate (ATP), RNA, and DNA. Only 5% to 10% is free, which is essential for the regulation of the intracellular Mg content.<sup>6</sup> The normal total plasma Mg concentration ranges from 0.62 to 1.02 mmol/L, where about 5% to 10% is complexed as salts (bicarbonate, citrate, phosphate, sulphate), 30% is protein bound, and 60% is present as free Mg ions, the biologically active form.<sup>7,8</sup> Magnesium functions as a cofactor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes.

### Magnesium Homeostasis

Magnesium balance, like that of other ions, is a function of intake and excretion (Figure 25.1). Magnesium is widely distributed in plant and animal foods, and geochemical and other environmental variables rarely have a major influence on its content in foods. Most green vegetables, legume seeds, peas, beans, and nuts are rich in magnesium, as are some shellfish, spices, and soya flour, all of which usually contain more than 500 mg/kg fresh weight. Although most unrefined cereal grains are reasonable sources, many highly refined flours,



**FIGURE 25.1.** Schematic representation of magnesium homeostasis.

tubers, fruits, and fungi and most oils and fats contribute little dietary magnesium (<100 mg/kg fresh weight). Corn flour, cassava and sago flour, and polished rice flour have an extremely low magnesium content. The average daily intake of magnesium is approximately 20 to 30 mEq (240–365 mg), and in order to maintain an optimal balance, a daily intake of 0.5 to 0.7 mEq/kg is critical.

Negligible amounts of Mg are absorbed in the stomach. The major site of digestive absorption of Mg is the small intestine, particularly in the proximal portions, but also occurs in the ileum as well as in the colon.<sup>9,10</sup> Absorption may involve active transport, facilitated diffusion, and a passive process by the electrochemical gradient and the solvent drag.<sup>11</sup> Under normal conditions, 30% to 50% of the ingested Mg is absorbed,<sup>12</sup> but gastrointestinal absorption can adapt to altered intake. Thus, under low Mg intake approximately up to 80% of Mg can be absorbed,<sup>9</sup> while only 25% is absorbed when intake is high.<sup>13</sup>

Scarce investigations have analyzed the intestinal absorption of Mg in chronic renal failure (CRF), with great controversy in the results. Early reports indicated that there were not significant differences in net Mg absorption between normal subjects and CRF patients.<sup>14,15</sup> Randall<sup>16</sup> reported only slightly reduced absorption of Mg in chronic renal disease, while other authors found average net absorption rates ranging between 17% to 38%.<sup>17,18</sup> Strictly controlled balance studies showed that the average net absorption of Mg in patients with CRF was significantly lower than the absorption in subjects with normal renal function,<sup>12</sup> results that have been confirmed using radioisotope techniques, even when age was taken into consideration.<sup>19</sup> Factors determining the reduced intestinal Mg absorption are not completely known. However, the

absorption of Mg in the jejunum has been demonstrated to be dependent on vitamin D, and that administration of 1-25-dihydroxyvitamin D<sub>3</sub> to patients with CRF resulted in an increased intestinal absorption of Mg.<sup>20</sup> Furthermore, successful renal transplantation is able to restore the impaired Mg absorption in these patients.<sup>16</sup> Therefore, deficient synthesis of the active metabolite of vitamin D by the kidneys may play a significant causative role in Mg malabsorption in CRF.

Another aspect of interest regarding Mg absorption in the context of renal disease is the effect of protein intake, because protein restriction is widely recommended in patients with renal insufficiency. Early works by McCance and colleagues<sup>21</sup> demonstrated that diets containing 0.7 to 0.9 g of protein/kg body weight were associated with a magnesium absorption of 32%, whereas when the diets contained 2.3 to 2.6 g of protein/kg body weight, magnesium absorption was 41%. These initial results were confirmed by later studies, indicating that when intake of protein is low, the intestinal absorption of Mg is reduced. All these studies were performed in subjects with normal renal function, and therefore, no data are available about the relationship between protein intake and intestinal Mg absorption in patients with CRF.

The kidney plays a critical role in Mg homeostasis and in determining the concentration of Mg in the extracellular fluid. Approximately 70% to 80% of plasma Mg is ultrafilterable, with about 2 g of Mg filtered daily. Under normal circumstances, 95% of the filtered load of Mg is re-absorbed by the kidney, and only 5% (100 mg) is excreted in the urine.<sup>1,22</sup> Magnesium transport differs from that of most other ions in that the proximal tubule is not the major site of re-absorption, because only 15% to 25% of the filtered Mg is re-absorbed in the proximal tubule. Micropuncture studies have showed that most of this re-absorption (60%–70%) occurs at the thick ascending limb of the loop of Henle, whereas the distal tubules and collecting ducts play a limited role in Mg handling.<sup>23,24</sup> Plasma Mg concentration is a major determinant of the renal handling. During significant hypermagnesemia, the kidney can excrete up to 70% of the filtered load. In contrast, during Mg deficiency the kidney can compensate by reducing the Mg excreted in the urine to less than 0.5% of the filtered load.<sup>25</sup> Furthermore, active re-absorption of magnesium has been reported to be influenced by acid–base balance.<sup>26</sup> Thus, diverse studies have showed that dietary changes which result in increased urinary pH and decreased titratable acidity are associated with reduced urinary magnesium output by 35%, despite marked increases in dietary magnesium input from vegetable protein diets.<sup>27</sup>

In CRF, although urinary Mg excretion may be normal or even increased in many patients with glomerular filtration rate (GFR) lower than 30 mL/min, it is usually low in advanced renal insufficiency.<sup>28</sup> As renal function deteriorates, the fraction of filtered Mg excreted increases as a result of impaired tubular re-absorption, which becomes more marked when GFR is below 10 mL/min. However, this compensatory rise of fractional Mg excretion is insufficient, and therefore the serum Mg concentration increases.

Another aspect of interest is that increased dietary calcium, particularly if associated with high sodium intakes, contributes to enhance urinary Mg output and to a shift toward negative Mg balance.<sup>26,29</sup> A close relationship between the urinary excretion of sodium and calcium and Mg has been also observed in patients with advanced CRF.<sup>30</sup> Thus, whereas in patients with polycystic kidney disease and a GFR higher than 20 mL/min there was no relationship between sodium and Mg or phosphate excretion, in patients with a GFR below 20 mL/min, the excretion of calcium, Mg, and phosphate was significantly related to that of sodium. These findings suggest that impairment of tubular re-absorption of these electrolytes is the result of a common mechanism.<sup>31</sup>

## Serum Magnesium Levels and Tissue Magnesium Content in Chronic Renal Failure

Because renal capacity for Mg elimination is impaired due to the reduction of the net Mg filtration, intestinal absorption will always exceed renal excretion despite a compensatory increase in the fractional excretion of Mg as renal function deteriorates. Thus, dietary Mg intake is a major determinant of serum and total body Mg levels because no other alternative routes for Mg excretion are available in patients with CRF.

Until severe reductions of GFR (<30 mL/min, chronic kidney disease stage 4), serum Mg levels are usually normal.<sup>32,33</sup> With lower rates of renal function, serum Mg is increased, with a greater rise of total one than that of ionic Mg. The typical patient with end-stage renal disease has a plasma Mg concentration between 1 to 1.5 mmol/L, but severe, symptomatic, and even fatal hypermagnesemia can be induced by the exogenous administration of antacids or laxatives containing Mg in usual therapeutic doses.<sup>34,35</sup> Other situations that have been reported to produce a rise in serum Mg are the administration of thiazide diuretics [an effect attributed to extrarenal mechanisms, such as the potentiation of parathyroid hormone (PTH) action on bone],<sup>36</sup> and treatment with 1- $\alpha$ -hydroxyvitamin D<sub>3</sub>.<sup>37</sup>

Hypermagnesemia (serum Mg >1.02 mmol/L) is not infrequent in dialysis population. Saha and colleagues<sup>38</sup> found that both total and ionized serum Mg were significantly higher in hemodialysis patients compared to control subjects. In patients of our unit dialyzed with a standard dialysate Mg (0.75 mmol/L), the mean serum Mg concentration was 1.15 mmol/L, and moreover, 68% of subjects had hypermagnesemia.<sup>39</sup> In uremic patients on hemodialysis the serum Mg concentration parallels the dialysate Mg level because Mg readily crosses the dialysis membrane with its movement determined by the gradient between the concentration of diffusible Mg in blood and the level of Mg in dialysate. Several authors have reported that a reduction in the Mg dialysate produces a significant decrease in the serum Mg concentration as early as the first month.<sup>40–42</sup> In contrast, in another study, changing the dialysate Mg from

0.75 to 1.5 mmol/L the mean serum Mg concentration increased from 1.25 to 1.7 mmol/L.<sup>43</sup> These data demonstrate that dialysate Mg plays a critical role in maintaining magnesium homeostasis in end-stage renal disease patients treated with hemodialysis.<sup>44</sup>

In peritoneal dialysis (PD) patients, elevated serum Mg levels have been also reported,<sup>45</sup> which largely depends on the concentration of the ion in the dialysate. Ahsan Ejaz and colleagues<sup>46</sup> studied the evolution of serum Mg concentration in 33 patients dialyzed with a low Mg PD solution (0.5 mmol/L). All subjects had serum Mg levels in the normal or elevated range prior to institution of PD (0.65–1.15 mmol/L). However, after dialysis with a low Mg fluid, 64% of them developed hypomagnesemia. Similar findings have been recently reported by Hutchinson and coworkers.<sup>47</sup> These authors found that the serum Mg concentration in 12 patients on dialysis with a standard PD fluid (Mg content, 0.75 mmol/L) was 1.24 mmol/L. However, 1 month after changing to a reduced Mg formulation (0.25 mmol/L), the mean serum Mg decreased to  $0.89 \pm 0.04$  mmol/L ( $p < 0.001$ ). These data are in accordance with initial studies in continuous ambulatory PD patients showing that mild hypermagnesemia was common when Mg concentration in the dialysate was 0.75 mmol/L.<sup>48</sup>

Results about tissue Mg content in uremic patients have shown conflicting results. Evaluation of the concentration of Mg in different tissues, such as skeletal muscle, has been reported to be either low, normal, or elevated.<sup>33</sup> On the other hand, normal Mg levels have been noted in lymphocytes of hemodialysis patients, in spite of enhanced serum Mg concentrations.<sup>49</sup> On the contrary to these findings, increased bone Mg content is an universal finding, being this excess of Mg distributed in both the rapidly exchangeable and the nonexchangeable pools.<sup>4,33</sup> Contiguglia and coworkers<sup>50</sup> reported a mean increase of 66% in the Mg content of bone.

## Consequences of Hypermagnesemia: Implications for Renal Patients

Mild hypermagnesemia (plasma Mg lower than 1.5 mmol/L) is usually asymptomatic. When plasma Mg is between 2 to 3 mmol/L, drowsiness, lethargy, and diminished deep tendon reflexes can be observed. The most consistent complication of hypermagnesemia is neuromuscular toxicity, which is due to a reduction in the impulse transmission across the neuromuscular junction, and decreased tendon reflexes is the initial clinical manifestation.<sup>51</sup> Hypermagnesemia with serum Mg level between 3 to 5 mmol/L can result in somnolence, loss of deep tendon reflexes, hypocalcemia, and cardiovascular effects including hypotension, bradycardia, and electrocardiographic changes such as prolongation of the P-R interval, increase in QRS duration, and Q-T interval.<sup>52</sup> Finally, extreme hypermagnesemia with plasma Mg concentration above 5 mmol/L is associated with muscle paralysis, potentially leading to flaccid quadriplegia and apnea, complete heart block, and cardiac arrest.

**TABLE 25.1.** Consequences of hypermagnesemia and potential effects in chronic renal failure.

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Mild hypermagnesemia
Drowsiness
Lethargy
Hyporeflexia
Moderate hypermagnesemia
Somnolence
Areflexia
Hypocalcemia
Hypotension
Bradycardia
Electrocardiographic changes (prolongation of P-R and Q-T interval, increase in QRS duration)
Severe hypermagnesemia
Muscle paralysis
Quadraplegia
Apnea
Complete heart block
Cardiac arrest
Potential effects in chronic renal failure
Retarding vascular calcifications
Reduction of nerve conduction velocity
Increased pruritus
Mineralization defects
Contribution to osteomalacic renal osteodystrophy
Bone pain
Impairment of parathyroid gland function
Pathogenic factor for adynamic bone disease

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In patients with CRF, hypermagnesemia has been implicated in both deleterious and beneficial effects (Table 25.1).<sup>44</sup> Purposed benefits of hypermagnesemia included suppression of PTH<sup>53</sup> and retarding vascular calcifications.<sup>54</sup> In contrast, potential harmful effects include altered nerve conduction velocity<sup>55</sup> and increased pruritus.<sup>56</sup> Initial studies reported that dialysis against a low Mg dialysate had beneficial effects on uremic itch,<sup>57</sup> although later prospective double-blind controlled trials failed to show any improvement of pruritus after Mg-free dialysis.<sup>58</sup> However, the most significant consequences of elevated Mg levels in renal patients have been related to osseous metabolism.

## **Magnesium, Bone Disease, and Parathyroid Hormone in Chronic Renal Failure**

Progressive CRF is accompanied almost universally by abnormalities in bone structure, with multiple factors involved in the pathogenesis of this disorder. Based on the histological characteristics, bone disease in patients with renal

insufficiency can be divided in two broad groups: (1) disorders characterized by normal or increased bone turnover, such as osteitis fibrosa, mixed bone pathology and other forms of hyperparathyroid bone disease; and (2) disorders characterized by low rates of bone remodelling, such as osteomalacia and adynamic bone disease.<sup>59,60</sup>

Magnesium ions are not incorporated directly into the apatite mineral structure of bone, but are concentrated in the hydration shell around hydroxyapatite crystals forming part of the surface-bound ion complex.<sup>44,61</sup> This physicochemical property of Mg influences the release of Mg from bone and its availability when Mg absorption is restricted.<sup>61</sup> Magnesium concentration in the physiological level (Mg/Ca ratio between 0.004–0.04) do not interfere with the crystallization of amorphous calcium phosphate to hydroxyapatite.<sup>62</sup>

Since the early 1970s, a high bone Mg content in patients with renal disease has been consistently reported by different authors.<sup>4,33,50</sup> In 1986, Brautbar and Gruber outlined the hypothesis that increased bone Mg arises from elevated serum Mg, and that increased bone Mg plays a direct role in mineralization defects and the pathogenesis of renal osteodystrophy.<sup>61</sup> Thus, high bone Mg content has been implicated in the pathogenesis of osteomalacic renal osteodystrophy.<sup>63,64</sup> In vitro studies have demonstrated that bone Mg excess can inhibit the formation of hydroxyapatite crystals by two mechanisms: (1) by interference with calcium, which produces a decrease in the calcium concentration in the mediating solution and reduces the amorphous calcium phosphate solubility<sup>61,62,65,66</sup>; and (2) by combination of Mg with pyrophosphate, which results in an insoluble salt that is resistant to hydrolysis by pyrophosphatase.<sup>44,67</sup> Furthermore, supportive data on the potential noxious effects of an increased bone Mg content are also suggested by the beneficial effects of a reduction in the dialysate Mg concentration. Brunner and Thiel<sup>63</sup> observed the resolution of clinical symptoms, such as bone pain, as well as healing of osteomalacic fractures in dialysis patients after reduction of dialysate Mg level. Moreover, Gonella and coworkers<sup>64</sup> reported a significant improvement in bone histology by normalizing serum Mg after 1 year in hemodialysis with a low Mg dialysate.

Besides these direct effects of increased Mg on bone histology, another aspect of special interest is the influence of hypermagnesemia on parathyroid gland function. It is well established that PTH production is stimulated by hypocalcemia and decreased by elevated serum calcium levels, with an inverse correlation between serum calcium and PTH concentrations in CRF patients. However, conflicting data have been reported about the relationship between Mg and PTH. Investigations in animal models<sup>53</sup> and non-uremic patients<sup>68,69</sup> have showed evidence for suppression of parathyroid gland function by hypermagnesemia. However, severe hypomagnesemia has been associated with profound hypocalcemia in patients with CRF, which has been commonly attributed to impaired PTH release and/or peripheral resistance to its action.<sup>70–72</sup> In this situation, Mg replacement was associated in most cases with increase in PTH levels, despite a concomitant increase in serum calcium.<sup>70,72</sup> Important

information has been obtained from dialysis patients, as well as uremic patients treated with Mg salts.

Kenny and colleagues<sup>73</sup> suggested that a low Mg dialysate might be expected to have a suppressive effect on parathyroid gland function. However, most of studies failed to confirm this hypothesis. Several trials on the successful use of Mg salts to control hyperphosphatemia and hyperparathyroidism<sup>74–77</sup> support the hypothesis that hypermagnesemia may have a suppressive effect on PTH secretion in dialysis patients.<sup>78,79</sup> O'Donovan and coworkers<sup>74</sup> compared the evolution of patients treated with Mg carbonate respect to a control group on therapy with aluminum hydroxide. After 2 years, the authors observed a significant reduction in the mean serum PTH concentration in patients receiving Mg salts, but not in controls. Morinière and coworkers<sup>75</sup> evaluated the effects of the administration of Mg hydroxide in association with moderate doses of calcium carbonate. They found that plasma concentration of Mg progressively increased concomitantly with a progressive reduction of PTH. Delmez and colleagues<sup>76</sup> observed that the administration of Mg carbonate allowed a reduction in the doses of calcium carbonate without any increase in the serum PTH level. Furthermore, these findings are concordant with the variation of PTH after modifications of dialysate magnesium content. Parsons and colleagues<sup>80</sup> and Nilsson and colleagues<sup>81</sup> reported that the correction of raised serum Mg concentration in patients dialyzed against a low dialysate Mg fluid resulted in a significant increase in serum PTH. In contrast, MacGonicle and coworkers<sup>82</sup> observed that in hypermagnesemic patients a further rise in serum Mg caused a subsequent fall of PTH.

We have analyzed the serum Mg concentration in dialysis patients classified according their PTH level.<sup>39,45,83</sup> We observed that patients with inadequately low PTH (<120 pg/mL) had a serum Mg concentration significantly higher than patients with adequate (120–250 pg/mL) or high (>250 pg/mL) PTH. Moreover, we found a significant negative linear relationship between the serum concentrations of PTH and Mg. Similar findings have been reported by Saha and colleagues,<sup>84</sup> who also found a significant inverse correlation between intact PTH and serum Mg. Finally, this relationship between Mg and PTH is independent of other factors that can affect the PTH synthesis or secretion. Thus, plasma Mg has been demonstrated as a significant determinant of serum PTH concentration independently of calcium and phosphorus.<sup>39,45</sup>

In summary, serum Mg concentration is frequently elevated in CRF. In these patients, hypermagnesemia is usually mild, without relevant acute clinical symptoms, although further increased plasma Mg level may have relevant consequences, especially on bone metabolism. Hypermagnesemia can have direct effects on bone histology, playing a significant pathogenic role in osteomalacic renal osteodystrophy. On the other hand, diverse evidences have been reported for suppression of parathyroid gland activity by hypermagnesemia. Further studies are necessary to clarify the mechanisms of action of hypermagnesemia on parathyroid gland function in uremic patients.



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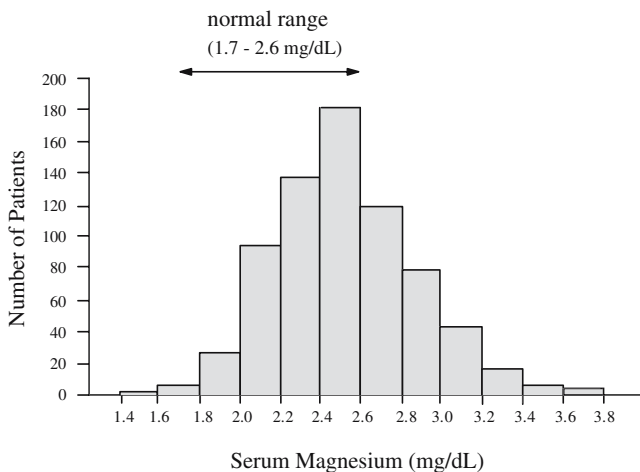
## Magnesium in Hemodialysis Patients

Senji Okuno and Masaaki Inaba

Magnesium (Mg) is the second most abundant intracellular cation and the fourth most abundant cation of the human body. Magnesium plays an essential role as a cofactor for a variety of enzymes, including those involved in several key steps of intermediary metabolism and phosphorylation. In addition, Mg is required for protein and nucleic acid synthesis, the cell cycle progression, cytoskeletal and mitochondrial integrity, and the binding of substances to the plasma membrane. Disorders of Mg homeostasis may lead to profound changes in the function and well being of the organism. Serum Mg concentration is normal in patients with early renal failure, but hypermagnesemia usually occurs in the advanced stage of renal failure due to the reduced urinary Mg excretion. Following the introduction of chronic hemodialysis or continuous ambulatory peritoneal dialysis (CAPD) treatment, the major factor to determine Mg balance is Mg levels in the dialysate. Patients with end-stage renal disease (ESRD) who are receiving dialysis may develop various complications including hypertension, atherosclerosis, dyslipidemia, and renal osteodystrophy. The disturbance of Mg balance in those patients maintained on dialysis may affect the development of these complications.

### Serum Magnesium Level

The total body Mg content is approximately 2000 mEq or 25 g. Like calcium (Ca), only a small fraction (about 1%–2%) of whole-body Mg store exists in the extracellular fluid compartment. Approximately 60% and 20% of total body Mg are found in bone and muscle, respectively. Circulating level of Mg is tightly controlled within narrow ranges between 1.7 to 2.6 mg/dL in healthy individuals. Although no single mechanism has been demonstrated for Mg homeostasis, the cellular availability of Mg is closely regulated by the kidney, gastrointestinal tract, and bone. On a normal dietary intake of Mg, urinary Mg excretion averages 100 to 150 mg/day. The kidney is able to respond rapidly to changes in Mg level in the extracellular fluid by modulating tubular Mg re-absorption. In patients receiving Mg-containing antacids, urinary Mg



**FIGURE 26.1.** Serum magnesium concentration in patients with maintained on hemodialysis ( $n = 714$ ). In 714 patients of our unit dialyzed with a standard dialysate Mg (1.0 mEq/L) the mean serum Mg concentration was 2.5 mg/dL, and moreover 35% of subjects had hypermagnesemia.

excretion can increase to 500 to 600 mg/day or more. Conversely, when dietary intake of Mg is restricted, urinary Mg excretion decreases to as low as 10 to 12 mg/day, indicating kidney as the major organ to maintain Mg homeostasis. Taken collectively, Mg disturbance may occur more easily as renal dysfunction progresses. However, the derangement of Mg homeostasis in chronic kidney disease has received less attention than that of Ca or phosphorus. Serum Mg level is usually normal in patients with early stages of renal failure, but hypermagnesemia becomes common in patients with progression of renal impairment (Figure 26.1). Hypermagnesemia becomes apparent when the glomerular filtration rate falls below 30 mL/min. Hypermagnesemia of chronic renal failure is usually asymptomatic, probably due to the gradual increase of serum Mg, although abrupt increase of serum Mg may occur when ESRD patients take Mg-containing antacids or laxatives.

Metabolic balance studies indicate that 25% to 60% of dietary Mg is absorbed. The preferential site of Mg absorption in the intestine may be different depending on the animal species studied. In humans, the temporal pattern of the appearance of radiolabeled Mg into the plasma following ingestion suggests that most of Mg absorption occurs in the small intestine. Although Mg shares with Ca the common pathways for intestinal absorption, most of the evidence suggests that Mg is absorbed mainly by ionic diffusion and solvent drag resulting from the bulk flow of water, in contrast to the fact that most of Ca absorption occurs by vitamin D-induced active transport at the gastrointestinal tract. The data available are not consistent with regard to whether the intestine is capable of modifying Mg absorption according to the Mg content of the diet. Although a few studies suggested that vitamin D and parathyroid hormone (PTH) may increase the intestinal absorption of Mg, other studies found no

effect. Whether intestinal absorption of Mg may be altered in uremia is controversial. Studies of net intestinal absorption of Mg in patients with uremia have yielded results that are either normal or only marginally decreased.<sup>1,2</sup> However, data from perfusion of different intestinal segments indicate that jejunal and ileal transport of Mg is reduced in uremic patients.<sup>3</sup> The reasons for the discrepancies between results obtained from intestinal perfusion studies and metabolic balance studies have yet to be determined.

The major factor in determining serum Mg level is a dietary Mg intake in ESRD patients who are not receiving dialysis, and Mg level in the dialysate in uremic patients undergoing dialysis. Mg readily crosses the dialysis membrane with its movement mainly determined by the gradient of diffusible Mg concentrations between blood and dialysate. The usual Mg level in the dialysate is 0.5 to 1.0 mEq/L. With the use of dialysate containing 1.5 mEq/L of Mg, serum Mg levels is increased up to 3.0 to 4.8 mg/dL. The use of dialysate containing 0.5 to 0.8 mEq/L of Mg results in serum Mg concentration near the normal upper limit or only slightly elevated. In the patients maintained on CAPD, the removal rate of Mg through peritoneal membrane can also be increased by lowering Mg level in the peritoneal dialysate. It was originally reported that CAPD patients showed mild hypermagnesemia when Mg level in peritoneal dialysate was 1.5 mEq/L. Because dialysate containing Mg level of 0.5 mEq/L are now commonly used, serum Mg level has become normalized with the body burden of Mg decreased.

Approximately 75% to 80% of the Mg in serum is ultrafiltrable, and the rest is bound to protein, mostly albumin. As the ultrafiltrable form of Mg in serum, Mg existed as ionized Mg, and in the form of complexes with small anions such as bicarbonate, citrate, and inorganic phosphate. It has been suggested that the relationship between ionized Mg and total circulating Mg is altered in dialysis patients, because Mg bind more to anionic compounds that accumulate in uremic conditions. However, the studies on the metabolism of the ionized Mg were conflicting in uremic patients. While some described a lower fraction of ionized Mg in uremic patients than in normal controls,<sup>4,5</sup> others did not.<sup>6,7</sup> This discrepancy could, in part, be attributed to analytical problems associated with the measurement of both total and ionized Mg.<sup>8</sup>

## Intracellular Level of Magnesium

Almost 99% of the body Mg exists inside the cells. It has been suggested that serum Mg levels poorly reflect the intracellular Mg content and that, in patients with renal failure, hypermagnesemia may be accompanied with normal or even depleted tissue Mg content. Muscle normally has 76 mEq of Mg per kilogram of fat-free solids, and much of this exists in a complex form with intracellular organic phosphate and protein. Erythrocytes contain normally about 4.6 mEq/L of Mg, of which 84% is thought to be complexed with adenosine triphosphate (ATP). As noted, bone is the principal source of Mg. The normal



Ca/Mg ratio in bone is 50, with the ratio consistently higher in trabecular bone than in cortical bone. Most of the Mg in bone exists in the form associating with apatite crystal rather than bone matrix. Approximately 30% of Mg in bone is present as a surface-limited ionized form on the bone crystal and thus is freely exchangeable. Investigation of the intracellular content of total Mg in uremic patients has given variable results in various tissues. In patients maintained on hemodialysis, Mg level in erythrocytes was found to be increased. The increase of Mg content in erythrocytes could solely reflect Mg retention due to high serum Mg, although other factors may contribute. An inverse correlation was found between erythrocytes Mg content and hematocrit in dialysis patients. Mg contents of erythrocytes appeared to be inversely related to the age of the cell, with the reticulocytes containing about two times more Mg than older erythrocytes. Therefore, the erythrocyte Mg content may not provide a reliable index of Mg status in dialysis patients. Magnesium content of muscle in ESRD patients has been reported to be either low, normal, or elevated. Normal Mg levels have been noted in lymphocytes of hemodialysis patients despite elevated serum Mg levels. In contrast, increased Mg content in bone has been a consistent finding in uremic patients.<sup>9</sup>

Nearly all intracellular Mg is bound or complexed with proteins or nucleic acids, or is confined within intracellular organelles. Intracellular ionized Mg, which constitutes only 1% to 10% of total intracellular Mg content, plays an important role in a wide range of cellular processes. These include energy production and utilization, protein and DNA synthesis, and membrane transport. Accordingly, under normal circumstances the intracellular levels of ionized Mg appears to be strictly regulated under tight homeostatic control. The main factors affecting intracellular levels of ionized Mg are the concentration of nucleotides and operation of transport systems in the plasma membrane and mitochondria.

Intracellular level of ionized Mg in dialysis patients is controversial. Kaupke and colleagues measured with molecular fluorescent probes intracellular level of ionized Mg in platelets from ESRD patients maintained on hemodialysis.<sup>10</sup> They found that ESRD patients exhibited significantly lower intracellular level of ionized Mg, despite higher serum Mg level, than the normal control group. Hemodialysis reduced serum Mg level without affecting intracellular level of ionized Mg. Nishida and colleagues reported that intracellular ionized Mg, measured with <sup>31</sup>P magnetic resonance spectroscopy, was normal in skeletal muscle of uremic patients.<sup>11</sup> Kister and coworkers determined cytosolic ionized Mg in lymphocytes from uremic patients and normal controls.<sup>12</sup> Serum total and ionized Mg concentrations were greater in uremic patient population, whereas the concentration of ionized Mg in lymphocytes was comparable in both groups. Huijgen and coworkers demonstrated that intracellular ionized Mg in mononuclear blood cells from hemodialysis patients was greater than that from the healthy population.<sup>13</sup> The reason for the discrepancy of these results is not well understood, but at least it may be attributed to different methods and tissues analyzed.

## Magnesium-Containing Phosphate Binder

Hyperphosphatemia is a common problem among ESRD patients. It is highly prevalent condition, since almost 40% of the hemodialysis population has serum phosphorus level greater than 6.0 mg/dL. Elevated serum phosphorus is associated with the progression of secondary hyperparathyroidism and metastatic calcification in soft tissue. Recently, Block and colleagues identified in multivariate analysis elevated serum phosphorus as an independent predictor for mortality.<sup>14</sup> Although 4-h hemodialysis treatment may remove about 1000 mg of phosphorus, this is generally inadequate to normalize phosphorus levels and/or phosphorus balance. Therefore, to completely re-establish normal level of phosphorus and its balance, dietary restriction and phosphate binders are also needed. Aluminum-based binders were originally used in the 1970s due to their efficient binding to phosphate. Patients receiving long-term therapy with aluminum-based binders were eventually recognized to be at risk for encephalopathy and bone disease associated with elevated aluminum levels in brain and bone tissue. In the 1970s and 1980s, concerns about aluminum toxicity led to a gradual decline in the use of aluminum-based phosphate binder. Although Ca salts are viewed as relatively benign and efficient binders of phosphate, ingestion of large amounts of Ca agents results in a large positive Ca balance, increasing the possibilities of hypercalcemia, progressive metastatic calcification, and adynamic bone disorders.<sup>15</sup>

Several investigators have evaluated the use of Mg-containing phosphate binders in dialysis patients. Fine and colleagues, in acute studies of normal subjects,<sup>16</sup> found a dose-dependent decrease in phosphorus absorption from 75% with placebo to 28% with 77 mEq/day of Mg acetate. Guillot and colleagues investigated the effect of  $\text{Mg}(\text{OH})_2$  as a phosphate binder in ESRD patients maintained on hemodialysis. Using doses of 0.75 to 3 g of  $\text{Mg}(\text{OH})_2$  per day for 3 to 5 weeks and dialysate Mg concentrations of 1.2 to 1.8 mg/dL, serum phosphorus decreased from 9.0 mg/dL to 8.1 mg/dL during the  $\text{Mg}(\text{OH})_2$  administration.<sup>17</sup> More favorable results were reported by O'Donovan and coworkers. They switched 28 patients undergoing hemodialysis from  $\text{Al}(\text{OH})_3$  to  $\text{MgCO}_3$  in combination with a Mg-free dialysate. Over the 2-year study period, Ca, phosphorus, and Mg levels were well controlled and not different from those during the control phase.<sup>18</sup> Delmez and coworkers examined the efficacy of  $\text{MgCO}_3$  as a phosphate binder in a prospective randomized crossover study.<sup>19</sup> The two therapeutic regimen were (1)  $\text{MgCO}_3$  plus half the usual dose of  $\text{CaCO}_3$  with a dialysate Mg of 0.75 mEq/L and (2)  $\text{CaCO}_3$  alone given in the usual dose with a dialysate Mg of 1.5 mEq/L. There was no difference in the serum concentrations of phosphorus, Ca, or Mg between the two different therapies. In addition, the  $\text{MgCO}_3/\text{CaCO}_3$  combination regimen permitted a greater amount of intravenous calcitriol to be used without the development of hypercalcemia. To administer oral Mg, serum Mg should have been lowered using the dialysate containing lower Mg concentration so as to avoid the development of hypermagnesemia. This is difficult to do in most units due to

centralized dialysate delivery systems. Further, frequent monitoring of serum Mg level is necessary. Because Mg-based binder is usually used at relatively high doses, side effects, such as diarrhea and hyperkalemia, may commonly occur. In the guidelines of the National Kidney Foundation-Dialysis Outcomes Quality Initiative (NKF/DOQI), the use of Mg-based phosphate binder may be justified only if all other compounds fail and the appropriate precautions are undertaken.

## Parathyroid Hormone Secretion

It is well known that PTH secretion is stimulated by hypocalcemia and decreased by hypercalcemia, and an inverse relationship between serum Ca and PTH levels is well established. A number of *in vivo* and *in vitro* studies have shown that Mg can modulate PTH secretion in a similar way to Ca. Perfusion of isolated parathyroid glands of experimental animals with varying concentrations of Mg showed that acute increases in extracellular Mg levels inhibit PTH secretion. The secretion of PTH from the parathyroid gland is controlled by the serum Ca via a specific G protein-coupled cell surface receptor, the Ca-sensing receptor (CaR). The CaR is not specific for Ca as it also responds to a variety of divalent and trivalent cations, including Mg, aluminum, and gadolinium, as well as polycations, all of which have in common a net positive charge at physiological pH. Because Mg is usually two- to three-fold less potent than Ca on a molar basis in suppressing PTH secretion, Mg may act as a partial agonist at the CaR. Although the characteristics of Mg-induced regulation of PTH secretion correlate well with the properties of Mg at the CaR, it is not clear whether the CaR is the only mediator of this regulation.

An increase in dialysate Mg concentration over 2 months produced a decrease in serum PTH, which was associated with a decrease in serum Ca and phosphorus levels.<sup>20</sup> Conversely, a reduction in dialysate Mg was associated with a marked increase in serum PTH levels. Navarro and colleagues reported that patients with low intact PTH had a higher serum Mg concentration than patients with normal or high intact PTH levels, and moreover, a negative linear correlation was observed between serum intact PTH and Mg concentration.<sup>21</sup> Based on these findings, sustained increase of serum Mg level decreases serum PTH levels, and it may have a role in the genesis of relative hypoparathyroidism and the resultant adynamic bone disease in dialysis patients.<sup>22</sup>

## Bone Metabolism

Renal osteodystrophy (ROD) is a common and difficult complication in dialysis patients. It is classified as either a high-turnover disorder, a low-turnover disorder, and a mixed bone disorder. Low-turnover disorders consist of adynamic bone disorder and osteomalacia, while high-turnover disorders consist

of osteitis fibrosa. The mechanisms of ROD development are multifactorial and complex, and include factors such as abnormality in Ca, phosphorus, PTH, and vitamin D levels. In addition to imbalances of hormone and ion, accumulation of trace elements such as fluoride, Mg, and aluminum may also influence bone quality in ROD because dialysis patients are unable to excrete excess elements.

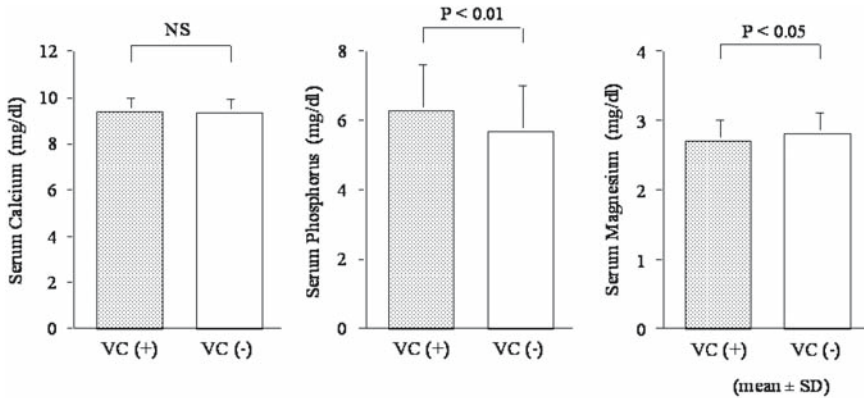
It has been suggested that Mg may inhibit the formation of apatite crystals and thereby inhibit bone formation *in vitro*. Thus, the increase in bone Mg content of dialysis patients may play a role in development of ROD.<sup>23</sup> However, Morinière and colleagues noted no significant changes in bone histomorphology after 8 to 20 months of  $\text{Mg}(\text{OH})_2$  therapy, during which time the serum Mg levels averaged 3.6 mg/dL.<sup>24</sup> Furthermore, Ng and coworkers demonstrated that the dialysis patients with adynamic bone disease had lower mean bone Mg content than those with osteomalacia or osteitis fibrosa.<sup>25</sup>

End-stage renal disease patients maintained on dialysis need serum level of intact PTH between two and five times the upper limit of the normal range to avoid both low- and high-turnover bone disease. Many investigators, who have studied the correlation between intact PTH level and bone biopsy finding, found that adynamic bone disease is associated with relatively low plasma PTH levels, that is, intact PTH in the normal range or slightly increased. In patients maintained on dialysis, PTH suppression induced by chronic hypermagnesemia may play a role in the pathogenesis of low-turnover ROD.

## Vascular Calcification

Vascular calcification has been recognized for many years as a common complication of chronic kidney disease. In hemodialysis patients, vascular calcification has already been related to increased arterial stiffening, high prevalence of cardiovascular disease, and cardiovascular mortality. Although the mechanisms responsible for vascular calcification are not well understood, abnormalities in mineral metabolism in general, and disturbances in phosphorus metabolism in particular, have traditionally been considered as important determinants.

It has been shown that Mg is a potent inhibitor of the calcification process and that experimental Mg deficiency appears to promote vascular calcification. It has also been suggested that high Mg intake may have an inhibitory effect on atherogenesis and acute cardiac ischemic attacks. Furthermore, oral administration of Mg hydroxide improved significantly the performance in patients with intermittent claudication. Meema and colleagues reported that serum Mg was significantly lower in dialysis patients with advanced arterial calcification than in those without.<sup>26</sup> Mitral annular calcification is common in hemodialysis patients, with prevalence varies from 9.5% to 52%. Tzanakis and colleagues reported that lower serum Mg is related with higher prevalence of cardiac valves calcifications among hemodialysis patients.<sup>27</sup> We examined



**FIGURE 26.2.** Comparison of serum calcium, phosphorus, and magnesium in 390 nondiabetic patients maintained by hemodialysis with or without vascular calcification (VC) in the hand plain X-P. Serum phosphorus was significantly higher and serum Mg was significantly lower in patients with VC than in patients without VC ( $6.3 \pm 1.3$  vs.  $5.7 \pm 1.3$  mg/dL in phosphorus and  $2.7 \pm 0.3$  vs.  $2.8 \pm 0.3$  mg/dL in Mg, respectively). (Source: Reproduced from Okuno et al.<sup>28</sup>)

the relationship between serum Mg concentration and vascular calcification of the hands in nondiabetic hemodialysis patients. Patients with vascular calcification showed their serum Mg concentration significantly lower than those without (Figure 26.2).<sup>28</sup> These observations support the notion that hypermagnesemia in dialysis patients may be a factor to retard the development of vascular calcification.

## Atherosclerosis

The prevalence of atherosclerotic coronary artery disease is increased in dialysis patients when compared with patients of similar age without renal impairment. In fact, cardiovascular disease is the most common cause of mortality among ESRD patients. The high prevalence of cardiovascular disease among patients on maintenance dialysis appears to result from a multiplicity of risk factors for atherosclerosis, such as hypertension, diabetes mellitus, hypercholesterolemia, and hyperhomocystinemia, rather than from dialysis per se. In keeping with the observation that atherosclerosis often is advanced in ESRD patients, some studies demonstrated an increased intima-media thickness at carotid artery and increased stiffening of these vessels in ESRD subjects when compared with patients of similar age without ESRD.<sup>29</sup>

A number of observations in both animals and humans suggest that Mg deficiency may be involved in the development of atheromatosis and vascular-related disease processes like stroke, peripheral artery disease, and ischemic heart disease. Oral supplementation of Mg salt to rabbits fed a high cholesterol diet lowered serum levels of cholesterol and triglyceride and resultantly atten-

uated the progression of atherosclerotic lesions in aortas and the intimal thickening.<sup>30</sup> Epidemiological studies suggested an inverse relationship between Mg content in drinking water and cardiovascular mortality. Lower level of tissue Mg is associated with greater occurrence of coronary heart disease. Gartside and colleagues reported an inverse relation between serum Mg and incidence of coronary heart disease.<sup>31</sup> Liao and coworkers also reported an inverse association in healthy middle-aged adults; relative risk of coronary heart disease across quartiles of serum Mg was 1.00, 0.92, 0.48, and 0.44 among women and 1.00, 1.32, 0.95, and 0.73 among men.<sup>32</sup> Amighi and colleagues found that low serum Mg levels were associated with an increased risk of neurological events in patients with symptomatic peripheral artery disease.<sup>33</sup> A few studies reported a correlation between Mg metabolism and atherosclerosis in dialysis patients. Tzanakis and colleagues showed that there was a negative linear correlation existed between intra- or extracellular Mg contents and intima-media thickness of common carotids in hemodialysis patients.<sup>34</sup> This observation suggested that Mg may play a protective role against the development and/or acceleration of arterial atherosclerosis in dialysis patients.

There is evidence enough to draw a conclusion on how low Mg is implicated in the pathogenesis of atherosclerosis. Magnesium is a biological competitor of Ca and inhibits its entry into the cells. Intracellular accumulation of Ca in smooth muscle as well as in vascular endothelial cells may induce vasospasm, endothelial dysfunction, arterial wall thickening, and, finally, atherosclerosis. Experimental and clinical data indicate that high serum Mg may result in decreased intracellular Ca and in attenuation of the above-mentioned consequences. In addition, Mg interferes with other mechanisms involved in atherosclerosis. It is noteworthy that Mg deficiency also stimulates the peroxidation of lipoprotein. In Mg-deficient rats, oxidation of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) becomes greater than in control rats, partly because the lipids of Mg-deficient rats were more susceptible to oxidative damage following iron incubation.<sup>35</sup> Similarly, when drinking water was fortified with Mg, serum levels of lipid peroxides were lower in mice fed an atherogenic diet.<sup>36</sup> Oxidized LDL is more atherogenic than the wild type of lipoprotein, and high-density lipoprotein (HDL), after oxidative modifications, loses its effect to stimulate efflux of cholesterol from foam cells. The role of Mg deficiency in inflammatory processes should also be considered. Magnesium deficiency in rats led to the sequence of events characteristic for the inflammatory response, that is, a significant increase in plasma interleukin-6 levels.<sup>37</sup> Inflammatory process was accompanied with an increase in plasma levels of acute-phase proteins. Increases in circulating levels of pro-inflammatory cytokines may activate macrophage and endothelial cells. The inflammatory response in Mg-deficient rats may contribute to atherogenesis by modifications of lipoprotein metabolism, oxidation of lipoproteins, inflammatory cell recruitment, and release of cytokine and growth factors that induce cell migration and proliferation.<sup>38</sup>

## Hypertension

Hypertension occurs in approximately 80% of ESRD patients. In ESRD patients, the relationship between blood pressure and cardiovascular events seems to be U shaped. Low predialysis systolic blood pressure is associated with decreased survival. Likewise, systolic blood pressure greater than 180 mm Hg is associated with poor clinical outcome. The pathogenesis of hypertension in ESRD patients is complex and probably multifactorial, including intravascular volume, renin-angiotensin system, vascular stiffness, nitric oxide formation, and uremic toxin such as PTH.

Magnesium deficiency may contribute to the development of hypertension in ESRD patients. Epidemiological studies have shown an inverse relationship between dietary Mg intake and blood pressure. Magnesium supplementation decreases blood pressure in several, but not in all, clinical studies. A few studies have evaluated the serum concentrations of Mg in hypertensive patients and have show a significant decrease or an inverse relationship between serum Mg and blood pressure. An abnormality of intracellular ionic homeostasis was proposed as a contributory pathophysiological factor for essential hypertension. Intracellular free Mg in vascular smooth muscle or circulating red blood cells has been found to be decreased in hypertensive patients. Irish and colleagues reported that there was a significant inverse correlation of intracellular ionized Mg in skeletal muscle with systolic and diastolic blood pressure in the uremic population.<sup>39</sup> This study suggested that intracellular ionized Mg played an important role for the hypertension in ESRD patients. End-stage renal disease patients are frequently associated with secondary hyperparathyroidism, leading to increased intracellular Ca in almost any organs. A relationship between intracellular Ca and blood pressure has been demonstrated in patients with essential hypertension as well as ESRD, suggesting that increased serum levels of PTH may be responsible for the increase of blood pressure in ESRD patients.

## Lipid Metabolism

End-stage renal disease is associated with dyslipidemia due to characteristic alterations of lipoprotein metabolism. The dyslipidemia characteristic for dialysis patients is increases of VLDL, VLDL remnant, and lipoprotein (a) [Lp(a)] as well as a decrease of HDL. Although both increased synthesis and decreased catabolism of VLDL contribute to increased VLDL-remnant level in dialysis patients, decreased catabolism of VLDL is the predominant mechanism. Plasma lipoprotein lipase, hepatic triglyceride lipase, and adipose tissue lipase activities are all impaired in dialysis patients. Decrease of plasma HDL levels in ESRD patients are probably caused by decreased synthesis that impairs the ability of HDL to esterify and transport free cholesterol from tissues and Apo B-100 containing lipoproteins. Although the mechanism of



increased Lp(a) in uremia is unknown, increased synthesis is the most likely explanation.

Magnesium deficiency has a possible role in the perturbation of lipid metabolism in the non-uremic population. In rat, dietary Mg restriction increases plasma levels of LDL and VLDL and reduces that of HDL. The lower concentration of HDL is accounted for by a decreases in the availability of apo E and apo A-I, the major apoproteins of HDL. In cholesterol-fed animals, oral Mg supplementation lowers serum cholesterol and triglyceride and attenuates the development of atherosclerotic lesions. Delva and colleagues reported that the ionized Mg inside lymphocytes from hypertriglyceridemic patients was decreased compared with normotriglyceridemic patients and that negative correlation was observed between ionized intralymphocyte Mg and plasma triglycerides levels.<sup>40</sup>

The association between abnormalities of Mg and lipid metabolism was also suggested in uremia. In a study by Inagaki and colleagues, Mg deficiency in five-sixths nephrectomized uremic rats resulted in an increase of serum triglyceride levels and a decrease of HDL cholesterol levels.<sup>41</sup> Robles and coworkers reported that there was a positive and significant correlation between serum levels of Mg and total cholesterol, LDL cholesterol, VLDL cholesterol, and apo-B in hemodialyzed uremic patients.<sup>42</sup> Nasri and coworkers also reported that there were a positive correlation between serum Mg and Lp(a) and also between serum Mg and triglyceride levels in hemodialysis patients. Kirsten and coworkers showed that Mg supplementation in patient with chronic renal failure resulted in a decrease of serum total and LDL cholesterol, and an increase of HDL cholesterol.

Several mechanisms have been proposed to explain the relationship between hyperlipidemia and Mg depletion. Magnesium-deficient rats developed hyperlipidemia as a result of reduction in plasma lipoprotein lipase activity and lecithin cholesterol acyltransferase activity. It has been considered that these enzymes require the Mg ion as an important cofactor. It is reported that, in Mg-deficient rats, there is increased HMG-CoA reductase activity and increased cholesterol synthesis. These factors may contribute of hyperlipidemia in Mg deficiency.<sup>43</sup>

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# Neurology

# 27

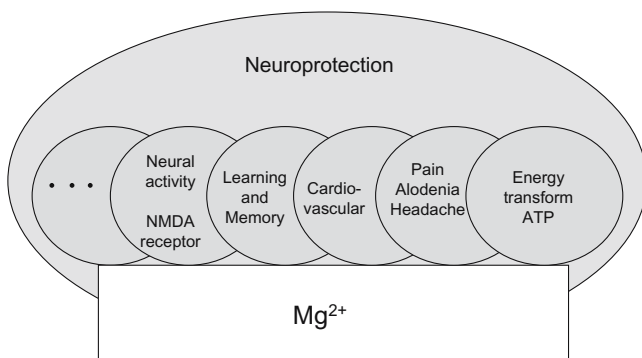
## Neurology Overview

Keiichi Torimitsu

The neurological effect of the magnesium ion ( $\text{Mg}^{2+}$ ) has long been well recognized as a blocker of the *N*-methyl-D-aspartate (NMDA) receptor. Recent reports indicate that  $\text{Mg}^{2+}$  plays an important role in brain disease and injury.  $\text{Mg}^{2+}$  deficiency causes severe brain function damage. Although the mechanism is unclear with respect to neurological functions, neuroprotective effects have been widely investigated. The neurological requirements of  $\text{Mg}^{2+}$  are still unknown, unlike those of  $\text{Ca}^{2+}$ . The recent development of analytical technologies has made it possible to investigate the effect of  $\text{Mg}^{2+}$  in real time. This chapter discusses a wide variety of neurological effects induced by  $\text{Mg}^{2+}$  and includes fundamental and clinical research.

$\text{Mg}^{2+}$  is an important ion with respect to various biological functions (see Figure 27.1). It has been long identified as an essential ion in cell and body functions because of the significant effects of magnesium deficiency. The consequences of following a diet deficient in  $\text{Mg}^{2+}$  have been widely investigated. The maintenance of a homeostatic condition is important for the prevention of various diseases, including brain disease. A low dietary magnesium intake leading to a hypomagnesium condition increases the risk of stroke, ischemia, and other cardiovascular diseases.<sup>1</sup> There have been many reports indicating the crucial role of  $\text{Mg}^{2+}$  in brain injuries and neurodegenerative disease. The neuroprotective effect of  $\text{Mg}^{2+}$  is also attracting considerable attention. The  $\text{Mg}$  blockade of the NMDA receptor ion channel that prevents ischemic damage to neurons has been well discussed. A pharmacological approach to neuroprotection using  $\text{Mg}^{2+}$  as a medicine has been extensively tested. In addition to  $\text{Mg}$ , glycine, zinc, and other antagonists are believed to be possible agents for reducing brain damage by keeping the NMDA receptor ion channels closed.<sup>2-4</sup>

As described above, a considerable amount is known about  $\text{Ca}^{2+}$ . However, the role of  $\text{Mg}^{2+}$  and the mechanism of the  $\text{Mg}^{2+}$  effects are not well understood. This is because of a lack of research, and a lack of a technique for measuring  $\text{Mg}^{2+}$  activity, such as fluorescent dye. Fura-2, Fluo-3, and Fluo-4 are widely used calcium fluorescent dyes that could be employed to investigate changes in intracellular calcium concentration. The role of  $\text{Ca}^{2+}$  has been well identified



**FIGURE 27.1.** Role of magnesium in the biological system.

using these dyes. In contrast, until recently there were only a few methods for investigating  $Mg^{2+}$  behavior in cells. Microdialysis is a commonly used measurement technique, especially with in vivo experiments. Although real-time measurement is possible if a selective detection system is employed, the target will be diluted 10 times or more because of the circulation of the medium inside the microdialysis tube. Chemical analysis in an external fluid also suggests the idea of determining magnesium content with a sampling procedure. A magnesium-selective electrode has also been used. However, the recent development of fluorescent dye to measure the intracellular magnesium concentration provides us with a great advantage when investigating  $Mg^{2+}$  activity. Magfura-2, magnesium green, and KMG-104 are the dyes that can be used for  $Mg^{2+}$  measurement.<sup>5</sup>

## Role of Magnesium in Neuronal Cells

As described above, the importance of magnesium ions can be seen in a wide variety of fields. The role of magnesium can be categorized as followings: (1) therapeutic importance; (2) neuroprotection; and (3) ion regulation (Figure 27.2).

### *Therapeutic Importance*

Magnesium deficiency causes various symptoms, such as cardiovascular problems, stroke, and headache. It may also induce cell death, resulting in a reduced neuroprotective effect as described below.

Headache is a typical symptom caused by the NMDA receptor-related hyperactivity of neurons. It is reported that magnesium reduces the pain of patients who suffer from headaches according to a dose-dependent manner. An increment in learning ability has also been observed in tests on animals.<sup>6</sup>

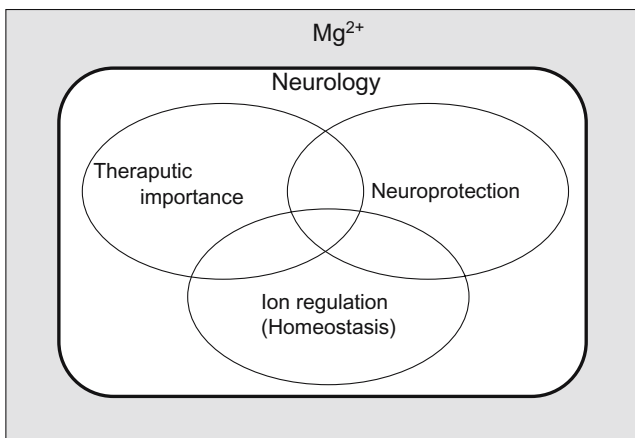
Recent research has identified that a secondary process is essential after primary brain injury or damage if cells are to survive. Cerebral ischemia and traumatic brain injury are representative examples of this phenomenon. Cerebral ischemia induces a decrease in energy supply because of a lack of oxygen. A breakdown of homeostatic conditions is induced by oxidative stress, the depolarization of membrane potential, and energy transduction failure. These phenomena increase the release of neurotransmitters, such as glutamate, which results in an intracellular calcium increase, and they finally induce apoptosis and/or necrosis leading to neuronal death.

An analysis of magnesium supplementation using an experimental model of animal cerebral ischemia indicates that reducing apoptotic neurons, and infarct volume, increases neuronal survival in CA1 region of the hippocampus.

### Neuroprotection

The neuroprotective effect of magnesium in relation to brain damage is widely recognized.<sup>4</sup> One interesting factor is the supportive function of magnesium during the process of recovery from injury and/or damage. Neurons are very sensitive to any kind of stress compared with other supportive cells, such as glial cells and Schwann cells. Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are well-known neuroprotective agents in neurons, and they use the tyrosine kinase pathway to provide damaged protection. Transient neurotransmitter release and intracellular calcium increase are also induced by these agents.<sup>7,8</sup>

On the other hand, magnesium compounds are essential in various enzyme processes, including DNA and protein synthesis. The adenosine triphosphate (ATP) energy process also depends on magnesium, but it does not require a specialized target, and this indicates its complexity.



**FIGURE 27.2.** Role of magnesium in neuronal cells.

In neural systems, the blockade of the NMDA receptor is a well-known phenomenon that regulates  $\text{Ca}^{2+}$  transport through a plasma membrane. The blockade is dependent both on membrane potential and on ionic strength and species. Neural activity depends on the magnesium concentration when glutamate is released under conditions of anoxia. In this respect, magnesium protects neurons from hyperactive situations as a neuromodulator.

The modulation of neural activity by magnesium may be related to memory and learning. Although increases in animal learning ability have been reported, further study, including an investigation of synaptic regulation, is required if we are to understand the effect of magnesium on memory and learning system. Magnesium could be effective for both neuroprotection and synaptic regulation.

### ***Ion Regulation***

Although the intracellular magnesium concentration is regulated at a certain level, its mechanism is still not well understood. Intracellular store site, such as mitochondria, and endoplasmic reticulum (ER), play a role in regulating the intracellular magnesium level together with ion channels and the ion exchange mechanism. The sodium (Na)—Mg exchange system<sup>9–11</sup> and transient receptor potential (TRPM)<sup>7,12,13</sup> are reported to be regulators of magnesium transport through plasma membranes. Another important role is Ca transport. An inhibitory effect on voltage-gated calcium channels might be involved in the neuroprotection system as described above.<sup>14</sup>

It is very common for cells to have a Na—Ca exchange system for controlling Ca transport. The Na—Mg system plays an important role in the regulation of magnesium transport. The system is considered to work along with the Na—Ca system to regulate magnesium homeostasis. However, it is unclear whether Na—Ca and Na—Mg systems are different independent systems. As the Na—Mg exchange system has yet to be identified and purified, the role of the system, especially its stoichiometry, is still under investigation.

There are some reports indicating a relationship between neurotransmitter release and intracellular magnesium concentration. Glutamate has been reported to increase intracellular magnesium in cultured cortical neurons.

### **Conclusion**

This chapter described the effect of magnesium on the nervous system, particularly that of the brain. Although magnesium has been recognized as a very important substance in various nervous systems whose deficiency causes severe damage, its role and mechanism are still not well understood. The recent development of measurement methods has provided us with more information on neuronal disease and protection. As the research is strongly related to clinical applications, we can expect significant pharmaceutical



developments in the near future. The following chapters will discuss the important role played by magnesium in the neural system.

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# 28

## Magnesium in the Central Nervous System

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It has been almost a century since a role for magnesium in the central nervous system (CNS) was first proposed. Despite intensive efforts, the subsequent 75 years saw few advances in our understanding of magnesium's precise role in brain function or the mechanisms by which the cation influences these functions. More recently, the advent of noninvasive techniques to measure intracellular free magnesium concentration, plus the recognition that magnesium plays a critical role in regulating neurotransmitter receptor function, have ushered in a new era for magnesium research in neuroscience. The result has been thousands of published studies describing various effects of magnesium in the CNS, ranging from effects on normal physiology and biochemistry to modulation of pathological events at the molecular level. The current review critically examines the evidence suggesting that alterations in intracellular free magnesium concentration may be an injury factor in acute and chronic CNS injury, as well as the potential for magnesium administration to be neuroprotective under these conditions. Finally, the reasons for contradictory results in the literature regarding therapeutic efficacy are discussed, with an emphasis on cellular energy state and how it may affect treatment, as well as dosage strategies and the potential for adverse side effects.

A role for magnesium in CNS function has been recognised since 1916, when magnesium sulphate was first described as a clinical anesthetic.<sup>1</sup> Despite this early recognition, an understanding of magnesium's role in cell physiology and metabolism has not been readily forthcoming largely because of the inherent difficulty in measuring free concentration of the ion. In contrast, calcium determinations have been possible for several decades and a critical role for calcium has now been well established. Despite several early reports supporting a similar role for magnesium, the evidence was difficult to substantiate and accordingly, magnesium became known as the forgotten cation. More recently, there has been a renewed interest in the role of magnesium in cell function, particularly in the obstetric, cardiovascular, and neural disciplines. With respect to this resurgence in interest in the neurosciences, the impetus can be arguably attributed to the ability to readily measure free magnesium concentration in the CNS, and to the finding that free magnesium regulates

N-methyl-D-aspartate (NMDA) channel activity. Over the past decade, substantial evidence has accumulated suggesting that magnesium changes may occur in the CNS and that these changes in magnesium concentration may have considerable effects on outcome. This recent evidence has never been summarized and consequently, the importance of the magnesium ion in CNS function is still poorly appreciated. The present review therefore examines the recent literature concerning magnesium's regulatory role in neuronal metabolism, particularly the evidence suggesting a role in acute and chronic injury.

## Basal Concentration

A number of methods exist to measure total magnesium concentration and these have been used clinically for several years.<sup>2</sup> These include spectrophotometric, atomic absorption, and mass spectrometer techniques. While these techniques are highly accurate, the diagnostic use of the values obtained is limited because clinical hypermagnesemia and hypomagnesemia overlap extensively with normal values. Moreover, it should be recognized that the free ion concentration is the critical physiological value and this value does not always correlate with the total concentration. Several methods have been used to determine free magnesium concentration.<sup>3,4</sup> Fluorometric techniques were first used to measure enzyme equilibria and thereby indirectly calculate free magnesium concentration. Similarly, ionophores were used in a null point titration technique to do likewise. The difficulty is that both these techniques were highly invasive and tissue destruction was inevitable. The development of ion-selective electrodes was far less destructive, and indeed could be used very successfully on body fluids to determine free magnesium concentration. However, it was still extremely difficult to determine free intracellular levels with these electrodes. The development of fluorescent probes for the measurement of free magnesium concentration has opened the way for intracellular magnesium determinations, however, the technique was limited to cell culture. The only truly noninvasive technique used to date is nuclear magnetic resonance (NMR) spectroscopy.<sup>5</sup> This technique takes advantage of an intracellular magnesium-binding ligand to provide information that can be used to calculate free magnesium concentration. Various ligands have been used with the most widely used being the endogenous ligand, adenosine triphosphate (ATP). The discussion of these techniques is beyond the scope of the present review and readers are referred to some excellent reviews of these NMR-based techniques.<sup>4,6</sup>

The use of NMR to demonstrate that brain intracellular free magnesium concentration can vary in response to an insult was pioneered by Vink and colleagues in the late 1980s.<sup>7,8</sup> Subsequently, a number of laboratories have demonstrated that both total and free brain magnesium concentration declines following injury and that these declines are associated with the development of functional deficits. Reported values for brain intracellular free magnesium

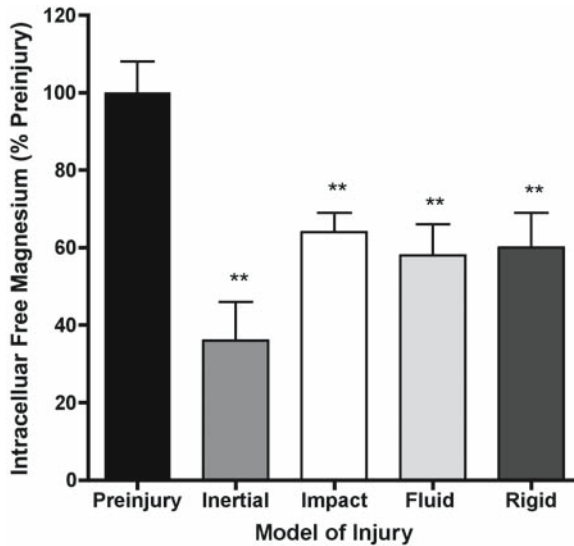
concentration range from 0.3 mM to 1.0 mM, depending on the species. Although some variability in this value can be attributed to small differences in the method of calculation from the NMR data, this can be largely avoided by using the specific equations available on the internet to calculate magnesium concentration by NMR.<sup>9</sup> In the rodent brain, the accepted mean resting value is now considered to be between 0.5 and 0.6 mM, while values on the order of 0.25 to 0.3 mM are considered to represent injured tissue. There is some evidence to suggest that magnesium is not homogeneously distributed throughout the brain with the concentration of the ion varying between cortex and subcortex and between structures.<sup>10,11</sup> Use of transgenic animals with magnesium deficiency may clarify why such variation exists.<sup>10</sup>

## **Changes in Disease States**

### ***Trauma***

Changes in brain magnesium concentration that could be shown to have functional consequences were first demonstrated in traumatic brain injury.<sup>7,8</sup> These authors used NMR to show that traumatic injury to the brain resulted in a 50% to 60% decline in the free intracellular concentration of the ion, and that this decline was associated with the development of neurological motor deficits. Subsequent studies by the same group demonstrated that depleting tissue magnesium concentration exacerbated neurological deficits while attenuation of the decline with parenteral magnesium administration prior to the injury significantly improved neurological outcome.<sup>12</sup> The decline in free magnesium after trauma was reflected in a much smaller decline in tissue total magnesium concentration that was limited to the injury zone<sup>7</sup> and did not extend to non-injured tissue.<sup>13</sup> Such a relationship between free and total magnesium changes was consistent with the previously described buffering role that total magnesium plays in the control of free concentration.<sup>14</sup> Thus, decline in magnesium concentration was thought to be a specific indicator of brain cellular damage.

Since those initial studies, a number of other laboratories have reported similar results suggesting that such declines in magnesium concentration were not limited to any specific model of brain trauma or to any particular species.<sup>15–18</sup> In all species and models, the declines reported in brain intracellular free magnesium concentration ranged between 40% and 60% (Figure 28.1). The fact that magnesium does decline following trauma irrespective of the injury model suggests that magnesium decline is a ubiquitous feature of CNS trauma. However, it is notable that the free magnesium decline never exceeds a minimum value of approximately 0.2 mM, suggesting that this concentration may be a threshold below which the ion cannot fall under physiological conditions. Because free magnesium changes were related to much smaller changes in total tissue magnesium,<sup>7</sup> buffering by intracellular ligands



**FIGURE 28.1.** Changes in brain intracellular free magnesium concentration as measured at 4 to 6 h after experimental brain injury induced using a variety of models. Inertial, inertial injury; Impact, impact acceleration injury; Fluid, fluid percussion injury; Rigid, rigid indentation injury. \*\* $p < 0.01$  versus pre-injury.

presumably prevents any further decline. The declines in brain free magnesium have been shown to persist for at least 4 days following traumatic brain injury and, with increasing severity of injury, out to 7 days.<sup>15,19</sup> Thus, it is not the magnitude of decline that is exclusively related to outcome but also the time period for which magnesium is depressed.

The availability of ion-selective electrodes for magnesium has meant that blood free magnesium concentration can also be readily measured after brain injury. Similar to brain free magnesium concentration, blood levels of free magnesium in rats significantly decline from a mean pre-injury level of approximately 0.50 mM to 0.35 mM by 30 min after traumatic brain injury.<sup>20</sup> This decline in magnesium persisted for at least 4 days following trauma recovering to pre-injury levels by 6 days after injury. This temporal profile of blood free magnesium change was similar to that observed in rat brain using magnetic resonance techniques. As with the brain determinations, there was a pattern whereby milder levels of injury resulted in transient declines in blood free magnesium while more severe injury caused persistent declines. However, unlike the brain determinations, a relationship between blood free magnesium and outcome only existed for the more severe injury levels. While the experimental evidence suggests that trauma to the brain specifically results in a decline in plasma magnesium concentration, there is little evidence to support the view that plasma magnesium reflects brain magnesium concentration, or that a clear relationship exists between blood free magnesium

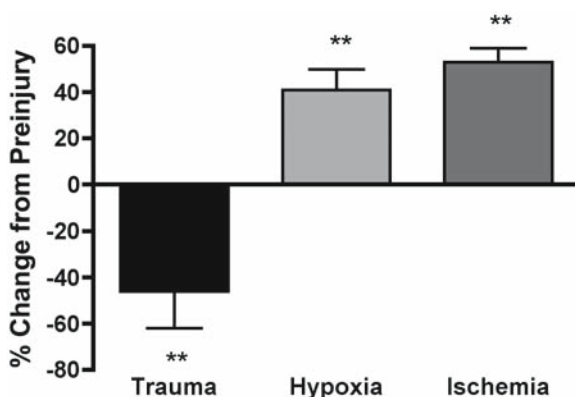
concentration and motor outcome following less severe injury. This is in contrast to findings in clinical trauma where blood hypomagnesemia is common<sup>21</sup> and is related to the severity of injury.<sup>22,23</sup> Nonetheless, contradictory reports do exist, with Frankel and colleagues reporting no correlation between blood magnesium and outcome in trauma patients.<sup>24</sup>

The declines in blood free concentration reported in experimental models have been associated with declines in total magnesium concentration,<sup>25</sup> although this is in contrast with observations in clinical brain trauma where there was always a decline in blood free magnesium concentration that was not associated with any decline in blood total levels.<sup>22</sup> Given that increased urinary excretion of magnesium occurs after clinical brain injury,<sup>26</sup> it would be expected that some loss of total blood concentration of the cation would be observed. A recent comprehensive study of total and free magnesium concentration in stress, mild, moderate, and severe brain injury<sup>23</sup> has confirmed that declines in blood total magnesium concentration are present in chronic or severe conditions but not in acute stress or following mild-to-moderate trauma. In contrast, declines in free magnesium concentration occur at all levels of injury.

Finally, extracellular magnesium changes have not been detected in trauma by microdialysis,<sup>27</sup> although the technique has been effective in detecting significant declines following ischemia.<sup>28</sup> This difference may be due to the detection methods used in the study, with the earlier study using ion chromatography as opposed to the more sensitive graphite furnace atomic absorption spectroscopy used in the later study.

### ***Ischemia/Stroke***

A number of studies have now reported changes in free magnesium concentration following stroke. Intracellular brain levels have been shown to increase and then decline following stroke,<sup>29</sup> with a number of studies implying that glutamate excitotoxicity may contribute to the initial increase. However, a rise in magnesium in response to glutamate exposure was shown to depend on high extracellular magnesium concentration,<sup>30</sup> and it is now appreciated that the reported magnesium increase in the *in vitro* studies was primarily a change in calcium. Therefore, any increase in free magnesium in stroke is now thought to be associated with declines in ATP concentration. The initial increase in intracellular levels following stroke is temporally correlated to approximately a 60% fall in extracellular magnesium levels as measured by microdialysis,<sup>28</sup> as well as by magnesium declines in blood and cerebrospinal fluid (CSF).<sup>31</sup> While a significant correlation was reported between CSF magnesium and infarct size and clinical outcome following acute stroke,<sup>32</sup> no correlation was observed between CSF magnesium and serum magnesium. Finally, magnesium deficiency has been noted to exacerbate injury from stroke<sup>33,34</sup> in a manner similar to that reported following traumatic brain injury.<sup>12</sup>



**FIGURE 28.2.** Change in brain intracellular free magnesium concentration as measured in the first 24 h following experimental trauma, hypoxia, or ischemia. \*\* $p < 0.01$  from pre-injury.

Related to ischemia is the condition known as hypoxia–ischemia. There are a number of similarities between ischemia and hypoxia–ischemia, particularly in relation to energy depletion with profound hypoxia–ischemia. However, with milder levels of insult, it has been possible to maintain ATP levels and determine free magnesium levels using magnetic resonance spectroscopy. Under these conditions, intracellular free magnesium declines during hypoxia–ischemia in neonatal rats.<sup>35</sup> Declines in brain magnesium by 18% over 4 h have also been reported after in utero hypoxia.<sup>36</sup> Finally, there is a deficiency in cord-blood magnesium following hypoxia–ischemia.<sup>37</sup>

Although there are many similarities in stroke and traumatic brain injury, there is the fundamental difference in stroke in that ATP levels decline and that this is associated with an increase in intracellular magnesium concentration, whereas following traumatic brain injury, ATP levels remain stable and that magnesium levels decline after injury (Figure 28.2). At the extracellular level, both injuries produce declines in CSF and serum magnesium concentration.

### **Neurodegenerative Disease**

There is a long history of reports associating low magnesium levels to neurodegenerative disease. While it is recognized that magnesium deficiency alone cannot account for neurodegenerative disease,<sup>38</sup> there is increasing evidence to suggest that in combination with other predisposing factors, altered magnesium homeostasis may play some part in the neurodegenerative condition. For example, in Parkinson's disease, lower concentrations of magnesium and copper were observed in specific brain regions associated with the disease.<sup>39</sup> Phosphorus magnetic resonance spectroscopy confirmed that the brain free magnesium concentration was significantly decreased in patients affected

with Parkinson's disease,<sup>40</sup> and that this low magnesium concentration was not apparent in multiple system atrophy (including the olivopontocerebellar atrophy and stiatonigral degeneration variants). A number of studies have also reported an association between low magnesium and high aluminium concentration in areas of brains affected by Parkinson's disease,<sup>41</sup> with recent studies in animal models demonstrating that high aluminium exacerbates tissue magnesium depletion,<sup>42</sup> and that reduced dietary magnesium intake plays a critical role in disease onset.<sup>43</sup> While the mechanism associated with low magnesium facilitating disease onset is unknown, Golts and coworkers<sup>44</sup> have recently shown that the reduced magnesium concentration may affect the conformation of  $\alpha$ -synuclein and thereby increase its aggregation in Lewy bodies.

Another neurodegenerative disease demonstrating an association with low magnesium levels is Alzheimer's disease. Early reports demonstrated a low plasma magnesium concentration in severe dementia of the Alzheimer's type.<sup>45</sup> Subsequently, decreased magnesium concentration in specific brain structures of Alzheimer's patients has been identified.<sup>11</sup> This magnesium deficit is particularly apparent in the hippocampus,<sup>46</sup> whose degeneration has been associated with short-term memory loss. Of interest is the fact that survivors of traumatic brain injury have an increased risk of Alzheimer's disease.<sup>47</sup> Part of the pathology of traumatic brain injury is decline in brain intracellular magnesium concentration and activation of amyloidogenic pathways that result in formation of the toxic  $\beta$  amyloid protein.<sup>48</sup> Administration of magnesium after brain trauma is thought to alter the post-translational processing of amyloid precursor protein away from the toxic form and toward the neuro-protective  $\alpha$  forms.<sup>48</sup>

Finally, magnesium has been shown to be increased in schizophrenia.<sup>49</sup> While increases in free magnesium concentration can be attributed to a decrease in ATP ligands that bind the cation, no decrease in ATP has been reported in schizophrenia. On the other hand, phosphorus magnetic resonance spectroscopy has shown decreased phosphomonoester/phosphodiester peaks in schizophrenia, implying changes in membrane structure. Because it is well known that the membrane is the major buffer for free magnesium concentration,<sup>14</sup> it could be hypothesized that the altered membrane structure would decrease the number of available binding sites for magnesium.

## Therapeutic Efficacy

Therapies targeting the restoration of magnesium homeostasis have demonstrated beneficial effects in a wide variety of CNS pathologies including focal<sup>50,51</sup> and diffuse traumatic brain injury,<sup>52–55</sup> sensorimotor lesions,<sup>56</sup> ischemia,<sup>57–59</sup> hypoxia,<sup>60</sup> subarachnoid hemorrhage,<sup>61,62</sup> and fetal brain damage following maternal seizures,<sup>63</sup> amongst others.

Nonetheless, there are reports that do not support a protective role for magnesium.  $\text{MgSO}_4$  at doses of 300 to 400 mg/kg is not protective in hypoxia–



ischemia<sup>64,65</sup> or in asphyxia in lambs.<sup>66</sup> When administered pre-injury, magnesium at a dose of 270 mg/kg is protective in hypoxia–ischemia, but not when administered postinjury.<sup>67</sup> Similarly, MgCl<sub>2</sub> does not protect in ischemia when used at doses of 235 mg/kg.<sup>68</sup> It is significant that dose optimization studies in both traumatic brain injury<sup>69</sup> and stroke<sup>70</sup> suggest that the optimal magnesium dose is between 30 and 90 mg/kg, and certainly less than 100 mg/kg. Higher doses of magnesium have the clear potential to cause hypotension and other cardiovascular effects which may adversely affect outcome. Our conclusion is therefore that studies that use more than 100 mg/kg IV run the risk of being negative.

A second factor when considering whether magnesium will be efficacious in the treatment of CNS injury is whether ATP declines during the insult. As stated above, ATP does decline in ischemia and in severe hypoxia. Under these conditions, the intracellular free magnesium concentration will increase as more of the cation is released from the binding ligand. Hence, the observations that magnesium initially increases in ischemia. This raises the issue of whether the increase in magnesium is an injury factor or a consequence of injury. In our opinion, any magnesium increase that accompanies energy depletion is more likely a consequence of injury rather than an injury factor in itself. Magnesium administration at this point may have little effect, particularly at the intracellular level where magnesium is already elevated. This does not preclude the fact that magnesium treatment may have beneficial effects at the extracellular level. However, in the absence of ATP, increasing the intracellular concentration of the cation will have little effect.

## Neuroprotective Properties

As stated above, magnesium administration can potentially be protective at both the intracellular and the extracellular level depending upon the ATP concentration during the insult. Where ATP levels fall, and free magnesium increases, there can only be a benefit from the extracellular effects of the cation. In contrast, where intracellular magnesium declines, there can be benefits from the combined intracellular and extracellular effects of the cation. Clearly, this will impact on the therapeutic efficacy.

## Extracellular Effects

Magnesium has been shown to cross the blood–brain barrier and enter the CSF,<sup>71</sup> a process that will be enhanced after acute CNS injury given that the blood–brain barrier is usually disrupted for extended periods of time. Having gained entry to the CNS, magnesium has a number of neuroprotective actions at the extracellular level, the most widely characterized being its effects on glutamate release and excitotoxicity. Increased extracellular magnesium is known to reduce glutamate release,<sup>72,73</sup> as well as the capacity of the

neurotransmitter to bind to the NMDA receptor.<sup>74</sup> In addition, intracellular and extracellular magnesium is a voltage-dependent blocker of this NMDA channel<sup>175,76</sup> whose activity is associated with entry of calcium into neurons and the initiation of an autodestructive cascade.<sup>72</sup> By blocking the NMDA channel, magnesium reduces calcium influx into the cell and protects against this injury cascade. This neuroprotective property of magnesium has now been extensively demonstrated both *in vitro* and *in vivo*.<sup>72,74,75,77–79</sup> Interestingly, injury reduces the magnesium blockade of the NMDA channel<sup>180</sup> and the block of the NMDA channel increases substantially with age,<sup>79</sup> the latter suggesting that the importance of the magnesium block increases as the complexity of the CNS increases.

The effects of magnesium on calcium entry into cells are not limited to the NMDA channel. Indeed, the magnesium ion has been described as nature's physiological calcium antagonist, having direct effects on calcium uptake.<sup>81</sup> Moreover, the modulation of ion fluxes is not limited to calcium, with the K<sup>+</sup> channel also being directly regulated by magnesium concentration.<sup>81</sup> In subarachnoid hemorrhage, the ability of magnesium to modulate ion fluxes and reduce depolarization time is thought to account, in part, for smaller lesion volumes.<sup>82</sup> Finally, with respect to neurotransmitters, magnesium has been shown to decrease the dopamine surge during anoxia,<sup>83</sup> suggesting that neurotransmitter release in general may be inhibited by extracellular magnesium, perhaps by inhibiting presynaptic calcium channels.

This combined effect on presynaptic neurotransmitter release and postsynaptic ion fluxes may therefore account for the observed ability of magnesium to inhibit membrane depolarization and cortical spreading depression.<sup>84</sup>

Magnesium's extracellular effects are not limited to neurotransmission, with a number of reports suggesting actions at the level of the vasculature. Magnesium can reverse vasoconstriction and increase cerebral blood flow in rats.<sup>85,86</sup> As a vasodilator, magnesium has the ability to inhibit cerebrovasospasms,<sup>87</sup> perhaps accounting for, in part, the reduced incidence of vasospastic episodes following subarachnoid hemorrhage, and the reduced lactate production after brain trauma.<sup>88</sup> Finally, magnesium has recently been shown to attenuate blood–brain barrier permeability following traumatic brain injury<sup>89</sup> and to reduce associated vasogenic edema formation.<sup>90,91</sup>

### ***Intracellular Effects***

Not only does magnesium enter the cerebrospinal fluid following parenteral administration,<sup>71</sup> it has also been shown to increase brain intracellular free magnesium concentration<sup>53</sup> and to influence a number of intracellular processes, including energy metabolism, free radical formation, membrane structure, apoptosis, and receptor activity.<sup>92</sup> For example, the effects of extracellular magnesium on the excitatory amino acids or ligand-gated ionotropic receptors described above are also mediated by intracellular magnesium, and in the case

of the NMDA channel, intracellular magnesium is the main regulator of channel activity.<sup>75</sup> Modulatory effects on receptor binding and activity have also been described for seven metabotropic, transmembrane receptors including serotonergic and opioid receptors. In these receptors, intracellular magnesium is required for the activation of G proteins and therefore the coupling of G protein-linked receptors to their effector mechanisms.<sup>93</sup>

In terms of energy metabolism, magnesium is an essential cofactor in all energy-producing and -consuming reactions, and therefore changes in the cation concentration will have profound consequences for all bioenergetic reactions, including those of oxidative phosphorylation.<sup>94</sup> After ischemia, magnesium is thought to reduce infarct size, in part, by preserving energy metabolism.<sup>73</sup> Specifically, magnesium preserves glucose and pyruvate levels after ischemia, while attenuating elevation of glutamate, lactate,<sup>73</sup> and malondialdehyde levels.<sup>95</sup> At the level of the mitochondrion, magnesium preserves mitochondrial membrane potential,<sup>96</sup> and after traumatic brain injury, improves mitochondrial ultrastructure.<sup>97</sup> It has also been shown to inhibit the opening of the mitochondrial permeability transition pore,<sup>98</sup> whose activity is thought to be integral to apoptosis.

Since the initial observations that intracellular magnesium deficiency enhances apoptosis<sup>99</sup> and that decline in blood magnesium causes an increase in pro-apoptotic intracellular ceramide,<sup>100</sup> a role for magnesium in apoptosis has become increasingly apparent.<sup>101</sup> Magnesium decreases nuclear oxidative damage and DNA fragmentation,<sup>102</sup> possibly through its ability to inhibit the induction of DNA fragmentation factor.<sup>103</sup> The cation also attenuates induction of p53 after trauma,<sup>104</sup> and reduces hippocampal apoptosis following hypoxia-ischemia,<sup>105</sup> although both of these studies suggest differential effects depending on the brain region. Whether this is dependent on the basal levels of magnesium and adenylates, which vary according to brain region,<sup>10,11</sup> is unknown. The work of Delivoria-Papadopolous and colleagues<sup>106</sup> has demonstrated that magnesium alters the balance between pro-apoptotic bax and the anti-apoptotic bcl-2, thus inhibiting the activation of caspases. These findings were consistent with others in hypoxia-ischemia, demonstrating magnesium inhibition of caspase-3 activation and MAP-2 immunostaining.<sup>107</sup> Clearly, magnesium has multiple effects on the apoptotic cell death pathway, although further studies are required to fully characterize the implications.

At the level of the cell membrane, magnesium is an integral component, with the membrane actually serving as a free magnesium buffer.<sup>14</sup> By reducing membrane lipolysis,<sup>108</sup> magnesium is thought to inhibit lipid peroxidation and generation of free radicals.<sup>109,110</sup> The reduction in free-radical production can also be mediated by magnesium's ability to inhibit reduced nicotinamide-adenine dinucleotide phosphate oxidase, an enzyme that produces superoxide radicals.<sup>111</sup> This inhibition is equivalent to other known inhibitors of this enzyme, such as copper. Magnesium also reduces free radicals by significantly increasing the levels of endogenous antioxidants after injury,<sup>88</sup> specifically

activating superoxide dismutase and catalase, as well as the concentration of glutathione.<sup>112</sup> Through its effects on the Na<sup>+</sup>/K<sup>+</sup> ATPase, magnesium can be considered as having an indirect effect on membrane potential.

While the critical role of magnesium in RNA aggregation, protein synthesis and the replication of DNA itself has been known for some time,<sup>113</sup> recent results suggest that the cation also reduces acute cytoskeletal alterations following traumatic brain injury,<sup>114</sup> perhaps through modulation of calpain. Finally, the cation is thought to regulate the processing of amyloid precursor protein (APP) away from the neurotoxic  $\beta$  amyloid form and toward the neuroprotective soluble APP $\alpha$  form.<sup>48</sup> This potentially has implications for Alzheimer's disease.

## Conclusion

A role for magnesium in both physiological and pathological states of the CNS has become increasingly evident, with considerable evidence now supporting the view that the intracellular free ion concentration is critical in normal cell functioning, and that the ion concentration can change in response to various stimuli. The change in magnesium concentration in pathological states can be considered as either an injury factor, or an injury marker, depending on the energy status of the cell. Where the cell does not have energy failure, the change is considered an injury factor and therapeutic approaches that restore magnesium homeostasis have shown considerable success in improving outcome. Because magnesium is involved in so many physiological and biochemical processes, administering a dose that restores homeostasis without producing adverse side effects is critical to therapeutic efficacy. While our understanding of magnesium's role in this multitude of processes has grown considerably over the last three decades, there is still a need to identify and characterize the transporter mechanisms that are responsible for maintaining normal magnesium concentration. Only then can the critical role for magnesium in biological processes, and in particular the central nervous system, be fully appreciated.

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# **Magnesium in Dental Medicine**

# 29

## Tooth and Magnesium

Masayuki Okazaki

Inorganic tooth materials are composed of hydroxyapatite. However, biological apatites contain many trace elements and have different crystallographic properties. Tooth enamel is well crystallized, contrary to poorly crystallized dentin and bone. Magnesium is richer in dentin than in enamel, and especially significantly affects the physicochemical properties of teeth. In general, magnesium decreases the crystallinity of hydroxyapatite and promotes solubility. Magnesium also has important roles in the relationship to cells. Divalent ions, such as magnesium, promote cell adhesion and contribute to the metabolism of hard tissues. This may affect the formation of teeth and be related to regulation during maturation.

### Tooth Structure and Its Chemical Composition

Teeth are hard tissues vital for human life because we maintain our health by chewing food. This biting action is also very important in maintaining brain activity through the impulse of the biting force. These teeth are sometimes destroyed by caries or periodontal diseases. Teeth are structured with enamel, dentin and cementum supported by alveolar bone, and periodontal membrane (ligament). Enamel is composed of almost 95 wt % well-crystallized hydroxyapatite containing a few percentages of noncollagenous proteins and water.<sup>1</sup> Dentine is composed of approximately 60 wt % to 70 wt % of poorly crystallized hydroxyapatite and 30 wt % to 40 wt % of collagen. These chemical compositions are shown in Table 29.1.<sup>2</sup> Magnesium is contained as a trace element and is rich in dentin.

Although the calcium and phosphate molar ratio approximately reflects their content in hydroxyapatite for enamel, it shows a higher value for dentin because several percentage of  $\text{CO}_3^{2-}$  ions are substituted for  $\text{PO}_4^{3-}$  positions in the apatite crystals of dentin.<sup>3</sup> The variety of these chemical compositions in hydroxyapatite crystals affects the physicochemical properties, especially the crystallographic and mechanical properties of teeth.

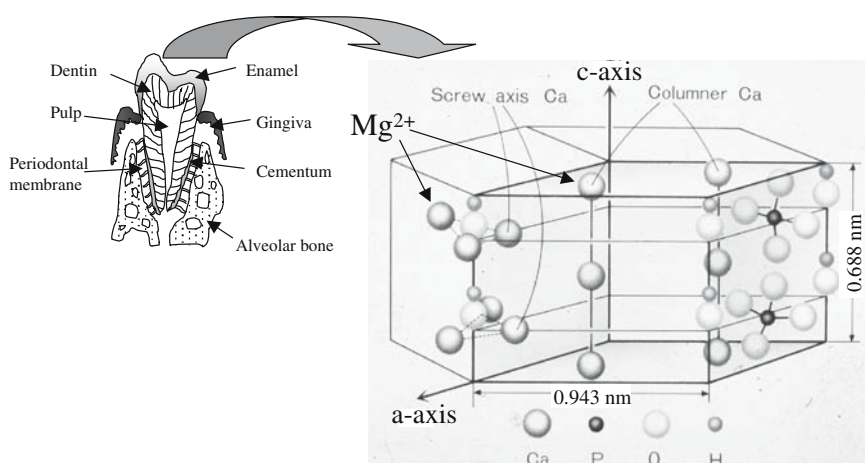
**TABLE 29.1.** Chemical compositions of human enamel, dentin, and bone (dry wt %).

Composition	Enamel	Dentin	Bone
Ash	95.7	70.0	57.1
Ca	37.9	25.9	22.5
P	17.0	12.6	10.3
Mg	0.42	0.82	0.26
Na	0.55	0.25	0.52
K	0.17	0.09	0.09
CO <sub>2</sub>	2.4	3.2	3.5
Cl	0.27	0.0	0.1
F	0.01	0.02	0.05

## Crystallographic Aspect

Apatites and related compounds are of fundamental importance in several areas, including the structural chemistry of hard tissue. Hydroxyapatite is the first compound to be examined in a program which calls, in part, for the detailed refinement of several of these materials.<sup>4</sup> The space group and unit cell dimensions are, respectively,  $P6_3/m$  and  $a = b = 9.432$ ,  $c = 6.881$ .

With constantly improving knowledge of structural detail now being provided by precision techniques in X-ray and neutron diffraction, the study of the structure–property relationships at the atomic level of organization seems to be especially promising. Atomic-scale mechanisms and the effects of substitution are particularly important in biological apatites which, apparently, always show considerable substitution and other crystalline defects.<sup>5</sup>

**FIGURE 29.1.** Tooth structure and hydroxyapatite crystal model.

Apatites are generally formulated as  $A_{10}(BO_4)6C2$ .<sup>6</sup> Hydroxyapatite is a kind of calcium phosphate and is the main inorganic component of hard tissues such as teeth and bones. Hydroxyapatite has a chemical formula of OH-containing apatites, and is an ionic compound in the hexagonal crystal group, theoretically expressed as  $Ca_{10}(PO_4)_6(OH)_2$  (Figure 29.1).<sup>4</sup> Recent computer graphics programs can draw the accurate hydroxyapatite structure.<sup>7</sup>

However, biological apatites contain many of trace elements such as magnesium.<sup>2</sup> The incorporation of magnesium in apatites is very limited unless carbonate or fluoride ions are simultaneously incorporated.<sup>8,9</sup> The amount of magnesium incorporated in apatite is proportional to the magnesium concentration in the solution. The incorporation of magnesium is increased by the simultaneous incorporation of fluoride or carbonate. In particular, the incorporation of magnesium accelerated with the increase of fluoride.<sup>10</sup> The limited incorporation of magnesium is also observed in apatites prepared at temperatures above 900°C by solid-state reaction.<sup>11</sup>

## Trace Elements in Teeth

It is well known that a number of elements can be substituted into the apatite crystal structure.<sup>6</sup> The behavior of many trace elements has been reported. It is said that most elements on the periodic table can be substituted into apatite crystals. Both cationic and anionic ions can substitute into the  $Ca^{2+}$  position and  $PO_4^{3-}$  or  $OH^-$  position. Furthermore, some of these substituted ions contribute to metabolism in the human body and cell adhesion. From the viewpoint of ionic charge balance, divalent ions are easy to substitute. Of course, this depends on the ionic radius.<sup>12</sup>

The most interesting ion is  $Mg^{2+}$ . Magnesium exists in human minerals, especially as a phosphorus complex in bone, and in chlorophyll in plants, and is known to be closely related to the metabolism and physiology of the human body.<sup>13</sup> Hexokinase, for example, which transfers a phosphoryl group from adenosine triphosphate (ATP) to a variety of six-carbon sugars, requires  $Mg^{2+}$  for activity in human cells.<sup>14</sup> In that human hard tissues contain certain amounts of magnesium, it may play an important role in the initial formation of tooth apatites and have a significant effect on their physicochemical properties. However, because magnesium is a minor constituent of human enamel, dentin, and bone, the nature of its association with the mineral phase, especially hydroxyapatite, and its contribution to the properties of biological apatites are still not clear. Because this ion has a smaller ionic radius (0.064 nm) than that of  $Ca^{2+}$  ion (0.099 nm),<sup>12</sup> it was previously thought that  $Mg^{2+}$  ion is hard to substitute into apatite crystals. Mostly, it was considered that it was adsorbed at the surface of the crystal<sup>15</sup> or combined with organic compounds.<sup>16</sup> However, LeGeros first suggested that some  $Mg^{2+}$  ions could substitute into apatite crystals.<sup>8</sup> Okazaki and colleagues<sup>9</sup> also demonstrated this considering the change of the *c*-axis dimension of magnesium-containing apatites. It is

estimated that the maximum magnesium content maintaining the apatite structure may be on fifth of calcium ions. In addition to the magnesium content, tricalcium phosphate [TCP;  $\text{Ca}_3(\text{PO}_4)_2$ ] with magnesium, which is a kind of calcium phosphate, is formed. By incorporating with magnesium, TCP is stabilized.

The presence of Mg in apatites decreases the *a*-axis dimension, decreases crystallinity, increases  $\text{HPO}_4^{2-}$  incorporation, and increases the extent of dissolution. Magnesium inhibits remineralization. In vitro, Mg suppresses the crystallization of apatite, stabilizes dicalcium phosphate dihydrate (DCPD;  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ), and promotes its formation even in neutral pH, stabilizes amorphous calcium phosphate (ACP), and increases the solubility of synthetic apatites.

## Magnesium Distribution in Teeth

Some trace elements such as magnesium, sodium, zinc, fluoride, and chloride have characteristic distribution patterns in human matured enamel and dentin.<sup>17</sup> For example, at the narrow surface layer of enamel, magnesium and sodium show a very steep decrease in concentration, whereas zinc, chloride, and fluoride show a peak concentration much higher than in the rest of enamel. It seems likely that these specific patterns of distribution are related to the progressive mineralization pattern observed during the maturation stage, which is characteristic of the layers. Suga and colleagues<sup>17</sup> indicated by electron microprobe analysis that in the matured enamel of cow, dog, rat, and guinea pig, magnesium, sodium, and chloride have almost the same distribution patterns as in human matured enamel.

Magnesium is distributed in dentin at a much higher level than in enamel.<sup>2,18</sup> Although the magnesium distribution difference between dentin and enamel has not been clarified, it can be speculated that this phenomena may be related to the association of magnesium with organic compounds such as collagen in dentin. Magnesium may also be related to caries susceptibility of teeth the same as carbonate.<sup>19</sup> Both magnesium and carbonate decrease crystallinity and increased the solubility of apatite crystals.

## Magnesium Incorporation with Cells

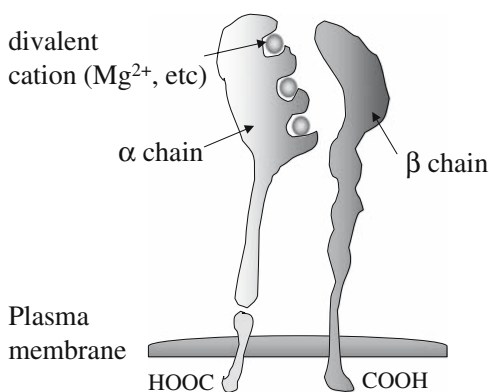
Although enamel develops from epithelial cells, dentin and bone develop from mesenchymal cells by epithelial-mesenchymal interaction.<sup>20</sup> The chemical composition and crystallinity of dentin and bone are relatively similar. Therefore, osteoblast behavior may be partially reflected in odontoblasts, which form dentin. Recently, adhesion molecules, such as those of the integrin family, were examined concerning cell structure and function. Divalent ions affect



cell adhesion in relation to the integrin molecule as an adhesion molecule at the cell surface,<sup>21</sup> and it has been especially, reported that  $Mg^{2+}$  ions promote cell adhesion.<sup>22</sup> Integrins are crucially important receptor proteins because they are the main way in which cells both bind to and respond to the extracellular matrix. They are composed of two noncovalently associated transmembrane glycoprotein subunits called  $\alpha$  and  $\beta$ , both of which contribute to the binding of the matrix protein. The binding of integrins to their ligands depends on extracellular divalent cations, reflecting the presence of three or four divalent cation-binding domains in the large extracellular part of the  $\alpha$  chain (Figure 29.2).<sup>21</sup>  $Mg^{2+}$  ions also play some role in cell adhesion. Thus, magnesium seems to be an important factor even in controlling *in vivo* bone metabolism because it plays a part in both bone formation and resorption.  $Mg^{2+}$  ions may contribute to the bone metabolism of osteoclasts and osteoblasts with integrins at their cell surfaces.

Recently, scaffold biomaterials have been studied in the tissue engineering field. In a continuation of those studies, functionally graded  $CO_3$ apatite containing magnesium, producing a negative gradient of magnesium concentration from the surface toward the core, was synthesized.<sup>23</sup> Second, the degree of cell adhesion to a composite made by mixing FGMgCO<sub>3</sub>-apatite and collagen to facilitate bonding and processing was investigated. Furthermore, the biocompatibility and effect of magnesium on bone formation by implanting the same FGMgCO<sub>3</sub>Ap-collagen composite into rabbit femur was examined.<sup>24</sup>

Electron Spectroscopy for Chemical Analysis (ESCA) analysis clearly showed a negative gradient distribution of Mg1s intensity (atomic concentration) of magnesium from the crystal surface toward the inner core. The WST cell growth assay of mouse MC3T3-E1 osteoblast cells incubated on the surface of the composites showed similar optical densities between the control



**FIGURE 29.2.** Schema of integrin as an adhesion molecule.

CO<sub>3</sub>Ap–collagen composite and the FGMgCO<sub>3</sub>Ap–collagen. This suggests that cell growth was good and that there was no significant inhibition in relation to the components of each sample. In the cell adhesion assay, after the nonadhering cells were rinsed off, the optical density of the FGMgCO<sub>3</sub>Ap–collagen composite was higher than that of the CO<sub>3</sub>Ap–collagen composite. Furthermore, the cell adhesion assay by radioisotope showed that the adhesion fraction of the FGMgCO<sub>3</sub>Ap–collagen composite was higher than that of the CO<sub>3</sub>Ap–collagen composite and much higher than that of the titanium plate as a control. After 4 weeks of incubation, many more osteoblasts adhered to the FGMgCO<sub>3</sub>Ap–collagen composite than to the CO<sub>3</sub>Ap–collagen composite and the layer they formed was thicker.

After 1 week of implantation into rabbit femurs, both the FGMgCO<sub>3</sub>Ap–collagen composite and the CO<sub>3</sub>Ap–collagen composite showed no clear difference from the control in histological results. However, hematoxylin–eosin stains of magnified new bone revealed the existence of many osteoblasts after 2 weeks of implantation. After 4 weeks of implantation, both the FGMgCO<sub>3</sub>Ap–collagen composite and the CO<sub>3</sub>Ap–collagen composite showed a clear bone formation, although the control hole with no implantation appeared to also have been repaired with a thinner layer of new bone. The bone density of the FGMgCO<sub>3</sub>Ap–collagen composite was higher than that of the CO<sub>3</sub>Ap–collagen composite.

Thus, the modification of gradational Mg<sup>2+</sup> ions on apatite crystals was successfully performed. The apatite crystals showed no special function on the surface but did promote cell adhesion. Furthermore, as a scaffold material, the FGMgCO<sub>3</sub>Ap–collagen composite was suggested to contribute to bone formation. Because this composite is easy to process into any form, it is extremely useful in the reconstruction of bone defects. Thus, trace elements in apatite crystals are strongly related to biological metabolism and physiology. Bone apatites contribute to store trace elements in addition to the skeletal structure.

These scaffold biomaterials are useful to replace the jawbone when it is lost by accident or maxillofacial disease and adsorbed after the loss of a tooth, and during the implantation of artificial teeth. Furthermore, it is speculated that Mg<sup>2+</sup> ions may contribute to the formation and maturation of enamel.

The latest researches have gradually clarified the unknown phenomena inside and outside cells with magnesium. Mg<sup>2+</sup> ions regulate numerous cellular functions, serving as a cofactor in many different enzymatic pathways. The cytoplasmic Mg<sup>2+</sup> ion, [Mg<sup>2+</sup>]<sub>c</sub>, is regulated by both passive (influx driven by the electrochemical gradient of this ion) and active (active efflux) transport.<sup>25</sup> It has been clarified that [Mg<sup>2+</sup>]<sub>c</sub> is tightly regulated by Mg<sup>2+</sup> efflux, depending on extracellular [Na<sup>+</sup>].<sup>26</sup> Therefore, we speculate that during the formation and maturation of teeth, [Mg<sup>2+</sup>]<sub>c</sub> in ameloblasts and odontoblasts may have significant roles. In conclusion, it can be speculated that magnesium is strongly related to the maturation of teeth and their physicochemical properties.

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# **Magnesium in Neurology and Psychiatry**

# 30

## Magnesium in Psychoses

Mihai Nechifor

Psychoses are severe psychiatric diseases. They affect a significant number of patients and have a various symptoms. The main psychoses are schizophrenia, major depression, and bipolar disorders. There are also other types of psychoses, such as interictal psychosis and postictal psychosis in epileptic patients.<sup>1</sup> These psychoses were less studied regarding the influence on the changing concentrations of magnesium and other cations. Magnesium and other bivalent cations play multiple roles in the central nervous system (CNS). Misbalances in intra- or extracellular concentrations of these cations are met in some neurological and psychiatric diseases and are involved sometimes in the pathogenic mechanisms of these diseases.

### Bipolar Disorder (BD)

This disease (also called manic–depressive psychosis) is included in the affective psychosis category. The clinical aspect of bipolar disorder is extremely different from patient to patient and this creates a difficult classification of this disease. There are patients with predominant manic manifestations and there are also patients with predominant depressive symptoms.

There are changes in the plasmatic and cellular concentrations of magnesium and certain other cations in case of BD patients.

Data existing about magnesium plasmatic concentration levels in this affective psychosis are heterogeneous. Certain authors detected an increase in magnesium intra- and extracellular concentrations, but others have reported results suggesting the contrary. It was found that erythrocyte  $Mg^{2+}$  level is significantly increased in case of BD nonhospitalized patients: this increase is not seen after hospitalization.<sup>2</sup> Some authors have reported that there are no changes in plasmatic and erythrocyte  $Na^+$  and  $Mg^{2+}$  levels in patients suffering from affective psychoses (including BD).<sup>3</sup> Also, other authors consider that in patients with BD there are not significant changes of  $Mg^{2+}$  levels in cerebrospinal fluid (CSF) and there are no correlations between magnesium concentrations and disease evolution.<sup>4</sup> Some differences between data obtained by

different authors might originate into the existence of more than one subtype of BD.<sup>5</sup> Other differences are due to distinct phases in disease evolution at the moment of taking cation samples. There are few data about the influence of pharmacotherapy on patient's magnesium concentrations in BD. Mood stabilizers represent the main pharmacotherapy for these patients. Beside lithium, there are used nowadays new mood stabilizers as lamotrigine, sodium valproate, carbamazepine, etc.<sup>6,7</sup>

This class of drugs is very heterogeneous and includes drugs used from a long time (such as lithium) as well as drugs recently introduced in the treatment of BD (such as some anti-epileptic drugs). In 1988, the possibility was discovered that a part of therapeutic effect of  $\text{Li}^+$  in BD is due to competition between  $\text{Li}^+$  and  $\text{Mg}^{2+}$  for the intracellular binding site of magnesium, because  $\text{Li}^+$  and  $\text{Mg}^{2+}$  have some similar physicochemical characteristics.<sup>8</sup> It was shown by nuclear magnetic resonance (NMR) spectroscopy that  $\text{Li}^+$  competes with  $\text{Mg}^{2+}$  for phosphates groups in GTP and GDP.<sup>9</sup> In this way, the transduction of biological signal is modified through second messenger inositol-1,4,5-triphosphate (IP3). There were identified some intracellular molecules, where  $\text{Li}^+$  competes for magnesium binding site (inositol, monophosphatase, inositol polyphosphate 1-phosphatase, etc.).<sup>10</sup> Lithium increases magnesium intracellular concentration; the intake of 1 to 2 nM lithium in the medium of culture significantly increases magnesium concentration in SH-SY5Y neuroblastoma cells.<sup>11</sup> It is considered that the mechanism of increasing this magnesium concentration is represented by a competition between  $\text{Li}^+$  and  $\text{Mg}^{2+}$  for magnesium intracellular binding sites. Increasing binding of lithium to these molecular sites decreases magnesium binding and raises the concentration of free intracellular magnesium.<sup>9</sup> An intracellular lithium concentration of 15 mm/L determines an important increase in  $\text{Mg}^{2+}$  concentration in neuroblastoma cells (approximately 158%).<sup>12</sup> The lithium/magnesium competition can also be noticed in case of therapeutic lithium concentration in BD patients. Only 72 h incubation at 1 to 2 mM extracellular  $\text{Li}^+$  concentrations determines a significant increase in magnesium intracellular concentration. Intracellular  $\text{Li}^+$  was found to be at therapeutic levels (between 1–1.5 nM) and its level may reach 2.5 nM in the neurons of  $\text{Li}^+$ -treated patients.

In BD and normal subjects, sodium (Na)–potassium (K)–adenosine triphosphatase (ATPase) activity is similar, but  $\text{Mg}^{2+}$  dependent activity of membrane Na-K-ATPase in erythrocytes is higher in BD patients than in normal patients.<sup>13</sup> Lithium treatment does not influence significantly activity of  $\text{Mg}^{2+}$ -dependent ATPase.

Carbamazepine therapy did not modify CSF concentration of this cation. The positive correlation between Na-K-ATPase activity and lithium concentration was emphasized in BD and also in the positive correlation between lithium and magnesium concentration in these patients.<sup>14</sup>

In neuroblastoma cells, an increase was observed in intracellular free  $\text{Mg}^{2+}$ , from  $0.39 \pm 0.04$  nM to  $0.6 \pm 0.04$  nM during  $\text{Li}^+$  incubation (when intracellular  $\text{Li}^+$  concentration increased from 0–5.5 nM).<sup>15</sup>

Verapamil is calcium-blocking channel drug, which proved to be useful as maintenance therapy of manic patients. The association between verapamil and magnesium was found to be significantly more effective than single verapamil in reducing manic symptoms in BD patients.<sup>16</sup> Administration of magnesium sulfate infusion in patients with severe therapy-resistant maniacal agitation improved their symptoms.<sup>17</sup>

In patients with severe maniacal agitation, the co-administration of  $Mg^{2+}$  allows the decrease of benzodiazepines or neuroleptic doses. Therapy with magnesium aspartate (40 mEq/day) leads to improvement in condition of BD patients.<sup>18</sup>

The fact that magnesium salts improved the symptoms of some BD patients argues for a role of the intracellular  $Mg^{2+}$  increment in BD drug mechanism. Changes in glutamatergic brain neurotransmission are involved in pathophysiology of psychoaffective psychoses, including BD.<sup>19</sup> We consider that symptoms in BD patients are alleviated by decreasing presynaptic release of glutamate, by partially blocking  $Ca^{2+}$  channel coupled with *N*-methyl-D-aspartate (NMDA) receptors, and by increasing intracellular concentrations of  $Mg^{2+}$ .

Calcium is an antagonist on carbamazepine effect used in the therapy of affective and schizoaffective psychoses, including BD.<sup>20</sup> This pleads for a magnesium role in supporting therapeutic effect of carbamazepine by decreasing calcium entrance through neuronal membrane. Decreasing magnesium concentrations could exacerbate the anxiety, weakness, and other symptoms in BD patients.<sup>21</sup> Increasing intracellular concentrations of magnesium in BD patients treated with lithium could not be the consequence of the effect on urinary elimination because  $Li^+$  increases  $Mg^{2+}$  urinary elimination. The  $Li^+$  effect is not the same in relation with all bivalent cations. For example, urinary elimination of  $Ca^{2+}$  is decreased.<sup>22</sup> Plasmatic concentrations of  $Mg^{2+}$  are not correlated with serum concentration other than  $Li^+$ .<sup>23</sup> Our data<sup>24</sup> have shown that treatment with carbamazepine and sodium valproate significantly increases  $Mg^{2+}$  intracellular concentration and plasmatic level of zinc. It is not known the effect of therapy in BD with atypic antipsychotics (quetiapine, ziprasidone, risperidone, olanzapine) on intracellular concentration of  $Mg^{2+}$ .<sup>25</sup>

## Major Depression (MD)

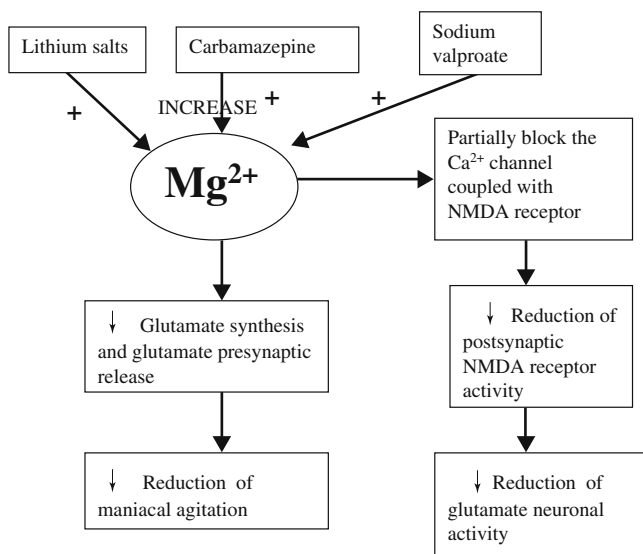
One of the difficulties linked with assessment of extra- and intracellular concentrations of bivalent cations in depression is the existence of substantial differences between MD and depressive states associated with other diseases.

Regarding only MD, data are heterogenic. In MD, in drug-free patient treatment low levels of plasmatic magnesium was found.<sup>26</sup> In patients with severe MD that committed suicide or attempted suicide,  $Mg^{2+}$  concentrations in CSF



were significantly lower.<sup>27</sup> Decreased  $Mg^{2+}$  and increased  $Ca^{2+}$  levels in the neocortex of the patients with severe MD was identified.<sup>28</sup> Decreasing plasmatic or erythrocyte magnesium concentration in patients with MD were accompanied by increasing plasmatic concentrations of calcium.<sup>29</sup> An increased Ca/Mg ratio in CSF was identified in patients with MD before treatment.<sup>30</sup> On contrary, other authors have found in drug-free depressed patients a higher plasma and erythrocyte magnesium concentration.<sup>2,31</sup> In patients with MD, changes in plasmatic or erythrocyte magnesium concentration were found, compared to healthy subjects.<sup>32-34</sup> A relationship between magnesium status and mood disorders is possible, but evidence remains inconsistent.<sup>35</sup>

There are few data referring to the influence of anti-depressant therapy on plasmatic and cellular concentration of magnesium and other cations. The effect of chronic treatment with imipramine was shown to increase plasmatic concentrations of zinc on experimentally induced depression in rats.<sup>36</sup> Difference in plasmatic  $Mg^{2+}$  concentrations in patients with MD or changes in concentrations after the drug therapy were not found.<sup>37</sup> It was shown that tricyclic anti-depressants inhibit voltage-dependent calcium channels and decrease intracellular concentrations of calcium.<sup>38</sup> Increasing magnesium concentration may contribute to reducing  $Ca^{2+}$  entrance in neurons. In our studies of drug-free patients diagnosed with MD (Hamilton score > 23), the intraerythrocytic magnesium level is significantly lower compared to control subjects. A decrease in plasmatic zinc level ( $0.68 \pm 0.09$  mg/L in MD subjects vs.  $0.99 \pm 0.11$  mg/L in control group;  $p < 0.01$ ) is associated with decreasing of magnesium intraerythrocytic level. Also, a significant increase in copper plas-



**FIGURE 30.1.**  $Mg^{2+}$  involvement in mechanism of action of mood stabilizers used in BD treatment.

**TABLE 30.1.** Influence of amitriptyline and sertraline on intraerythrocytic  $Mg^{2+}$  level in MD patients.

Group	Control	MD patients before treatment	MD patients + amitriptyline	MD patients + sertraline
Intraerythrocytic magnesium (mg/L)	59.1 ± 3.2*	44 ± 2.7	57.6 ± 4.5*	56.9 ± 5.32*

\* $p < 0.01$  versus MD group before treatment.

matic level can be noticed in MD patients. Magnesium plasmatic concentration suffered no significant variations in MD. Calcium plasmatic concentrations are not influenced in MD. Our data show that amitriptyline therapy (tricyclic anti-depressant drug),  $3 \times 25$  mg/day per os, 4 weeks, as well as sertraline (Zoloft®) (serotonin re-uptake inhibitor), 150 mg/day per os, 4 weeks, determine a significant increase in magnesium intraerythrocytic levels in two different groups of patients with MD, the patients being in a drug-free state before the initialization of this treatment.<sup>39</sup> This effect is shown in Table 30.1. We consider that the increasing magnesium intraerythrocytic and zinc plasmatic level effect of amitriptyline and sertraline represents a component of its anti-depressant mechanism of action. Some anti-depressant drugs decrease  $Ca^{2+}$  concentrations. It is considered that this might be a mechanism of action for these drugs.<sup>40</sup> The fact that anti-depressant drugs (with different chemical structures and different mechanism of action) has a similar influence on the concentration of bivalent cations (significantly increases intraerythrocytic  $Mg^{2+}$  and plasmatic  $Zn^{2+}$ ) pleads for including changes in some bivalent cations concentrations in the anti-depressant mechanism of action of these drugs.

## Schizophrenia (SCH)

There are various and sometimes divergent data about plasmatic and intracellular concentrations of  $Mg^{2+}$  and other cations in patients with schizophrenia. In some studies, the schizophrenic patients shown lower  $Mg^{2+}$  plasmatic levels compared to normal subjects.<sup>21,41</sup> Plasmatic level of magnesium and calcium were not found modified in patients with untreated schizophrenia in other studies.<sup>14,42,43</sup> On the contrary, other authors found an increased magnesium level in CSF in patients with schizophrenia.<sup>27,31</sup>

Our research<sup>43</sup> show that both typical (haloperidol, 8 mg/kg/day, 3 weeks) and atypical (risperidone, 6 mg/kg/day, 3 weeks) anti-psychotic drugs produce a significantly increase in intracellular concentrations of magnesium in adult patients with paranoid schizophrenia. Our data are in agreement with authors that shown haloperidol (typical anti-psychotic) increases intraerythrocytic level of magnesium.<sup>45</sup> There are several hypotheses regarding schizophrenia pathophysiology. One of these hypothesis is the glutamatergic hypothesis, which suggests that schizophrenia is determined by an imbalance between

glutamatergic and dopaminergic mediation in some brain areas.  $Mg^{2+}$  ions block the  $Ca^{2+}$  ion channel of NMDA receptors<sup>46</sup> and reduces the activation of glutamatergic systems in the brain. It is considered by some authors that a high level of free radicals is involved in the pathogeny of acute psychoses. Other possible way that increased intracellular  $Mg^{2+}$  and plasmatic  $Zn^{2+}$  might influence SCH evolution is decreasing formation of peroxidic radicals.

We consider that increasing intracellular magnesium concentration is an important part of the mechanism of action of some typical and atypical antipsychotics. Intra- and extracellular concentrations of magnesium and other bivalent cations may be considered as a biological marker for some psychoses. We consider that a low level of intracellular  $Mg^{2+}$  correlated with a raised plasmatic ratio ( $Cu^{2+}/Zn^{2+}$ ) might be a severity indicator of MD and SCH.

In all of the psychoses described above, we have observed a good correlation between normalization of intra-erythrocytic magnesium level/plasmatic zinc level and clinical evolution of the patient during drug therapy.<sup>47</sup> Periodical assesment of intraerythrocytic magnesium level correlated with plasmatic zinc and copper concentrations may offer a guidance for the efficiency of applied pharmacotherapy.

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# **Veterinary Medicine**

# 31

## Significance of Magnesium in Animals

Tohru Matsui

Magnesium (Mg) metabolism differs among animal species because the digestive system and feeds are different. The diseases related to Mg nutrition are rare in pigs and poultry under practical conditions because their diets are formulated as containing an appropriate level of Mg. On the other hand, Mg deficiency is not rare in grazing animals because Mg in pasture is affected by several factors such as soil and plant species and maturity, and thus Mg concentration is largely varied in pasture. Grass tetany in ruminants is induced by the reduction of Mg absorption resulting from low Mg intake with high potassium and nitrogenous compounds, and with the reduction of ruminal fermentation. Additionally, cold stress stimulates the incidence of tetany through decreasing Mg concentration in the cerebrospinal fluid. Excess Mg is one of the factors inducing urolithiasis in cats and cattle, and enterolithiasis in horses. However, Mg level in the practical diets alone cannot induce these diseases. Cat urolithiasis is developed in combination with alkaline urine, and cattle urolithiasis and horse enterolithiasis are developed in combination with high phosphorus intake. The diseases related to Mg nutrition are mainly developed in combination with other dietary factors and/or environmental factors in ruminants, horses, and cats.

### Comparative Aspects of Magnesium Metabolism

The digestive system is widely different among animal species. Herbivores consume high fibrous feeds (rich in cellulose) originated from plants and their digestion is largely owing to microbial fermentation in the rumen (a forestomach) of ruminants, such as cattle and sheep, or in the large intestine of monogastric herbivores, such as horses and rabbits. These sites of microbial digestion are large because longer transit time is necessary for sufficient fermentation. Carnivores natively obtain most of their feed by eating other animals, and their digestion is mainly owing to digestive enzymes and microbial digestion is minimal. Thus, the alimentary tract is short and simple in carnivores. In omnivores such as dogs, pigs, and rats, both enzymatic and microbial



digestion are important and the structure of digestive tract is intermediate between carnivores and herbivores.

Apparent absorption of magnesium (Mg) is different among animal species (Table 31.1). Magnesium absorption is generally lower in ruminants than in other animals. In ruminants, sheep absorb Mg 1.7-times more efficiently than cattle because the ratio of surface area to content is higher in the major site of Mg absorption of sheep.<sup>1</sup> The efficiency of Mg absorption is higher in horses than in ruminants. Magnesium absorption is less in pigs than in rats. Dietary phytate (inositol hexaphosphate) is known to decrease Mg solubility in intestinal digesta of monogastric animals and suppresses Mg absorption.<sup>2</sup> Practical diets of pig contain whole grains, oilseed meals, and bran that are rich in phytate, but rats are usually given semipurified diets without containing phytate. Thus, Mg absorption may be different between these omnivore species. Because cats have a relatively short intestine resulting in a rapid passage rate of digesta, the digestibility for many natural feedstuffs is generally lower in cats than in rats and dogs.<sup>3</sup> The absorption of Mg also may be less in cats than in rats.

Ruminants mainly absorb Mg from the rumen.<sup>4</sup> The major site of Mg absorption is the distal small intestine<sup>5</sup> or the ileum and the colon<sup>6</sup> in rats. There have been few reports showing the major site of Mg absorption in other domestic animals. Pigs were reported to absorb Mg in the ileum and the colon.<sup>7</sup> Cats and dogs predominantly absorbed Mg from the large intestine.<sup>8</sup> The major site of Mg absorption was the small intestine in horses<sup>9,10</sup> and rabbits.<sup>11</sup>

There are two pathways for Mg absorption, that is, paracellular route and transcellular route.<sup>6</sup> The paracellular route consists of tight junctions and intercellular space between the epithelial cells, which depends on the passive driving force and the permeability of this route for Mg. The transcellular route consists of the influx through the apical membrane of epithelial cells and the

**TABLE 31.1.** Magnesium absorption in some species.

	Apparent absorption (% of intake)	Major site of absorption
Cattle	23.3 ± 7.8 <sup>a</sup>	Forestomach (rumen)
Sheep	32.8 ± 9.0 <sup>b</sup>	Forestomach (rumen)
Horse	51.2 ± 5.6 <sup>c</sup>	Small intestine
Pig	39.2 ± 9.7 <sup>d</sup>	Ileum and colon
Rat	60.8 ± 14.5 <sup>e</sup>	Ileum and colon
Cat	39.3 ± 12.9 <sup>f</sup>	Large intestine

Values are mean ± standard deviation (SD).

<sup>a</sup>Calculated from 35 publications in dairy cattle given diets containing appropriate amounts of magnesium and potassium.

<sup>b</sup>Calculated from 37 publications in lambs given diets containing appropriate amounts of magnesium and potassium.

<sup>c</sup>Calculated from seven publications in horses given diets containing sufficient amounts of magnesium.

<sup>d</sup>Calculated from 12 publications in growing pigs given diets containing sufficient amounts of magnesium.

<sup>e</sup>Calculated from 27 publications in growing rats given AIN diets.

<sup>f</sup>Calculated from 11 publications in cats given diets containing sufficient amounts of magnesium.

efflux across the basolateral membrane. The Mg concentration was 5 mM in the liquid phase of ileal digesta of pigs given a conventional diet<sup>12</sup> and the luminal Mg concentration ranged between 4 and 13 mM in the rumen.<sup>13</sup> The intracellular concentration of ionized Mg ranged between 0.5 and 1.0 mM in ruminal epithelial cells<sup>14</sup> and between 0.4 and 0.7 mM in Caco-2 intestinal cells.<sup>15</sup> Additionally, ionized Mg concentration is 0.4 to 0.6 mM in blood. Therefore, the intracellular concentration of ionized Mg is generally considered lower than its luminal concentration and is close to its concentration in blood. The entry of ionized Mg into the epithelial cells does not require energy but the efflux is energy dependent.<sup>8</sup>

Coudray and colleagues<sup>16</sup> suggested that the active transport of Mg was important only under conditions of extremely low dietary Mg in rats because the amount of absorbed Mg linearly increased with increasing dietary Mg up to the requirement level. Some researchers also reported that Mg was primarily absorbed by a passive diffusion at usual Mg intake in rats.<sup>17,18</sup> Additionally, metabolic inhibitors and an adenosine triphosphatase (ATPase) inhibitor did not affect transepithelial Mg transport, which also supported passive transport as the major route of Mg absorption.<sup>19</sup>

As reviewed by Schweigel and Martens,<sup>8</sup> ruminants mainly absorb Mg through transcellular route across the rumen epithelium by the secondary active transport. Ruminants obtain dietary energy as volatile fatty acids produced by ruminal microbes. Additionally, ruminal microbes degrade dietary nitrogenous components (protein and nonprotein nitrogen) to ammonia and they reconstitute protein from ammonia. The microbial protein largely contributes to protein nutrition of ruminants. Magnesium absorption increases with increasing readily fermentable carbohydrates in diets. The ingestion of readily fermentable carbohydrates rapidly raises ruminal concentration of volatile fatty acids and  $\text{CO}_2/\text{HCO}_3^-$ , which stimulate directly Mg uptake by the epithelial cells. The high intake of nitrogenous substances increases ruminal ammonia concentration through the fermentation because the degradation of dietary protein is higher than microbial protein synthesis in this condition, which transiently reduces Mg absorption. Additionally, the reduction of ruminal pH increases Mg absorption through rising Mg solubility. The concentrations of volatile fatty acids and ammonia affect ruminal pH and Mg absorption. The high concentration of potassium (K) in the rumen largely and directly suppresses Mg uptake by the epithelial cells.

Magnesium is endogenously excreted in both urine and feces. Urinary Mg excretion was largely more than the endogenous fecal excretion in sheep<sup>20</sup> and cats<sup>21</sup> given Mg at the requirement level. On the other hand, the endogenous fecal excretion was as much as urinary excretion in rats.<sup>16</sup> Horses secreted a half of absorbed Mg into the large intestine<sup>10</sup> or the endogenous fecal loss of Mg was more than urinary excretion in horses given Mg at its requirement level.<sup>22</sup> Urinary Mg excretion increases with dietary Mg in most animals and it is known that there is a good correlation between Mg absorption and its urinary excretion. The endogenous excretion into feces was reported to

increase with increasing dietary Mg in rats<sup>16</sup> and in sheep.<sup>23</sup> However, the relationship between dietary Mg and its endogenous excretion into feces is still controversial.

The concentration of Mg is approximately 5 mM in cow's milk. A cow producing 30 kg of milk would loss 150 mmol Mg/day into the milk, which approximately corresponds to half of absorbed Mg.<sup>24</sup> The Mg concentration in milk was relatively stable in Mg-deficient cows but the milk production decreased.<sup>25</sup> Rats secreted Mg into milk at approximately 40% of apparently absorbed Mg.<sup>26</sup> The Mg concentration was approximately 1.6 mM in mare's milk and mares lost Mg at 12 to 40 mmol/day into milk.<sup>27</sup> Lactating mares absorb 160 mmol Mg/day when dietary Mg is satisfied with its requirement.<sup>28</sup> Therefore, the absorbed Mg is fourfold more than its secretion into milk in horses. Lactation stimulates Mg absorption due to increasing feed intake and efficiency of Mg absorption. The positive balance of Mg may be maintained even in lactating cows when a sufficient amount of Mg is given.

## Magnesium Deficiency

The main manifestation of Mg deficiency induces retarded growth, hyperirritability and tetany, peripheral vasodilation, anorexia, muscular incoordination, and convulsion.<sup>29</sup> Typical ingredients of feeds contain sufficient amounts of Mg and thus practical diets usually contain adequate Mg in many species and Mg deficiency is rare. Although the Mg concentration in forages generally satisfies its requirement of ruminants, the Mg concentration varies largely with plant species, maturity of plants, and with the soil and climate in which plants are grown. Therefore, Mg in forages is occasionally low and hypomagnesemia is observed in grazing herbivores. Hypomagnesemia in ruminants is classified into a rapidly developing type and a slowly developing type.<sup>30</sup>

### *Acute Type of Hypomagnesemia (Grass Tetany)*

Grass tetany results from hypomagnesemia that occurs suddenly in early spring just after the initiation of grazing.<sup>29</sup> Grass tetany is seldom developed in horses grazing pastures that develop grass tetany in cattle.

The reduction of Mg absorption is considered as a major factor of the pathogenesis, which results from low Mg in diets and factors reducing Mg bioavailability. Grass tetany is found in areas where dairying or beef production is highly developed. Pastures in areas with intensive livestock production are generally rich in K and nitrogenous components due to frequent fertilization with manure. Grazing cattle on such pastures entails the risk of hypomagnesemia, primarily due to K-suppressing Mg absorption.<sup>31</sup> Additionally, excess nitrogenous components raise ruminal ammonia concentration, which reduces Mg absorption.<sup>31</sup>

The feed intake is reduced by the rapid change of environment after the initiation of grazing, which decreases Mg absorption. Lactating cows are more susceptible to development of grass tetany because of Mg secretion into milk. Furthermore, the susceptibility to grass tetany is increased in order ruminants. Bone Mg concentration is lower in order animals. The reduction of available Mg in bone was possibly related to the higher incidence of grass tetany in older cows.<sup>32</sup> The administration of a pyrophosphate analogue suppressed bone resorption in sheep, which did not affect plasma Mg concentration in sheep given an adequate amount of Mg<sup>33</sup> but the pyrophosphate analogue enhanced the reduction of plasma Mg concentration in sheep given a Mg-deficient diet.<sup>34</sup> On the other hand, Robson and colleagues<sup>35</sup> reviewed the relationship between bone resorption and the plasma Mg concentration and they suggested that bone Mg was not important for maintaining the plasma Mg concentration. Therefore, the relationship between bone resorption and the incidence of hypomagnesemia is not clear in old ruminants. The Mg absorption was low in old ruminants,<sup>36</sup> which may be related to higher incidence of grass tetany in older animals.

The onset of grass tetany is more closely associated with the Mg concentration in the cerebrospinal fluid (CSF) than with blood Mg. The Mg concentration in CSF was lower in clinically affected cows than in nontetanic cows but plasma Mg concentration was almost similar between them.<sup>37</sup> The low Mg concentration in CSF was associated with alterations in monoamine concentrations in the central nervous systems that played an important role in both voluntary and involuntary motor function.<sup>38</sup> Therefore, the disturbance of monoamine concentrations was considered to play a role in the etiology of hypomagnesemic tetany.

The Mg concentration is higher in CSF than in plasma and the difference of Mg concentration is generated by its active transport. Mild hyperkalemia lowered Mg concentration in CSF of sheep.<sup>39</sup> Therefore, Mg influx into CSF may be inhibited by high concentration of K in blood. The initiation of grazing in early spring stresses animals through the rapid changes in environment. A stress reaction involving the adrenal–glucocorticoid axis increased circulating K concentration and lowered Mg transport across the choroidal plexus, which was one of causes of this disease.<sup>35</sup> Thus, dietary and environmental factors synergistically develop grass tetany.

### ***Slow Type of Hypomagnesemia***

Subclinical hypomagnesemia is observed in cattle for several months especially during winter. When a plasma Mg concentration reaches critically low level, it is accompanied by clinical symptoms such as moderate incoordination, tetany, and hyperirritability. This disease is called winter tetany.<sup>40</sup> Winter tetany is developed by low dietary Mg, low quality of feeds, and environmental stresses such as extremely cold and wet weather.<sup>40</sup> The reduction of energy intake suppresses ruminal fermentation, which decreases volatile fatty acids

and CO<sub>2</sub> concentrations, and elevates ammonia concentration in the rumen. Additionally, the reduction of energy intake increases ruminal pH because of high ammonia and low volatile fatty acid concentrations in the rumen. These changes decrease Mg absorption in the rumen.

Hypomagnesemia is observed in calves consuming whole milk for an extended period, particularly calves suckling cows that are subclinically hypomagnesemic. This disease is called milk tetany.<sup>40</sup> The etiology of this disease is a simple deficiency of Mg. The Mg requirement was calculated as 1.3 g/kg dry matter in a 75-kg suckling calf gaining at 1 kg/day.<sup>41</sup> Magnesium concentration was 1 g/kg dry matter in milk.<sup>42</sup> Therefore, the fast-growing calves may be susceptible to development of milk tetany. Additionally, Mg absorption decreases with growth, that is, 70% for a 50-kg calf and 30% for 75-kg calf<sup>41</sup> and hypomagnesemia occurs in older suckling calves.

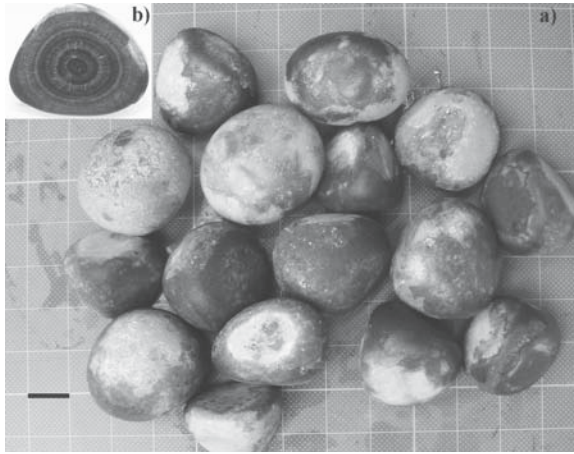
Spontaneous atherosclerosis (AS) is considered to occur in almost all animal species, including wild ruminants. Although AS is rare in adult ruminants, AS is developed in calves consuming whole milk for an extended period. A sclerosis of arteries was also found in calves given artificial magnesium-deficient diets. The margarine-fed calves developed hypomagnesemia and severe arteriosclerosis that could be prevented with Mg supplementation. Thus, Mg deficiency is considered as a trigger of AS in calves.<sup>43</sup>

## Excess Magnesium

Magnesium toxicosis has not been reported and does not appear in many animals given natural feedstuffs but would be most likely to occur using excess supplementation with Mg. On the other hand, excess Mg may induce urolithiasis of ruminants (Figure 31.1) and cats, and enterolithiasis of horses (Figure 31.2) in practical conditions. Excess Mg-induced uroliths are com-



**FIGURE 31.1.** Uroliths in the bladder and urinary duct of beef cattle. (Courtesy of H. Yano, Kyoto University, Kyoto, Japan.)



**FIGURE 31.2.** Enteroliths from the large intestine of a horse. Transverse section of an enterolith. Scale bar = 2 cm. (Courtesy of Y. Tajima and T. Ueno, Equine Research Institute, Japan Racing Association, Utsunomiya, Japan.)

posed of Mg ammonium phosphate (P) and are determined as struvite (Mg ammonium P hexahydrate) in cats and dogs. Crystallization of Mg ammonium P depends on the urinary concentration of its components, that is, the product of  $[Mg^{2+}] \times [NH_4^+] \times [PO_4^{3-}]$  and urinary pH affecting  $NH_4^+$  and  $PO_4^{3-}$  concentrations.<sup>44</sup>

Urolithiasis is an important clinical problem, especially in dogs and cats. Uroliths are mainly composed of struvite or calcium oxalate in these animals. Although the formation of struvite stone in dogs usually results from a urinary tract infection with urea-splitting microbes that increase urinary ammonium concentration, the majority of struvite uroliths are observed in sterile cats forming sterile urine.<sup>45</sup> Thus, the diet is the major causal factor inducing urolithiasis in cats. Since the cat evolved as a desert animal, it has the capacity to produce highly concentrated urine in order to conserve water. Cats usually consume diets rich in animal protein, which produces net amounts of acid from sulfur amino acids and acidifies urine. Urinary P and ammonia concentrations are higher in cats than in other animals, although urinary pH and Mg may be lower in cats than in other animals (Table 31.2).

Urinary Mg excretion increases with Mg intake in animals including cats. Excess Mg is linked to struvite urolithiasis. The dietary Mg over 0.35%

**TABLE 31.2.** Urinary concentrations of factors affecting struvite formation in some species.

	Magnesium mM	Phosphorus mM	Ammonia mM	pH	References
Cow	19	0.9	10.5	8.3	Vagnoni et al. <sup>46</sup>
Rat	19	12	45	6.97	Amanzadeh et al. <sup>47</sup>
Cat	3.1	81	118	6.37	Cottam et al. <sup>48</sup>

produced struvite urolithiasis.<sup>49</sup> Pet food industries reduce Mg content in commercial cat foods and Mg concentration ranges from 0.05% and 0.3% in many commercial cat foods. Thus, dietary Mg is not critical at present. Nevertheless, excess Mg should be avoided and low-Mg diets are considered advantageous in the prevention of struvite urolithiasis. Cats produce acidic urine with a pH of 6.0 to 7.0 in the normal situation. Struvite remains largely in solution below pH 6.6, while the crystallization may occur spontaneously if the urinary pH rises above 7.1.<sup>50</sup> High levels of dietary Mg did not induce urolithiasis when acidic urine was produced.<sup>51</sup> Therefore, urinary pH may be more important factor than the urinary Mg concentration in cats. However, urine acidification together with a low Mg intake increases the risk of calcium oxalate urolithiasis in cats.<sup>52</sup>

Ruminants ingest plant materials that contain large amounts of K and organic anions. Organic anions are protonated during catabolism and then oxidized to water and CO<sub>2</sub> (base forming), leaving K and HCO<sub>3</sub><sup>-</sup> to be excreted into urine. Therefore, urinary pH is higher in ruminants than in some other animals (Table 31.2). The alkaline urine may also stimulate the development of urolithiasis in ruminants. Although the urinary Mg concentration is not high in cattle, excess Mg increases its concentration. Urinary P and ammonia concentrations are low in ruminants because they excreted mainly P and ammonia into the digestive tract via saliva (P and ammonia) or via transport across ruminal wall (ammonia). However, excess P and protein increase urinary P and ammonia. A field survey indicated that dietary Mg was positively correlated with both the morbidity and mortality rates due to urolithiasis in fattening lambs.<sup>53</sup> However, some researchers suggested that high dietary Mg per se did not develop urolithiasis and that high dietary P was required for the urolith formation in calves<sup>54</sup> and lambs.<sup>55</sup>

Enterolithiasis is a serious problem in horses. The enteroliths consist primarily of Mg ammonium P. Prominent clinical features were recurrent mild abdominal pain, gaseous distension, and minimal intestinal motility. Most obstructing enteroliths were found near the beginning of the small colon. Horses with enterolithiasis represented 15.1% of patients admitted for treatment of colic, and 27.5% of patients undergoing celiotomy for treatment of colic.<sup>56</sup> Enteroliths ranged from 200 g to 9 kg, but generally weighed 450 g to 3 kg.<sup>57</sup> Horses secrete Mg and P into the large intestine, which may stimulate the incidence of enterolithiasis. Wheat bran was reported as a dietary factor inducing enterolithiasis because of its high concentration of P and Mg.<sup>58</sup>

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