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*The contents in this manual are being continuously updated*

# RESEARCH MANUAL

# INNER7®

## BIO-MINERAL COMPLEX

INNOVATION LABO
Next Stage >>

Innovation Labo, Kanaya Bldg 5F,
4-11-3 Hatchobori Chuo-ku Tokyo 104-0032 JAPAN

# CLINICAL TRIALS AND TESTINGS LABORATORIES

**JAPAN FOOD RESEARCH LABORATORIES**

52-1 Motoyoyogi-cho Shibuya-ku

Tokyo 151-0062 Japan

**MEDICA TOKYO LABORATORIES**

20-1, 3Chome Nishi-Shinjuku

Shinjuku-ku, Tokyo JAPAN

Managed by: Dr. Taro Hirata

**INNOVATION LABO TOKYO**

Kanaya Bldg 5F, 4-11-3 Hatchobori

Chuo-ku Tokyo 104-0032 JAPAN

Managed by: Dr. Ikeda Yuki

SUMMARY

# 1. INNER7® COMPLEX

INNER7® is a balanced complex of minerals from deep sea water from a depth of more than 300 meters.

INNER7® plays 2 crucial roles in the body:

- Supports a balanced PH in the body, also reduce the size of intestine and belly by flushing out excess water and smooth transit.

- Supports body's fat burning system (without minerals, our bodies would be unable to efficiently use the carbohydrates, proteins, and fats in our diet).

## 1.1. GENERAL INFORMATION

INNER7® contains bio-minerals (mainly Magnesium, Calcium, Potassium, Selenium, Zinc and Copper) which tend to be deficient in our modern-day bodies. Each mineral is essential for proper functioning of the body and has it owns benefit and type of action:

- Magnesium helps convert blood sugar to energy, and is a natural anti-stress, it attract water to the intestines as soon as digested and reduce constipation by having this water into the intestines.
- Calcium has an effect on reducing body acidity.
- Selenium has effect on Vitamin C regeneration and protects micro-circulation.
- Potassium regulates the heart and muscles, assuring smooth signal transmission.
- Copper regulates blood cholesterol.

INNER7® contains the magnesium as core ingredient for immediate efficacy that customers can feel from the first intake (over dosage will cause diarrhea). Its minerals come from deep see water which offer an unmatched quality and purity. It has a much smaller chance of exposure to pollution from bacteria or chemical substances. Deep see water contains over than 60 minerals and trace mineral like chromium, vanadium which have great impact on sugar metabolism. The complex has high bio-availability and gives results within 10-15 minutes.

## 1.2. INNER7® ORIGIN

INNER7® water is collected in Japan sea territory, at a distance of 2200m off Muroto City, Kochi Prefecture. The collection of Deep-Seawater is done through a 2,650m pipe from a depth of 321m.

This area has been designated by Japanese government to be a model zone for research and development of deep-seawater.

**Production**

Pure deep sea water is condensed by evaporation during 1 week, which gives a condense salin water. The solution is concentrated 6 times the sea water. During this process the sodium chloride is eliminated by ions exchange filtration where the positive ions of sodium are separated from the solution.

Then a slow process of boiling during 48H is carried out, to get a final concentrate which contains more than 60 minerals, with magnesium as main mineral. 100L of Deep Sea Water produce 1L of INNER7®.

## 1.3. INNER7® COMPOSITION

INNER7® contains more than 60 minerals: Chloride, Potassium, Sodium, Calcium, Magnesium, Phosphorus, Strontium, Boron, Silicon, Iron, Chromium, Lithium, Antimony, Zinc, Copper, Molybdenum, Selenium, Nickel, Phenium, Manganese, Tungsten, Thallium, Tin, Platinum, Thorium, Titanium, Palladium, Praseodymium, Vanadium, Neodymium, Aluminum, Barium, Erbium, Tellurium, Gold, Beryllium, Europium, Terbium, Lanthanium, Samarium, Dysprosium, Lutetium, Rhodium, Silver, Scandium, Thillium, Bismuth, Germanium, Indium, Cobalt, Gadolinium, Cerium, Tantalum...

Taking only one type of mineral in large dosages is not healthy. Since the minerals influence each other, it is important to take many minerals in the proper amount.

| Mineral | For 1L |
|---|---|
| Magnesium | 55,000 mg / L |
| Potassium | 17,000 mg / L |
| Calcium | 110 mg / L |
| Selenium | <0.05 mg / L |
| Zinc | 0.12 mg / L |
| Iron | <1 mg / L |
| Copper | 0.06 mg / L |
| Phosphorus | <1 mg / L |
| Manganese | 0.03 mg / L |
| Sodium | 44,000 mg / L |

## 1.4. DOSAGE AND BIOAVAILABILITY

INNER7® recommended dosage is 2ml per day. It can be mixed in a bottle of 1L ~ 2L of plain or aromatized water.

The magnesium allows an immediate efficacy that customers can feel from the first intake (over dosage will cause diarrhea). As product have fast activity, it is better to take it on wake-up time, as the first thing to do in the morning, "an Internal Shower" this will speed up going to WC before leaving home.

Minerals in INNER7® are in a natural form easily absorbed by the body and are balanced for a complete and immediate assimilation in the digestive tract.

Magnesium in INNER7® is totally ionized as free magnesium. This magnesium chloride form presents the higher bioavailability. Uses of poorly ionized forms of magnesium result in ineffective magnesium supplementation (only 4% on average). If we change the formulation, the mineral complex balance will be affected and the absorption of magnesium will decline.

Magnesium chloride (formula $MgCl_2$) is totally ionized form of magnesium. It means that we assimilate magnesium not as the magnesium chloride compound, but rather as free magnesium and chloride ions, close to magnesium in our diet, allowing great bioavailability. Sea water have a similar concentration of magnesium to human body, and this explain the importance of magnesium to humans metabolism as its used for a lot of enzymatic reactions.

INNER7® is not only composed of magnesium chloride as other products in the market, it is a complex of minerals which works in synergy for better efficacy, also the fact that it is a complex of several type of minerals allow the intestines to absorb them in optimal dosage. When we used heat to dry INNER7® and get it into powder form, the chloride will crystalize and absorption of magnesium is reduced.

## 1.5. SIDE EFFECTS AND RECOMMENDATIONS

Minor side effects of taking these magnesium supplements are common, and may include light diarrhea and abdominal cramping, and nausea. Magnesium supplements may possibly be unsafe when taken in large doses or when used by people who have specific health problems.

**When you should avoid taking INNER7®**

Magnesium supplements are contraindicated when used by people with following health problems:

- **Kidney Failure:** Special precautions should be taken by people who have kidney disease, since these conditions may cause failure for the kidneys to eliminate excess magnesium from the body. Magnesium competes with calcium for absorption and can cause a calcium deficiency (hypomagnesaemia) if calcium levels are already low.
- **Heart block:** Another condition that requires precaution is for people who have heart blocks, a type of heart rhythm irregularity, since magnesium influences electrical conduction and contraction of the heart. Same for other types of heart rate irregularities: slow heart rate, atrial fibrillation and myasthenia.
- **High blood pressure:** INNER7® magnesium supplementation associated with high blood pressure increase the risk of negative effects, such as abnormal heart rhythms and changes in mental status

**Drug interactions**

INNER7® supplementation may not be safe for people under medication due to possible drug interactions: Magnesium may reduce the absorption and the effects of some antibiotics (take the antibiotics at least 2 hours before, or 4 to 6 hours after taking magnesium supplements).

INNER7® should not be taken with medications for high blood pressure (specifically calcium channel blockers), medications for muscle relaxation, potassium-sparing diuretics or "water pills".

**Special precautions**

Not recommended for pregnant or lactating women. Do not exceed the recommended daily dosage

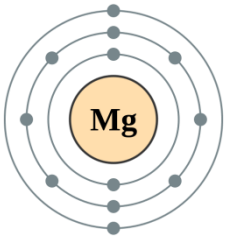
# 2. MAGNESIUM - CHARACTERISTICS

## 2.1. MAGNESIUM UNIQUE PROPERTIES

**Magnesium** is a chemical element with symbol Mg and atomic number 12. It is an alkaline earth metal and the eighth most abundant element in the Earth's crust and ninth in the known universe.

**Chemical Properties of Magnesium**

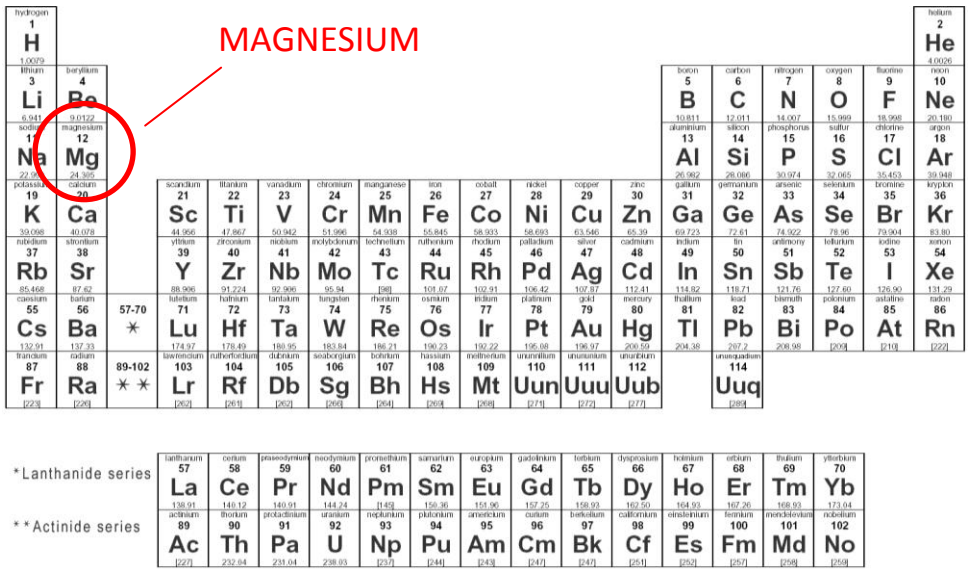
Magnesium is located among the alkaline earth metals on the periodic table. This element belongs to the group 2 and period 3 of the periodic table and has the atomic number 12. The average atomic mass of the element magnesium is 24.305.




*Electron configuration*

Because of its ready solubility in water, magnesium is the third most abundant mineral in sea water, after sodium and chloride. In the human body, magnesium is the eleventh most plentiful element by mass—measuring about two ounces. Most magnesium contained in the body is found in the skeleton and teeth—at least 60 to 65 percent of the total. Nearly the entire remaining amount resides in muscle tissues and cells, while only one percent is contained in our blood.

**PERIODIC TABLE**



| Magnesium | Properties |
|---|---|
| **Atomic number** | 12 |
| **Atomic mass** | 24.305 $g.mol^{-1}$ |
| **Electronegativity according to Pauling** | 1.2 |
| **Density** | 1.74 $g.cm^{-3}$ at 20 °C |
| **Melting point** | 650 °C |
| **Boiling point** | 1107 °C |
| **Vanderwaals radius** | 0.16 nm |
| **Ionic radius** | 0.065 nm |
| **Isotopes** | Mg has 21 known isotopes ranging from Mg-20 to Mg-40. Mg has 3 stable isotopes: Mg-24, Mg-25 and Mg-26 |
| **Electronic shell** | [Ne] $3s^2$ |
| **Energy of first ionisation** | 737.5 $kJ.mol^{-1}$ |
| **Energy of second ionisation** | 1450 $kJ.mol^{-1}$ |
| **Standard potential** | - 2.34 V |
| **Element Classification** | Alkaline Earth Metal |
| **Appearance** | lightweight, malleable, silvery-white metal |
| **CAS Registry Number** | 7439-95-4 |
| **Discovered by** | Magnesium was discovered by Joseph Black in England in 1775. The element was isolated by A.A.B. Bussy and Sir Humphry Davy in 1808. |
| **Name Origin** | Greek: From Magnesia a district of Thessaly |

**Physical properties of magnesium**

About 2.1 percent of the earth's crust contains magnesium. This volume makes magnesium the 6th most found element. One of the biggest deposits of magnesium is found in the sea water. It has been calculated by the scientists, that a cubic mile of seawater contains about six million tones of magnesium. As mentioned in the chemical properties, magnesium is also present in many other compounds like dolomite, magnesium carbonate that is also known as magnesite and magnesium sulfate which is also known by the name epsomite.

One readily accessible and easily absorbed form of magnesium is magnesium chloride. Because it is soluble in water, magnesium chloride readily dissociates, increasing rate of absorption.

**Magnesium in body**

Magnesium is the fourth most abundant mineral in the body and is essential to good health. Approximately 50% of total body magnesium is found in bone. The other half is found predominantly inside cells of body tissues and organs. Only 1% of magnesium is found in blood, but the body works very hard to keep blood levels of magnesium constant.

Certainly one of the most important nutrients needed by the human body, magnesium is needed for more than 300 essential biochemical reactions, including protein synthesis, testosterone production, insulin sensitivity, calcium absorption, and regulation of the sympathetic nervous system.

**Magnesium function in body**

Magnesium is the second most abundant element inside human cells. Within the body's cells, it serves literally hundreds of functions. Magnesium is a macro-mineral, which, unlike trace minerals, is needed by the body in large amounts. Calcium, sodium, and potassium are also macro-minerals. The average human body contains about 25 grams of magnesium, one of the six essential minerals that must be supplied in the diet.
Once magnesium enters the body through food, supplements, or topical applications, it is broken down and released to form independent magnesium atoms, or "ions". In its ionic form, magnesium has a positive charge, commonly noted as $Mg^{2+}$.

The importance of magnesium ions for all life itself, as well as for overall vibrant health, is hard to overstate. Magnesium is required to give the "spark of life" to metabolic functions involving the creation of energy and its transport (ATP, the body's fundamental energy currency), and the creation of proteins—the nucleic acid chemistry of life—RNA and DNA, in all known living organisms. In plants, a magnesium ion is found at the center of every chlorophyll molecule, vital for the creation of energy from sunlight. Magnesium is an essential element for both animals and plants, involved in literally hundreds of enzymatic reactions affecting virtually all aspects of life.

Every single cell in the human body demands adequate magnesium to function, or it will perish. Strong bones and teeth, balanced hormones, a healthy nervous and cardiovascular system, well-functioning detoxification pathways and much more depend upon cellular magnesium sufficiency. Soft tissue containing the highest concentrations of magnesium in the body include the brain and the heart—two organs that produce a large amount of electrical activity, and which can be especially vulnerable to magnesium insufficiency.

Magnesium works in concert with calcium to regulate electrical impulses in the cell—magnesium concentration inside healthy cells is ten thousand times greater than calcium, and there are crucial reasons for this safeguard. Cellular calcium channels allow that mineral to enter the cell only as long as needed to conduct an impulse; it is ushered out immediately by magnesium once its task is

fulfilled. This vigilance is necessary to prevent calcium accumulation in the cell, which could cause dangerous hyper-excitability, calcification, cell dysfunction and even cell death. When excess calcium enters the cells because of insufficient magnesium, muscle contraction is sustained for too long, and we suffer, for example, twitches and tics in mild cases. When magnesium deficiency becomes chronic, we suffer the symptoms of heart disease such as angina pectoris, hypertension and arrhythmia, or the spasms and contractions characteristic of asthma, migraine headache or painful menstrual cramping.

Magnesium operates as a natural calcium channel blocker and is responsible for relaxation—counter to calcium's contraction. Thus magnesium is pivotally important to the healthy functioning of our parasympathetic nervous system. It may be hard to believe, but our bodies were actually designed to operate for the most part in a calm, relaxed parasympathetic state, rather than in the heart-pounding, stress- and adrenaline-driven mode of sympathetic nervous system dominance that is nearly constant for many of us today, and which uses up great quantities of magnesium.

Magnesium is so important to so many vital body functions, and its deficiency is integrally involved in so many diseases, that more than one researcher has dubbed magnesium a miracle in its ability to resolve or improve numerous disorders. The current list of disorders with direct and confirmed relationships to chronic and acute magnesium deficiency is long, and includes many diseases whose conventional medical treatment does not commonly address magnesium insufficiency.

## 2.2. MAGNESIUM CHLORIDE

Since the body has no magnesium reserves, it needs a regular intake to meet its requirements. Not all forms of magnesium have the same bioavailability. The most effective form is magnesium chloride, which works by regulating chemical reactions in the body.

**INNER7® natural origin and body recognition**

INNER7® is made from marine magnesium minerals which come from deep see water collection done through a 2,650m pipe from a depth of 321m. It has a much smaller chance of exposure to pollution from bacteria or chemical substances and offer unmatched quality and purity.
Human body has used marine salts from nature during thousands of years, the body can recognize them and have efficacy without side effects.

**What is magnesium chloride?**

Magnesium in INNER7® is totally ionized as free magnesium. This magnesium chloride form presents the higher bioavailability. Uses of poorly ionized forms of magnesium result in ineffective magnesium supplementation (only 4% on average). If we change the formulation, the mineral complex balance will be affected and the absorption of magnesium will decline.
Magnesium Chloride is a totally ionized form of magnesium that is highly soluble in water and highly absorbable.

**How magnesium chloride improves digestion efficacy?**

Magnesium chloride provides ions of magnesium and chloride which are both required to stimulate the activity of digestive enzymes and produce hydrochloric acid (HCL) in the stomach. Chloride combines with hydrogen in the stomach to make hydrochloric acid, responsible for maintaining the strong acidity of the *stomach* necessary the breakdown of proteins.
A common problem is having much too little stomach acid for appropriate digestion, which produces gastric stress and health issues related to metabolism and nutrient absorption.
INNER7® magnesium chloride helps increase production of HCL, enhances the absorption of vitamins and minerals, and improves the digestion efficacy.
Intake in juice or during meal is best to not feel the bitter taster of product.

## 2.3. MAGNESIUM DEFICIENCY

**Why are we magnesium deficient?**

Why are so many people lacking in magnesium these days? A poor diet of processed foods and a stressful lifestyle can lead to mineral deficiencies, including magnesium, very common in many individuals.

57% of the US population does not meet the US RDA for dietary intake of magnesium. A less than recommended dietary intake does not necessarily imply magnesium deficiency in an individual. The kidneys are very efficient at maintaining body levels, but not in cases where the diet is deficient.

- Unlike our ancestors, our water no longer comes from streams, rivers, or springs. We get our water from the tap, from filters, or from pre-bottled water. All the vast majority of the minerals our bodies require are filtered out.
- Magnesium is constantly depleted in our soil because of years of replanting on farm lands without replenishing nutrients. Chemical fertilizers also contribute to this depletion by altering the way plants can absorb magnesium.
- When we boil, steam or broil food, the magnesium can be removed.
- High carb and fat foods increase our need for more magnesium as well as when we suffer from physical and emotional stress.

**What Factors Reduce the Body's Use of Bioavailable Magnesium?**

An easy way to think about how magnesium levels are affected is to consider the "ins" vs. the "outs". Levels are decreased when either less magnesium comes in or more magnesium goes out of the body.

The following will raise magnesium levels:

- Higher intake — eating more magnesium-rich foods, using magnesium bath salts and magnesium oil, or taking oral magnesium supplements.
- Higher absorption of magnesium in the small intestines, in the case of oral and dietary magnesium
- Lower elimination as waste through the gastrointestinal "GI" tract (the stomach, intestines and colon) — transdermal magnesium avoids this issue
- Lower excretion by the kidneys

As seen above, the digestive system works alongside another pair of organs, the kidneys, which are equipped both to eliminate wastes and to handle excessive nutrient intake. In doing so, the kidneys help to maintain “homeostasis”, a Latin word that literally translates as “the position of sameness”. Homeostasis is the process by which the internal systems of the body maintain a balance — essentially a set of internally programmed healthy levels for temperature, pH, nutrient levels, etc. — by adjusting its physiological processes.

In maintaining homeostasis, several systems of the body work together like an internal thermostat. In healthy individuals, two kidneys filter all of the blood in the human body. All of the contents of the blood, including nutrients, ultimately pass through the kidneys’ filters and can be excreted out of the body at any time.

**Magnesium Deficiency Symptoms**

Because of magnesium’s diverse roles in so many different cellular metabolic functions, magnesium deficiency symptoms are varied and can include:

| | |
|---|---|
| Insomnia / Fatigue | Anxiety, hyperactivity, restlessness |
| Nervousness, apprehensiveness and irritability | Disorientation and confusion |
| Constipation | Muscle spasms, twitches, soreness |
| Gastrointestinal disorders | Back aches Dizziness |
| Headaches | Chest tightness and difficulty breathing |
| Heart palpitations | High blood pressure |
| Irregular heart rhythm | Rapid heartbeat |
| Depression | Confusion Irritability |
| Poor memory / Reduced ability to learn | Bone and teeth problems |
| Difficulty swallowing | Osteoporosis |

Most commonly when we see people with magnesium deficiency they are suffering from restless leg syndrome, where they get twitchy legs in bed at night. They may also be having difficulties

waking up in the night and may have an irritating tick in their eye. Also if you stick out your tongue you can see a slight tremor, which indicates deficiency or you may be suffering from muscle cramps. Expert Naturopath Jane Cronin explains that she has also noticed “a number of people who are being investigated for stomach issues and have a feeling round their oesophagus and throat of tightness and feel like they are being strangled. This can be to do with lack of magnesium tightening in the digestive system muscles and those round the throat.” Other signs are fatigue, mental confusion, irritability, weakness, heart disturbances, loss of appetite, and a predisposition to anxiety.

# 3. MAGNESIUM – MECHANISMS OF ACTION

**What does magnesium do for the body?**

Magnesium is a mineral that is essential for overall physical health. Approximately half of the magnesium in our bodies is found in our bones. The rest serves to help cell functioning throughout the body's various systems. Magnesium plays an important role in muscle function, heart rhythm, blood pressure, immune system functioning and blood sugar level. In general, healthy individuals have enough magnesium in their system and do not need to take a magnesium supplement.

## 3.1. IMPROVE INTESTINAL HEALTH

Having one to three bowel movements daily is normal. Constipation status is defined as having a bowel movement less than 3 times per week. It usually is associated with hard stools or difficulty passing stools.

Constipation is partly due to the lack of fibres and not enough water in our diets. The longer undigested or discarded food matter remains in the large intestine, the more it putrefies and creates harmful wastes that can be reabsorbed into the bloodstream. These toxins and poisons can circulate in the bloodstream, affect the liver, and cause dozens of symptoms such as headaches, fatigue, itchy skin, insomnia, irritability, and joint stiffness. Some of these poisons are even carcinogenic.

The causes of constipation can also include sensitivity to certain foods such as dairy or medications including antidepressants, codeine, certain calcium supplements, and aluminum antacids.

**Magnesium anti-constipation 2 mechanisms**

The anti-constipation effect of magnesium appears to come through two different mechanisms:

- Magnesium relaxes the muscles of the gastro-intestinal tract. It helps to establish a smoother rhythm that helps eliminate constipation.
- Magnesium draws water into the gut. It increased amount of water in the colon and add extra moisture to over-dehydrated fecal matter. The stool is soften, making easier to pass and thus removing constipation.

Since your intestines will be absorbing this excess water from your body it is very important to drink plenty of water after taking magnesium. This will keep you from becoming dehydrated.

If you suffer from constipation predominant irritable bowel syndrome (IBS-C), you may have come across the recommendation to take a magnesium supplement as a way of regulating your bowel movements and easing constipation.

Inflammatory bowel disease (IBD) includes two chronic conditions—Crohn's disease and ulcerative colitis. Both can cause cramping, weight loss, bloody stools, and other health problems. Chronic diarrhea is a common symptom of both. However constipation can be a problem too.

## 3.2. REDUCE FLUID RETENTION

**What is water retention?**

Fluid retention occurs when the body cannot get rid of excess water. Fluid retention can occur for a variety of reasons. It can be caused by a number of medical conditions and some medications. Sometimes, it is caused by premenstrual syndrome in women, or too much salt in your diet.

As a result of fluid retention, there may be some swelling of the body especially in the legs, ankles, hands, feet and abdomen. Magnesium supplementation is a natural ways of treating fluid retention by expelling excess water from the body and hydrating properly.

**Fluid retention causes**

Fluid retention, occurs when the body accumulates excessive fluids. Fluid retention means that the cells in the body are hydrated more than they need to be. Causes include:

• Hormonal imbalances such as low thyroid levels or an imbalance involving the hormones produced by the kidneys and nervous system , all of which affect the size and absorption capacity of the blood vessels
• Lack of minerals in the blood stream which means that fluid does not pass through cell membranes
• Excess salt or sugar which has the effect of pulling water into the cells
• Substances such as drugs, cigarette by-products an d other manufactured chemicals which have a similar effect because the body tries to dilute them
• A heart problem can obstruct flow through the veins and force fluid back through the walls of the tiny blood vessels
• Food allergy may create a toxic state within the tissues that causes water to be ‘pulled in’ for dilution
• Dehydration. If not enough water is taken in, the body cells attract fluid into themselves and when replete build up an impermeable outer wall to protect themselves from further fluid loss. Where there is not enough water circulating in the system, hormonal reactions trigger the storage of even more fluid in the body tissues, particularly the fatty tissues, causing further water retention

**Symptoms of fluid retention**

Symptoms include swelling of body parts such as feet, hands and ankles, a feeling of stiffness or aching and weight fluctuations:

- Swelling of affected body parts
- Feet, ankles and hands are commonly affected
- The affected body parts may ache
- The joints may feel stiff
- Rapid weight gain over a few days or weeks
- Unexplained weight fluctuations
- When pressed, the skin may hold the indent for a few seconds (pitting oedema)
- In other cases, the skin may not indent when pressed (non-pitting oedema).

**Magnesium helps reduce bloating and fluid retention**

There are several methods to reduce the excess water retention in the body. This can be achieved through dietary changes. Supplementing the body with vital minerals such as magnesium may also help get rid of water retention.

Magnesium is known to act as mild diuretics to help the body release excess water. Magnesium helps increase the amount of urine the body would normally excrete. Ordinarily, the kidneys make urine by filtering out water and sodium and potassium ions from the blood. Through a complex process, the kidneys return an exact amount of sodium and potassium ions and some water to the blood stream so these levels will remain constant. The rest of the water goes into the bladder as urine.

When blood flow to the kidneys declines due to illness, they respond by retaining water, which is why magnesium is needed. Magnesium helps reduce bloating due to water retention.

## 3.3. ANTI-DIABETES

Diabetes is a disease that is characterized by high blood sugar levels and is associated with a high risk of heart related complication and heart disease. Insulin is the hormone that helps with the regulation of glucose (sugar) metabolism. Glucose is the basic fuel burned by all of the body's cells and is vital to life. In order for glucose to be used it must first get into the cells. Insulin is the hormone secreted by the pancreas, which enables glucose to move from the blood into the cells where it can be used for energy. Magnesium is needed for insulin to deliver glucose into the cells.

**Type I Diabetes**

In people with diabetes, this system has broken down. With type I diabetes insulin is not produced in adequate amounts causing blood glucose to rise to dangerous and even lethal levels if insulin is not administered. The kidneys must excrete the excess glucose, which causes the kidneys to also eliminate magnesium which leads to a deficiency in magnesium and a further risk of heart disease and heart related complications.

People with type I diabetes need and respond to insulin injections. They also need magnesium supplementation to avoid the higher risk of heart disease.

**Type II Diabetes**

People with type II diabetes produce plenty of insulin, but their cells do not respond to it, their muscle and fat cells cannot take in normal amounts of glucose from the blood. Their glucose delivery to their cells is blocked even though they secrete enough insulin. This condition is called insulin resistance. Magnesium is needed for insulin to bring glucose into the cells, therefore it follows that a deficiency in magnesium in the cells would be a contributing factor to if not a cause of insulin resistance.

The incidence of type II diabetes is growing as the consumption of modern processed foods increases throughout the world. While this disease has a genetic component, it mostly becomes visible after years of living on a processed food diet that is high in refined foods, including sugar and low magnesium. When such a lifestyle results in a low magnesium content within the cells, the cells become resistant to insulin so that glucose cannot enter the cells. A sustained high blood glucose results as in people with type I diabetes, which further increases the excretion of magnesium as well as glucose. This situation further exacerbates the health issues associated with this condition.

Magnesium has been found to improve insulin's response to dietary sugar and to improve the action of insulin in regulating blood sugar levels.

According to the American Diabetes Association, projections of a continued rapid growth in the incidence of type 2 diabetes requires a cost-effective approach that can be widely employed to prevent or delay this major disorder. Published in the journal Diabetes Care, two recent studies suggest that an increased intake of magnesium could have a role in reducing the risk of type 2 diabetes.

In people with either type of diabetes, the tendency toward low magnesium levels increases the risk of heart disease. People with type I diabetes often have a low cellular magnesium level because of constantly high blood glucose levels. This results in increased excretion of magnesium through the urine. In addition, the utilization of glucose by the cells depends on magnesium, so when there is a large amount of glucose that needs processing, there is an increased need for magnesium.

For people with type I diabetes, magnesium supplements can help prevent heart disease, but they can never make insulin secretion normal. However, they can improve the response to any insulin that is secreted or to the insulin administered by injection.

The sustained high blood sugar in type II diabetes also results in high urinary loss of magnesium. This intensifies the magnesium deficiency that caused the resistance of cells to insulin in the first place, making type II diabetes a progressive disease if not treated. Supplementation with magnesium can improve response to insulin and can arrest the disease process, especially if combined with exercise and weight loss.

**Magnesium reduces cholesterol levels**

Magnesium regulates blood cholesterol by playing an important role in the function of the enzyme responsible for its synthesis in the body: HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase). Magnesium deactivates this enzyme, which is the rate-limiting enzyme controlling the conversion of HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) into the compound mevalonate (a fatty acid derivative). When this first step in the mevalonate pathway is inhibited by the deactivation of HMG-CoA reductase by magnesium and ATP (magnesium-ATP complex), then cholesterol production correspondingly is reduced. The final result is less formation of cholesterol.

When magnesium levels remain sufficiently high, then the body can inhibit the enzyme HMG-CoA reductase whenever necessary. However, when there is magnesium deficiency, cholesterol synthesis increases because the conversion of HMG-CoA to mevalonate is enhanced.

Sufficient magnesium levels in the body do not prevent the synthesis of cholesterol needed for health (e.g., inactivated HMG-CoA reductase can be reactivated by other enzymes, some of which require magnesium for proper function). Yet, by selectively inhibiting the enzyme HMG-CoA reductase, magnesium can prevent the overproduction of cholesterol, a risk factor in cardiovascular health conditions.

## 3.4. WEIGHT MANAGEMENT

Magnesium and the B-complex vitamins are energy nutrients: they activate enzymes that control digestion, absorption, and the utilization of proteins, fats, and carbohydrates. Lack of these necessary energy nutrients causes improper utilization of food, leading to such far-ranging symptoms as hypoglycemia, and obesity.

Food craving and overeating can be simply a desire to continue eating past fullness because the body is, in fact, craving nutrients that are missing from processed food. You continue to eat empty calories that pack on the pounds but get you no further ahead in your nutrient requirements.

Magnesium is also necessary in the chemical reaction that allows insulin to usher glucose into cells, where glucose is involved in making energy for the body. If there is not enough magnesium to do this job, both insulin and glucose become elevated. The excess glucose gets stored as fat and contributes to obesity. Having excess insulin puts you on the road toward diabetes.

The connection between stress and obesity cannot be overlooked. The stress chemical cortisol signals a metabolic shutdown that makes losing weight almost impossible. It's as if the body feels it is under an attack such that it must hoard all its resources, including fat stores, and won't let go of them under any inducement. Magnesium can effectively neutralize the effects of stress.

Every metabolic function in the body requires vitamins and minerals - without them, symptoms develop. Therefore, the first step in treating nonspecific symptoms is diet and dietary supplements, not drugs.

**Abdominal obesity**

Gaining weight around your middle is related to magnesium deficiency and an inability to properly utilize insulin. As more and more insulin is produced to deal with a high-sugar diet, abdominal girth increases to process the extra insulin (half the insulin in the bloodstream is directed at abdominal tissue).

**Metabolic syndrome**

Metabolic syndrome describes a set of conditions that includes high cholesterol, hypertension and obesity. It also encompasses elevated triglycerides and elevated uric acid. High triglycerides are usually found when cholesterol is elevated but most often when someone has a high-sugar diet.

High uric acid is due to incomplete breakdown of protein from lack of B vitamins and digestive enzymes. This complex collectively appears to be caused by disturbed insulin metabolism, called insulin resistance, and eventually can lead to diabetes, and heart attack.
As previously noted, magnesium is required in the metabolic pathways that allow insulin to usher glucose into cells, where glucose participates in making energy for the body. If magnesium is deficient, the doorway into the cells does not open to glucose, resulting in the following cascade of events:

1. Glucose levels become elevated.
2. Glucose is stored as fat and leads to obesity.
3. Elevated glucose leads to diabetes.
4. Obesity puts a strain on the heart.
5. Excess glucose becomes attached to certain proteins (glycated), leading to kidney damage, neuropathy, blindness, and other diabetic complications.
6. Insulin-resistant cells don't allow magnesium into the cells.
7. Further magnesium deficiency leads to hypertension.
8. Magnesium deficiency leads to cholesterol buildup, and both these conditions are implicated in heart disease.

Magnesium deficiency is a major factor in the origins of each of its signs and symptoms, from elevated triglycerides and obesity to disturbed insulin metabolism.

**Insulin resistance**

Insulin's job is to open up sites on cell membranes to allow the influx of glucose, a cell's source of fuel. Cells that no longer respond to the advances of insulin and refuse the entry of glucose are called insulin-resistant. As a result, blood glucose levels rise and the body produces more and more insulin, to no avail. Glucose and insulin rampage throughout the body, causing tissue damage that results in overuse and wasting of magnesium, an increased risk of heart disease, and adult onset diabetes.

One of the major reasons the cells don't respond to insulin is lack of magnesium. Some studies show that chronic insulin resistance in patients with type II diabetes is associated with a reduction of magnesium; magnesium is necessary to allow glucose to enter cells. Additional studies confirm that when insulin is released from the pancreas, magnesium in the cell normally responds and opens the cell to allow entry of glucose, but in the case of magnesium deficiency combined with

insulin resistance the normal mechanisms just don't work. However, the higher the levels of magnesium in the body, the greater the sensitivity of the cells to insulin and the possibility of reversing the problem.

## 3.5. REDUCE HIGH BLOOD PRESSURE

A well-established body of research indicates that nutrients such as magnesium are highly effective in treating and—even more importantly—preventing high blood pressure. Because this metallic element is not plentiful in foods, magnesium supplementation may be effective in both preventing and controlling high blood pressure.

**How Lifestyle Affects Blood Pressure**

Many factors contribute to the development of hypertension, and lifestyle choices play a central role. Maintaining a healthy weight is very important. Extra pounds mean extra work for the heart, which must exert additional pressure to push the blood through the extra mile of blood vessels that come with each pound of excess fat.

Regular exercise and stress reduction also help keep blood pressure normal. Tobacco and stimulant drugs should be avoided, as these substances boost blood pressure and can injure the sensitive endothelium that lines the blood vessels.

Caffeinated beverages are a controversial topic among hypertension specialists. Caffeine appears to raise the blood pressure of some people but not others. Caffeine certainly does not appear to help control blood pressure and thus probably should be avoided.

Similarly, some people's hypertension is sensitive to salt, meaning that a high or even moderate salt intake will raise their blood pressure. Other people are not sensitive to salt. Nevertheless, experts generally recommend moderation in the use of salt.

**What is high blood pressure level?**

This table lists the levels of hypertension associated with different blood pressure readings.

| | **Systolic Blood Pressure (mm Hg)** | **Diastolic Blood Pressure (mm Hg)** |
|---|---|---|
| Normal | less than 120 | less than 80 |
| Prehypertension | 120-139 | 80-89 |
| Stage 1 hypertension | 140-159 | 90-99 |
| Stage 2 hypertension | 160 or higher | 100 or higher |

TABLE 1: BLOOD PRESSURE CLASSIFICATIONS

It is vitally important to keep blood pressure low, especially for people have a genetic tendency toward hypertension.

**High blood pressure associated risks**

High blood pressure is a stronger predictor of cardiovascular risk. Scientific studies directly correlate high blood pressure with decreased longevity. Yet most mainstream physicians and their patients ignore this risk until life-threatening hypertension has already developed.

Developing high blood pressure, or hypertension, is a major risk factor for heart attacks, strokes, congestive heart failure, circulatory failure, kidney disease, and loss of vision.

Hypertension greatly reduces longevity, because high blood pressure is a much stronger indicator of cardiovascular risk than high cholesterol. The damage to blood vessels caused by high blood pressure leads to hundreds of thousands of heart attacks and strokes each year. Moreover, people with high blood pressure have a much greater risk of developing adult-onset diabetes, and most people with diabetes sustain their greatest harm from the hypertension that frequently accompanies it.

**How magnesium reduces hypertension ?**

The vasodilator action of parenteral magnesium has been well known for over a century. The hypotensive action of parenteral magnesium may be used in hypertensive patients, particularly in pre-eclampsia but its pharmacological mechanisms are observed irrespective of magnesium status. Therefore such pharmacological effects should not be used as a diagnostic tool in the investigation of magnesium deficit because its pharmacological actions are observed irrespective of magnesium.

Magnesium influences blood pressure regulation by modulating vascular tone and reactivity. The direct vascular effect of magnesium was first suggested in the early 1900s when it was observed in clinical studies that magnesium salt infusion lowers blood pressure via a reduction in peripheral vascular resistance in spite of a slight increase in myocardial contractility. Experimental studies support these clinical observations and confirm that acute magnesium administration induces hypotension through vasodilatory actions. Increased concentrations of extracellular magnesium cause vasodilation, improve blood flow, decrease vascular resistance, increase capacitance function of peripheral, coronary, renal, and cerebral arteries, and attenuate agonist-induced

vasoconstriction, whereas, decreased concentrations cause contraction, potentiate agonist evoked asoconstriction, and increase vascular tone. Exact molecular mechanisms underlying magnesium vascular actions are unclear, but magnesium probably influences intracellular free Ca2+ concentration ([Ca2+]i), which is fundamental in myocardial regulation, endocrine and renal secretion, and smooth muscle contraction. In vascular smooth muscle cells, magnesium antagonizes Ca2+ by inhibiting transmembrane calcium transport and calcium entry. It also acts intracellularly as a calcium antagonist thereby modulating the vasoconstrictor actions of [Ca2+]i, a major determinant of vascular contraction. Low magnesium causes an increase in [Ca2+]i with associated vascular contraction and increased tone.

Since [Mg2+]i influences many enzymes in the signal transduction pathways involved in vascular contraction, low [Mg2+]i could have important implications in vascular smooth muscle cell function in hypertension. Intracellular magnesium depletion has been demonstrated in many tissues (heart, lungs, kidney, bone, and muscle) and cell types (vascular smooth muscle cells, fibroblasts, erythrocytes, platelets, and lymphocytes) in both human and experimental hypertension. Underlying mechanisms for cellular magnesium changes in hypertension are unclear, but magnesium-deficient states, decreased membrane permeability, altered Na+–Mg2+ exchange, defective membrane binding as well as altered cellular responsiveness have been implicated. We recently suggested that altered activity of vascular TRPM6/7, the major transcellular magnesium transporter, may also play a role in cellular magnesium deficiency in hypertension.

## 3.6. REDUCE INFLAMMATION AND PAIN RELIEF

**Magnesium deficiency elevates CRP**

C-reactive protein is an inflammatory marker in the blood that predicts who is likely to suffer a heart attack or stroke. Higher blood levels of C-reactive protein mean greater risk of cardiovascular disease.

Most scientists now accept that inflammation plays a role in the development of atherosclerosis, cancer, Alzheimer's disease, and other age-related disorders. The best way to assess whether a person suffers from chronic inflammation is the C-reactive protein blood test.

A new study showed that adults who consume less than the recommended amount of magnesium are 1.48 to 1.75 times more likely to have elevated C-reactive protein. This finding offers yet another reason why those who are magnesium deficient have increased rates of cardiovascular disease—their C-reactive protein levels are likely to be higher!

### Magnesium and joint health

Muscle, joint, and connective tissue pain can be exacerbated by higher intakes of calcium. An overabundance of calcium flushes magnesium out of cells, reducing its availability for reducing pain. In such extreme states of deficiency, magnesium simply is not available to block pain effectively.

In normal condition, magnesium is highly effective for relieving pain, by acting as a non-competitive antagonist of the N-Methyl-D-Aspartate (NMDA) receptor site. The NDMA receptor plays a critical role in the bodily mechanisms relating to central sensitization in the spinal cord. This sensitization is involved in the establishment of chronic neuropathic pain. A centrally positioned magnesium ion has the ability to block the NMDA receptor site.

NMDA receptor activation and release of pro-pain substances including substance P, nerve growth factor, brain derived nerve factor, and nitric oxide are believed to "drive the process of central sensitization" in the spinal cord. Magnesium can increase the magnesium concentration gradient between cell membranes and extracellular fluid, and thereby blocks the NMDA receptor. This is one of the mechanisms that allows magnesium to bring immediate pain relief.

When low levels of magnesium result in reduced capacity to block the NMDA receptor site, central sensitization of the spinal cord is more likely to occur. Under such circumstances, wherein the

NMDA receptor site is not blocked adequately, it is evident that low magnesium status not only can contribute to higher levels of acute or immediate pain, but can also lead to the establishment of chronic neuropathic pain.

## 3.7. IMPROVE BRAIN HEALTH

In our modern world, we often have chronic stress and chronic magnesium deficiency. And the depleted magnesium content in our soil and food is not helping either.

People that suffer from hyperactive nervous systems, anger issues, or even just spasms can all benefit from magnesium's effects on the muscles. Magnesium is perhaps the most important mineral in relation to stress balancing. When taken, it has a calming effect on your heart and muscles.

This is especially helpful for people that have trouble sleeping. Magnesium's calming effect has helped many people relax enough to be lulled into deep, replenishing sleep that their body needed.

**Connection between magnesium and brain health**

Studies have confirmed that better magnesium levels help protect brain tissue from the damage that can be caused by any head trauma. Magnesium acts as a natural calcium channel blocker, which makes it a principle nutrient for helping to control blood pressure. In the Dietary Approaches to Stop Hypertension (DASH) study, researchers found that people who took in enough magnesium while on a calorie-controlled diet were able to lower their blood pressure. And this same calcium channel blocking effect plays a role in protecting the brain. The brain operates on a balance between "excitatory" and "inhibitory" activity. A neurotransmitter called glutamate is the primary trigger for the excitatory activity. When brain cells are activated by glutamate, calcium ions rush in. This is fine as long as the excitation is kept under reasonable control. But too much calcium entering the cells can be deadly to brain tissue. That's where the calcium channel blocking effect of magnesium comes in.

## 3.8. REGULATE BODY pH

Magnesium helps return the body's pH balance. Magnesium reduces lactic acid, which is partly responsible for post-exercise pain (Delayed Onset Muscle Soreness).

**Acid Alkaline Imbalance**

Over acidity, which can become a dangerous condition that weakens all body systems, is very common today. It gives rise to an internal environment conducive to disease, as opposed to a pH balanced environment which allows normal body function necessary for the body to resist disease. A healthy body maintains adequate alkaline reserves to meet emergency demands. When excess acids must be neutralized our alkaline reserves are depleted leaving the body in a weakened condition. A pH balanced diet, according to many experts, is a vital key to health maintenance.

The concept of acid alkaline imbalance as the cause of disease is not new. In 1933 a New York doctor named William Howard Hay published a ground-breaking book, A New Health Era in which he maintains that all disease is caused by autotoxication (or "self-poisoning") due to acid accumulation in the body:

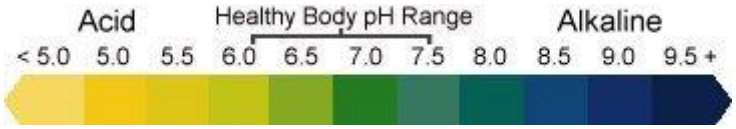
Now we depart from health in just the proportion to which we have allowed our alkalies to be dissipated by introduction of acid-forming food in too great amount... It may seem strange to say that all disease is the same thing, no matter what its myriad modes of expression, but it is verily so.—William Howard Hay, M.D.

More recently, in his remarkable book Alkalize or Die (see recommended reading), Dr. Theodore A. Baroody says essentially the same thing: The countless names of illnesses do not really matter. What does matter is that they all come from the same root cause...too much tissue acid waste in the body!—Theodore A. Baroody, N.D., D.C., Ph.D.

**Understanding pH**

pH (potential of hydrogen) is a measure of the acidity or alkalinity of a solution. It is measured on a scale of 0 to 14—the lower the pH the more acidic the solution, the higher the pH the more alkaline (or base) the solution. When a solution is neither acid nor alkaline it has a pH of 7 which is neutral.

Water is the most abundant compound in the human body, comprising 70% of the body. The body has an acid-alkaline (or acid-base) ratio called the pH which is a balance between positively charges ions (acid-forming) and negatively charged ions (alkaline-forming.) The body continually strives to balance pH. When this balance is compromised many problems can occur.




It is important to understand that we are not talking about stomach acid or the pH of the stomach. We are talking about the pH of the body's fluids and tissues which is an entirely different matter.

Most people who suffer from unbalanced pH are acidic. This condition forces the body to borrow minerals—including calcium, sodium, potassium and magnesium - from vital organs and bones to buffer (neutralize) the acid and safely remove it from the body. Because of this strain, the body can suffer severe and prolonged damage due to high acidity - a condition that may go undetected for years.

## 3.9. FIGHT STRESS

Magnesium is also very important as calming or anti-stress mineral. Magnesium deficiency can lead to fatigue, irritability, poor sleep, stress, anxiety and insomnia.

Stress can come in many forms, both physical and mental. Our bodies respond similarly to both types by increasing the use of and need for magnesium to manage the stress.

### Magnesium and stress level

Magnesium status is highly associated with stress levels, with both stress and hypomagnesaemia potentiating each other's negative effects. Indeed, stress and hypomagnesaemia potentiate each other's negative effects in a veritable pathogenic vicious circle. Low Mg/Ca ratios augment the release of catecholamines in response to stress. Fatty acids resulting from adrenergic induced lipolysis form undissociated Mg soaps which further exacerbates Mg depletion. Indeed, hypomagnesaemia has been associated with stressful conditions such as photosensitive headache, fibromyalgia, chronic fatigue syndrome, audiogenic stress, cold stress, and physical stress, amongst others.

Magnesium deficiency makes you feel more stressed. When you are stressed, your adrenal glands release the stress hormones adrenaline and cortisol into your bloodstream. These hormones cause the loss of magnesium through urine. This means that anyone who has experienced long term stress will probably end up magnesium deficient unless they take a supplement. People who are magnesium deficient feel more stressed. This leads to a negative spiral: stress causes magnesium deficiency and a lack of magnesium magnifies stress.

### Magnesium affect the NMDA receptor

Magnesium affects a number of neurotransmitter systems. It inhibits the release of excitatory neurotransmitters and also acts as a voltage gated antagonist at the glutamate, NmethylDaspartate (NMDA) receptor.

Magnesium is a natural blocker of a receptor in the brain called NMDA. This receptor is stimulated with calcium, that leads to over-excitation and stimulation of the brain and irritability, depression. Magnesium is a natural NMDA receptor antagonist, which helps to really calm the nervous system overall.

**Magnesium reduces insomnia and improves sleep quality**

Lack of magnesium has shown to alter electrical activity in the brain, causing agitated sleep and frequent awakenings. Magnesium has a calming effect on the nervous system, decreasing chronic stress levels and improving sleep quality. It allows people to be less fatigued, make brain work better, and reduce stress levels.

## 3.10. DETOXIFICATION

Magnesium is utilized by the body for all sorts of detoxification pathways and is necessary for the neutralization of toxins, overly acidic conditions that arise in the body, and for protection from heavy metals. It plays a vital role in protecting from the onslaught of man-made chemicals all around us.

Glutathione, an antioxidant normally produced by the body and a detoxifier of mercury, lead and arsenic among others, requires magnesium for its synthesis. A deficiency of magnesium increases free radical generation in the body and causes glutathione loss, which is not affordable because glutathione helps to defend the body against damage from cigarette smoking, exposure to radiation, cancer chemotherapy, and toxins such as alcohol and just about everything else.

Maintaining high levels of magnesium can prevent deficiencies that lead to breakdowns in detoxification systems and reduce the body's functional capacity to detoxify metals. When the body does not detoxify metals at an optimal rate, then metals accumulate and result in toxicity.

When bodies are replete with magnesium (and in balance with the other essential minerals) we are protected from heavy metal deposition and the development of associated neurological diseases. Research indicates that ample magnesium will protect brain cells from the damaging effects of aluminum, beryllium, cadmium, lead, mercury and nickel. We also know that low levels of brain magnesium contribute to the deposition of heavy metals in the brain that heralds Parkinson's and Alzheimer's. It appears that the metals compete with magnesium for entry into the brain cells. If magnesium is low, metals gain access much more readily.

There is also competition in the small intestine for absorption of minerals. If there is enough magnesium, aluminum won't be absorbed.

Magnesium is a foundational nutrient on which detoxification systems depend for their function. The health of detoxification systems also is critical because exposure to environmental contaminants, synthetic chemicals, and pollution is a reality in our industrial world. Magnesium detoxification is one weapon in the arsenal against chemical toxicity.

## 3.11. SPORT AND MUSCLE RECOVERY

Lack of magnesium plays a role long before you ever feel pain, and continues to play a role while your new and then chronic Tendonitis lingers and gets worse over time.

1. Muscles require Calcium to fire, and Magnesium to relax/stop firing.
2. You don't eat enough quality sources of magnesium, so your muscles slowly start to suffer as the ecology of your body and the specific area starts to fail to work properly.
3. You perform some repetitive activity, whether sports like baseball, a hobby like knitting, or work activities like typing and mousing. This means that you are eating through a lot of calcium and magnesium that you aren't replacing appropriately.
4. This helps your muscles 'lock' into being tight. Different parts of your muscles can also get 'confused' and not be sure whether to fire or let go, and not really able to because they don't have the right balance of necessary nutrients, so they get stuck into tiny bits of spasm, which basically starts to create a pain dynamic of tightness, waste product and irritant, and danger signals sent to the nervous system.
5. This all happens under the radar, slowly starting to ache, then hurt a little, then hurt a lot, until you hit the point where you realize that you are desperate for a tendonitis remedy.

**Sports Nutrition - Magnesium and Energy Nutrition**

Exercisers who feel weak and tired may be suffering from magnesium deficiency. A loss of magnesium through sweat can bring fatigue and muscle cramps because of the role the mineral plays in controlling muscle contraction and regulating the conversion of carbohydrates to energy.

Athletes, runners and those who perform strenuous exercise or physical work are advised to make sure their cellular magnesium levels are up.

Magnesium is responsible--together with calcium--for the production of adenosine triphosphate (ATP), our most important high-energy phosphate compound. In addition, good magnesium levels are needed for optimal muscle contraction and to sustain the high oxygen consumption necessary for athletic performance. Research indicates that magnesium facilitates oxygen delivery to working muscle tissue.

In addition to its contribution to multiple enzyme systems, including ATP metabolism in the production of energy, magnesium plays a role in protecting us against ischemic heart disease,

myocardial infarction, cardiac arrhythmias, high blood pressure, asthma, preeclampsia and alcohol withdrawal.

Magnesium is vital for converting glycogen into glucose for use as the body's fuel.

**Magnesium For Tendonitis Prevention and Healing**

The benefits of magnesium for Tendonitis prevention, treatment, curing, and healing cannot be overlooked (but regularly is).

If you have tendonitis pain, then you have muscles that are chronically too tight. This points to being or becoming magnesium deficient. If you notice that your muscles spasm sometimes or constantly, then you are calcium and/or magnesium deficient.

If you feel like your muscles just won't 'let go', and/or if you feel like the pain has a 'tension' element, then it's safe to say that you could benefit from more magnesium.

Pain creates tightness (and vice versa). Tightness uses up magnesium. Your diet is unlikely to provide you the magnesium you need.

## Muscle Cramping and Magnesium

Magnesium is also essential in addressing an issue common to almost all of the athletes: muscle cramping. As you sweat, you’re going to be losing magnesium, which is water soluble. In addition, you’ll be sweating out electrolytes, and of course water too. These losses mean that the ratio of calcium to magnesium will be changing in the body: the percentage of calcium will increase; and since calcium is a muscle contractor, the muscles cramp and that’s it.

# 4. OTHER MINERALS

## 4.1. CALCIUM

### *4.1.1. Properties & Formulation*

The chemical element Calcium (Ca), is the fifth element and the third most abundant metal in the earth's crust. The metal is trimorphic, harder than sodium, but softer than aluminium. It is less chemically reactive than alkaline metals.

| | |
|---|---|
| **Atomic number** | 20 |
| **Atomic mass** | 40.08 $g.mol^{-1}$ |
| **Electronegativity according to Pauling** | 1.0 |
| **Mass volume** | 1.6 $g.cm^{-3}$ at 20°C |
| **Melting point** | 840 °C |
| **Boiling point** | 1484 °C |
| **Vanderwaals radius** | 0.197 nm |
| **Ionic radius** | 0.099 nm |
| **Isotopes** | 10 |
| **Electronic configuration** | [ Ar ] $4s^2$ |
| **Energy of first ionisation** | 589.6 $kJ.mol^{-1}$ |
| **Energy of second ionisation** | 1145 $kJ.mol^{-1}$ |
| **Standard potential** | - 2.87 V |
| **Discovered** | Sir Humphrey Davy in 1808 |

**Where is calcium found in the body?**

Calcium is the most abundant mineral in the body. 99% of the body's calcium supply is stored in the bones and teeth where it supports their structure and function.

The remaining 1% is found in the blood, muscles, and other tissue and is required to support critical metabolic functions, like vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signaling and hormonal secretion.

Calcium in the bones can be used as a reserve that can be released into the body as needed. The concentration of calcium in the body tends to decline as we age because it is released from the body through sweat, skin cells, and waste. In addition, as women age, absorption of calcium tends to decline due to reduced estrogen levels. Calcium absorption can vary depending on race, gender, and age.

**Why is calcium needed?**

The body needs calcium to maintain strong bones. Almost all calcium is stored in bones and teeth, where it supports their structure and hardness. Bone itself undergoes continuous remodeling, with constant resorption and deposition of calcium into new bone. The balance between bone resorption and deposition changes with age. Bone formation exceeds resorption in periods of growth in children and adolescents, whereas in early and middle adulthood both processes are relatively equal. In aging adults, particularly among postmenopausal women, bone breakdown exceeds formation, resulting in bone loss that increases the risk of osteoporosis over time.

The body also needs calcium for muscles to move and for nerves to carry messages between the brain and everybody part. In addition, calcium is used to help blood vessels move blood throughout the body and to help release hormones and enzymes that affect almost every function in the human body.

### *4.1.2. Mechanisms of action*

Calcium's potential benefits in health promotion and disease prevention and treatment included: bone health and osteoporosis; cardiovascular disease; blood pressure regulation and hypertension; cancers of the colon, rectum, and prostate; kidney stones; and weight management.

Calcium is an important nutrient and is needed for many of the body's functions, including blood clotting and the proper function of the heart, muscles, and nerves. Calcium is also critical for the health and strength of bones. Not getting enough calcium can contribute to the development of osteoporosis (porous bones).

**Osteoporosis**

Bones are always breaking down and rebuilding, and calcium is needed for this process. Taking extra calcium helps the bones rebuild properly and stay strong.

Calcium is the most abundant metal in the human body: is the main constituent of bones and thees and it has keys metabolic functions.

Calcium is sometimes referred to as lime. It is most commonly found in milk and milk products, but also in vegetables, nuts and beans. It is an essential component for the preservation of the human skeleton and teeth. It also assists the functions of nerves and muscles. The use of more than 2,5

grams of calcium per day without a medical necessity can lead to the development of kidney stones and sclerosis of kidneys and blood vessels.

A lack of calcium is one of the main causes of osteoporosis. Osteoporosis is a disease in which the bones become extremely porous, are subject to fracture, and heal slowly, occurring especially in women following menopause and often leading to curvature of the spine from vertebral collapse.

Unlike most of the people think, there is an intense biological activity inside our bones. They are being renewed constantly by new tissue replacing the old one. During childhood and adolescence, there's more production of new tissue than destruction of the old one, but at some point, somewhere around the 30 or 35 years of age, the process is inverted and we start to loose more tissue than what we can replace. In women the process is accelerated after the menopause (he period marked by the natural and permanent cessation of menstruation, occurring usually between the ages of 45 and 55); this is because their bodies stop producing the hormone known as estrogen, one of which functions is to preserve the osseous mass.

Calcium and Bone Mass

Bones may seem like hard and lifeless structures, but they are, in fact, living tissue. Old bone is constantly broken down (through a process called resorption) by our bodies, and new bone is deposited. Anytime bone is broken down faster than it is deposited, bone weakness and osteoporosis can occur.

Bones are made from collagen and noncollagen proteins and are fortified with calcium. If a person does not take in enough calcium from their diet, the body extracts calcium from the bones, resulting in loss of bone strength and mass. This can ultimately lead to thin, fragile bones and osteoporosis.

More than 90% of a person's bone mass develops before 20 years of age, and half of that bone mass develops from 11-15 years of age. To have strong bones, children and adolescents need to consume enough calcium to build up the bone mass that they will need throughout their lives.

Even after age 20, a person can help protect his or her bones. Bone mass can still be built up until the early 30s. After that, protecting the amount of bone that already exists comes from consuming enough calcium because calcium is essential in maintaining bone mass.

Calcium works like this:

- After calcium is consumed, several nutrients, especially vitamin D, help the body absorb the calcium.
- The blood transports the calcium that is not needed for other body processes to the bones where it adds to the bone mass and is stored for when it is needed in the rest of the body.
- Sometimes a lack of calcium comes from not consuming enough in the diet or because the body is not absorbing enough into the blood. When this happens, calcium is removed from the bones into the blood to keep a constant level of calcium in the blood.

Adequate calcium intake is important to keep a normal amount of calcium in the blood and to protect the bones from calcium loss. If enough calcium is not regularly consumed and the calcium continues to be taken from the bones, a person's bone mass decreases. Decreased bone mass can lead to osteoporosis, fractures, and disability.

Adequate calcium intake is also important because the body cannot produce calcium on its own. Every day, the body loses calcium through shedding hair, skin, and nails and through sweat, urine, and feces. Every day, this lost calcium must be replaced by what a person eats.

How Calcium Helps Prevent Osteoporosis

Calcium makes bones strong. In fact, bones and teeth contain 99% of the body's total calcium, with the remaining 1% in intracellular and extracellular fluids. Bones act as a storehouse for calcium, which is used by the body and replaced by the diet throughout a person's life. If enough calcium is not consumed, the body takes it from the bones. If more calcium is removed from the bones than is consumed in the diet, the bones become fragile and weak as a person gets older, leading to osteoporosis and fractures.

Osteoporosis prevention begins during childhood and adolescence by getting enough exercise and the proper nutrients, including calcium and vitamin D. However, adults can help prevent osteoporosis in the same ways.

The importance of calcium in developing and maintaining bone mass (bone density) varies throughout a person's life. At times of rapid and significant bone growth (during the teenage years) or rapid bone loss (after age 50 years), calcium is more important. Therefore, to reduce the risk of osteoporosis, calcium intake should be the highest during adolescence and after 50 years of age.

*Bone health and osteoporosis*

Bones increase in size and mass during periods of growth in childhood and adolescence, reaching peak bone mass around age 30. The greater the peak bone mass, the longer one can delay serious bone loss with increasing age. Everyone should therefore consume adequate amounts of calcium and vitamin D throughout childhood, adolescence, and early adulthood. When calcium intake is low or ingested calcium is poorly absorbed, bone breakdown occurs as the body uses its stored calcium to maintain normal biological functions. Bone loss also occurs as part of the normal aging process, particularly in postmenopausal women due to decreased amounts of estrogen.

Osteoporosis, a disorder characterized by porous and fragile bones, is a serious public health problem for more than 10 million U.S. adults over the age of 50, 80% of whom are women. (Another 34 million have osteopenia, or low bone mass, which precedes osteoporosis.) Many factors increase the risk of developing osteoporosis, including being female, thin, inactive, or of advanced age; smoking cigarettes; drinking excessive amounts of alcohol; and having a family history of osteoporosis.

Various bone mineral density (BMD) tests are available to measure the amount of calcium and other minerals in bones in the spine, hip, and/or forearm. The T-score from these tests compares an individual's BMD to an optimal BMD (that of a healthy 30-year old adult). A T-score of -1.0 or above indicates normal bone density, -1.0 to -2.5 indicates low bone mass (osteopenia), and lower than -2.5 indicates osteoporosis. Although osteoporosis affects individuals of all races, ethnicities, and both genders, women are at highest risk because their skeletons are smaller than those of men and because of the accelerated bone loss that accompanies menopause.

Osteoporosis is most associated with fractures of the hip, vertebrae, wrist, pelvis, ribs, and other bones. An estimated 1.5 million fractures occur each year in the United States due to osteoporosis. Supplementation with calcium plus vitamin D has been shown to be effective in reducing fractures and falls (which can cause fractures) in institutionalized older adults. However, among community-dwelling postmenopausal women with no symptoms of bone disease such as osteoporosis, daily supplementation with 1,000 mg or less of calcium and 400 IU or less of vitamin D will not help to prevent bone fractures. Among these healthy individuals, intakes of both nutrients at recommended levels are important to overall bone health as one ages, but greater amounts appear to provide no additional benefits to bone.

Regular exercise and adequate intakes of calcium and vitamin D are critical to the development and maintenance of healthy bones throughout the life cycle. Both weight-bearing exercises (such as

walking, running, and activities where one's feet leave and hit the ground and work against gravity) and resistance exercises (such as calisthenics and that involve weights) support bone health.

In 1993, the U.S. Food and Drug Administration authorized a health claim related to calcium and osteoporosis for foods and supplements. In January 2010, this health claim was expanded to include vitamin D. Model health claims include the following: "Adequate calcium throughout life, as part of a well-balanced diet, may reduce the risk of osteoporosis" and "Adequate calcium and vitamin D as part of a healthful diet, along with physical activity, may reduce the risk of osteoporosis in later life".

**Cardiovascular disease**

Calcium helps reduce cardiovascular disease (CVD) risk by decreasing intestinal absorption of lipids, increasing lipid excretion, lowering cholesterol levels in the blood, and promoting calcium influx into cells. In the Iowa Women's Health Study, higher calcium intake from diet and/or supplements was associated with reduced ischemic heart disease mortality in postmenopausal women. Conversely, in a cohort of older Swedish women, both total and dietary calcium intakes of 1,400 mg/day and higher were associated with higher rates of death from CVD and ischemic heart disease than intakes of 600–1,000 mg/day.

**Blood pressure and hypertension**

Several clinical trials have demonstrated a relationship between increased calcium intakes and both lower blood pressure and risk of hypertension. In the Women's Health Study, calcium intake was inversely associated with risk of hypertension in middle-aged and older women. However, other studies have found no association between calcium intake and incidence of hypertension. The authors of a systematic review of the effects of calcium supplements for hypertension found any link to be weak at best, largely due to the poor quality of most studies and differences in methodologies.

Calcium's effects on blood pressure might depend upon the population being studied. In hypertensive subjects, calcium supplementation appears to lower systolic blood pressure by 2–4 mmHg, whereas in normotensive subjects, calcium appears to have no significant effect on systolic or diastolic blood pressure.

**Kidney stones**

Kidney stones in the urinary tract are most commonly composed of calcium oxalate. Some studies suggest a positive association between supplemental calcium intake and the risk of kidney stones, and these findings were used as the basis for setting the calcium UL in adults. High intakes of *dietary* calcium, on the other hand, do not appear to cause kidney stones and may actually protect against developing them.

**Weight management**

Several studies have linked higher calcium intakes to lower body weight or less weight gain over time. Two explanations have been proposed. First, high calcium intakes might reduce calcium concentrations in fat cells by decreasing the production of two hormones (parathyroid hormone and an active form of vitamin D) that increase fat breakdown in these cells and discourage fat accumulation. Secondly, calcium from food or supplements might bind to small amounts of dietary fat in the digestive tract and prevent its absorption.

## 4.2. POTASSIUM

### *4.2.1. Properties & Formulation*

Potassium is the third most abundant mineral in the body and is considered an electrolyte. The human body has about a 4 1/2 ounce supply and most of it is located inside muscle cells.

| | |
|---|---|
| **Atomic number** | 19 |
| **Atomic mass** | 39.0983 g.mol -1 |
| **Electronegativity according to Pauling** | 0.8 |
| **Density** | 0.86 g.cm -3 at 0 °C |
| **Melting point** | 63.2 °C |
| **Boiling point** | 760 °C |
| **Vanderwaals radius** | 0.235 nm |
| **Ionic radius** | 0.133 (+1) |
| **Isotopes** | 5 |
| **Electronic shell** | [ Ar ] 4s1 |
| **Energy of first ionisation** | 418.6 kJ.mol -1 |
| **Discovered by** | Sir Davy in 1808 |

Potassium serves as the ionic counterpart to other electrolytes sodium and chloride, and needs a balance of these minerals for many essential body functions. Studies have shown that potassium may help to prevent high blood pressure and may enhance the effect of antihypertensive medications. Both physical and mental stress can lead to a deficiency in potassium. Alcohol, coffee, and sugar deplete potassium levels in the body.

### *4.2.2. Mechanisms of action*

Potassium assists in muscle contractions and in maintaining appropriate levels of fluid and the electrolyte balance in the body cells. It is critical to maintaining a normal heartbeat or heart rhythm. Potassium also functions in the conduction of nerve impulses and enables the body to convert glucose into energy, which is then stored in reserve by the muscles and liver.

Curing Dry Skin

One of the reasons why many people suffer from dry skin is due to deficiency of potassium. Potassium keeps the skin moisturized and hydrated internally. So, to cure dry skin, start eating potassium rich fruits and vegetables like bananas, potatoes, etc.

Potassium and sodium

Work together to maintain the body's water balance. Potassium also is involved in the function of nerves, muscles, and certain enzymes. Potassium may have a protective effect in hypertension; increased potassium intake results in increased sodium excretion. Potassium also helps to regulate blood pressure and to maintain normal muscle contraction, as well as maintaining water balance in tissues and cells. Potassium deficiency results in fatigue and muscle weakness; these are the first signs of potassium deficiency.

The kidneys regulate the body's potassium levels. A potassium deficiency is not common but may result from severe diarrhea, poor control of diabetes, very-low-calorie diet (less than 800 calories/day), chronic alcoholism, or the use of some diuretics or laxatives.

Diuretics, which are taken to eliminate excess sodium from the body, also result in loss of potassium, so you should eat potassium-rich foods when taking diuretics. You also may be prescribed a potassium supplement while taking diuretics.

Potassium plays a number of important roles in the human body also. It helps control the proper balance of fluids in cells and body fluids. It is involved in the transmission of chemical messages between nerve cells and in the contraction of muscles. Potassium also helps in the digestion of food and in the proper function of the eyes. In many of these reactions, potassium and sodium work together to keep these functions performing properly.

The health benefits of potassium include relief from stroke, blood pressure, heart and kidney disorders, anxiety and stress, enhanced muscular strength, metabolism, water balance, electrolytic functions, and nervous system.

Potassium, the third most abundant mineral in human body, is the synonym for health insurer. It contains the qualities for maintaining a high level of human well-being and a cheerful lifestyle. There is no way one should overlook the inclusion of potassium in routine diet plan.

Apart from acting as an electrolyte, this mineral is required for keeping heart, brain, kidney, muscle tissues and other important organs of human body in good condition. Potassium chloride is the main variety of this mineral amongst others. It works in association with sodium to perform a number of critical body tasks.

Potassium is a simple mineral with a crucial job: helping your heart beat. A hundred thousand times a day, potassium helps trigger your heart's squeeze of blood through your body.

If you have high blood pressure, heart failure, or heart rhythm problems, getting enough potassium is especially important. Although potassium and cholesterol aren't directly related, eating a potassium-rich diet just might lower your cholesterol, too.

Potassium exists in abundance in soil and seawater. A healthy amount of potassium is essential to all plant and animal life. A critical electrolyte, potassium allows our muscles to move, our nerves to fire, and our kidneys to filter blood. The right balance of potassium literally allows the heart to beat.

Most people get plenty of potassium just by eating a normal American diet. The main source of potassium in our food is fruits and vegetables. Dairy products, whole grains, meat, and fish also provide potassium.

Eating a diet rich in fruits and vegetables is the best way to get enough potassium. You'll also get the other benefits of a high fruits-and-veggies diet. Those include:

- Reduction of heart disease risk
- Lower cancer risk
- Lower risk for obesity

**Potassium and Your Heart**

In healthy amounts, potassium is a heart-friendly mineral. Potassium doesn't treat or prevent heart disease, but studies have shown that getting enough potassium benefits the heart in several important ways.

**Potassium and high blood pressure**

In one major study of people with high blood pressure, taking potassium supplements reduced systolic blood pressure (the top number) by about 8 points.

But you don’t have to pop potassium pills to get the heart-healthy benefits. A diet high in fruits and vegetables (good sources of potassium) and fat-free or low-fat dairy foods can help lower systolic blood pressure by more than 10 points in people with hypertension.

**Potassium and high cholesterol**

A direct link between potassium and cholesterol hasn't been established. But it's interesting that many diets proven to lower cholesterol are also high in potassium.

If you have abnormal cholesterol levels, you're at higher than average risk for heart disease. The same goes for anyone with any of the other risk factors for atherosclerosis:

**Potassium and abnormal heart rhythms (arrhythmias)**

For people with abnormal heart rhythms, potassium may be even more important. Potassium is hiding inside every heartbeat. Each heart muscle needs just the right potassium balance in order to contract in a coordinated fashion.

People who've had abnormal heart rhythms -- arrhythmias or dysrhythmias -- are at risk for an uncoordinated heart rhythm. Some abnormal heart rhythms include:

- Atrial fibrillation
- Atrial flutter
- Ventricular tachycardia
- Ventricular fibrillation
- Supraventricular tachycardia

People with a history of arrhythmias should see a doctor on a regular basis. A periodic potassium check might be part of your routine doctor’s visits.

**Potassium and heart failure**

For many people with heart failure (also called congestive heart failure), getting enough potassium is especially important. Some diuretics -- water pills -- for heart failure can cause you to lose potassium in the urine. Potassium supplements or a potassium-rich diet can put it back. Ask your doctor before starting a potassium supplement on your own, because it may not be necessary.

## 4.3. SELENIUM

### *4.3.1. Properties & Formulation*

Selenium is a non-metallic chemical element, member of the group XVI of the periodic table. Selenium appears in red crystalline material, and a gray crystalline metallike.

| | |
|---|---|
| **Atomic number** | 34 |
| **Atomic mass** | 78.96 $g.mol^{-1}$ |
| **Electronegativity according to Pauling** | 2.4 |
| **Density** | 4.79 $g.cm^{-3}$ at 20°C |
| **Melting point** | 217 °C |
| **Boiling point** | 688 °C |
| **Vanderwaals radius** | 0.14 nm |
| **Ionic radius** | 0.198 nm (-2) ; 0.042 nm (+6) |
| **Isotopes** | 9 |
| **Electronic shell** | [ Ar ] $3d^{10}$ $4s^2$ $4p^4$ |
| **Energy of first ionisation** | 940,7 $kJ.mol^{-1}$ |
| **Energy of second ionisation** | 2045 $kJ.mol^{-1}$ |
| **Energy of third ionisation** | 2973.7 $kJ.mol^{-1}$ |
| **Standard potential** | - 0.77 V |
| **Discovered by** | Jons Berzelius 1817 |

Selenium is among the rarer elements on the surface of this planet, and is rarer than silver. Selenium is present in the atmosphere, and is occasionally found in rare minerals. Selenium occurs naturally in food: it is present in grains, cereals and meat.

### *4.3.2. Mechanisms of action*

Selenium is an antioxidant that is present in human cell, and is mostly found in the liver, kidney, testes, pancreas and spleen.

**Antioxidant properties**

Selenium has attracted attention because of its antioxidant properties. Antioxidants protect cells from damage. There is some evidence that selenium supplements may reduce the odds of prostate

cancer. Selenium does not seem to affect the risk of colorectal or lung cancer. But beware: selenium also seems to *increase* the risk of non-melanoma skin cancer.

Selenium helps to limit the activities of free radicals, which can be harmful to the body. Selenium promotes the release of an enzyme called glutathione peroxidise, which can eliminate peroxides that destroy essential lipids. When the body experiences selenium deficiency, a variety of health problems can develop, including thyroid disorder, psoriasis, heart illness and viral infections.

**Cancer prevention**

Because of its effects on DNA repair, apoptosis, and the endocrine and immune systems as well as other mechanisms, including its antioxidant properties, selenium might play a role in the prevention of cancer.

Studies have suggested an inverse association between selenium status and the risk of colorectal, prostate, lung, bladder, skin, esophageal, and gastric cancers. Several mechanisms have been suggested to explain the cancer-preventive activities of selenium. Selenium has been shown to induce DNA repair and synthesis in damaged cells, to inhibit the proliferation of cancer cells, and to induce their apoptosis, the self-destruct sequence the body uses to eliminate worn out or abnormal cells. In addition, selenium is incorporated at the active site of many proteins, including glutathione peroxidase, which is particularly important for cancer protection. One of the body's most powerful antioxidant enzymes, glutathione peroxidase is used in the liver to detoxify a wide range of potentially harmful molecules. When levels of glutathione peroxidase are too low, these toxic molecules are not disarmed and wreak havoc on any cells with which they come in contact, damaging their cellular DNA and promoting the development of cancer cells.

**Support of the thyroid gland**

Selenium is also a critical mineral for maintaining proper function of the thyroid gland. In order for the thyroid to produce the most active form of its hormone (thyroid hormone T3), selenium is essential to regulate the amount of hormone that is produced.

Selenium concentration is higher in the thyroid gland than in any other organ in the body, and, like iodine, selenium has important functions in thyroid hormone synthesis and metabolism.

## 4.4. ZINC

### 4.4.1. Properties & Formulation

Zinc is a lustrous bluish-white metal. It is found in group IIb of the periodic table.

| | |
|---|---|
| **Atomic number** | 30 |
| **Atomic mass** | 65.37 $g.mol^{-1}$ |
| **Electronegativity according to Pauling** | 1.6 |
| **Density** | 7.11 $g.cm^{-3}$ at 20°C |
| **Melting point** | 420 °C |
| **Boiling point** | 907 °C |
| **Vanderwaals radius** | 0.138 nm |
| **Ionic radius** | 0.074 nm (+2) |
| **Isotopes** | 10 |
| **Electronic shell** | [ Ar ] $3d^{10}$ $4s^{2}$ |
| **Energy of first ionisation** | 904.5 $kJ.mol^{-1}$ |
| **Energy of second ionisation** | 1723 $kJ.mol^{-1}$ |
| **Standard potential** | - 0.763 V |
| **Discovered** | Andreas Marggraf in 1746 |

Zinc is found in cells throughout the body. It helps the immune system fight off invading bacteria and viruses. The body also needs zinc to make proteins and DNA, the genetic material in all cells. During pregnancy, infancy, and childhood, the body needs zinc to grow and develop properly. Zinc also helps wounds heal and is important for proper senses of taste and smell.

### 4.4.2. Mechanisms of action

**Immune system and wound healing**

Many types of immune cells appear to depend upon zinc for optimal function. Among all the vitamins and minerals, zinc shows the strongest effect on our all-important immune system.

Zinc plays a unique role in the T-cells. Low zinc levels lead to reduced and weakened T-cells which are not able to recognize and fight off certain infections. An increase of the zinc level has proven effective in fighting pneumonia and diarrhea and other infections. Zinc can also reduce the duration and severity of a common cold.

The main job of zinc is to help white blood cells, and bodies natural infection-fighters to work properly. This includes skin infections that result from wounds. Zinc is used to treat many skin wounds, including bed sores, skin ulcers, cold sores, canker sores, surgical incisions and burns. Besides fighting infections, zinc is also important in your body's production of the connective tissue collagen. It is also supports the enzymes that allow your flesh to heal.

Researchers have studied the effects of zinc deficiency (and zinc supplementation) on immune response and number of white blood cells, including specific studies on T lymphocytes, macrophages, and B cells (all types of white blood cells). Zinc deficiency has been shown to compromise white blood cells numbers and immune response, while zinc supplementation has been shown to restore conditions to normal.

**Age-related macular degeneration (AMD)**

High concentrations of zinc are found in the retina. With age, the retinal zinc declines and seems to play a role in the development of age-related macular degeneration (AMD), an eye disease that gradually causes partial or complete loss of vision.

Research suggests that zinc might help preventing loss of visual acuity, and keep early AMD from worsening into advanced AMD. Zinc may also protect from night blindness and prevent the development of cataracts.

In a large study, older people with AMD who took a daily dietary supplement with 80 mg zinc for about 6 years had a lower chance of developing advanced AMD and less vision loss than those who did not take the dietary supplement. In the same study, people at high risk of the disease who took dietary supplements containing only zinc also had a lower risk of getting advanced AMD than those who did not take zinc dietary supplements.

## 4.5. COOPER

### 4.5.1. Properties & Formulation

Copper is a reddish metal with a cubic crystalline structure found in group Ib of the periodic table, together with silver and gold.

| | |
|---|---|
| **Atomic number** | 29 |
| **Atomic mass** | 63.546 $g.mol^{-1}$ |
| **Electronegativity according to Pauling** | 1.9 |
| **Density** | 8.9 $g.cm^{-3}$ at 20°C |
| **Melting point** | 1083 °C |
| **Boiling point** | 2595 °C |
| **Vanderwaals radius** | 0.128 nm |
| **Ionic radius** | 0.096 nm (+1) ; 0.069 nm (+3) |
| **Isotopes** | 6 |
| **Electronic shell** | [ Ar ] $3d^{10}$ $4s^{1}$ |
| **Energy of first ionisation** | 743.5 $kJ.mol^{-1}$ |
| **Energy of second ionisation** | 1946 $kJ.mol^{-1}$ |
| **Standard potential** | + 0.522 V ( $Cu^{+}$/ Cu ) ; + 0.345 V ($Cu^{2+}$/ Cu ) |
| **Discovered by** | The ancients |

Copper is a trace mineral that doesn't get a lot of attention today. Besides iron and zinc, copper is the third most abundant mineral in the body. It is found in all tissues, but is stored primarily in the liver. Copper offers both internal and external benefits. Getting enough copper is quite important to good health.

### 4.5.2. Mechanisms of action

Approximately 90% of the copper in the blood is incorporated into a compound called ceruloplasmin, which is a transport protein responsible for carrying copper to tissues that need the mineral. In addition to its role as a transport protein, ceruloplasmin also acts as an enzyme, catalyzing the oxidation of minerals, most notably iron.

The oxidation of iron by ceruloplasmin is necessary for iron to be bound to its transport protein (called transferrin) so that it can be carried to tissues where it is needed. Because copper is necessary for the utilization of iron, iron deficiency anemias may be a symptom of copper deficiency.

**Elimination of Free Radicals**

Superoxide dismutase (SOD) is a copper-dependent enzyme that catalyzes the removal of superoxide radicals from the body. Superoxide radicals are generated during normal metabolism, as well as when white blood cells attack invading bacteria and viruses (a process called phagocytosis). If not eliminated quickly, superoxide radicals cause damage to cell membranes. When copper is not present in sufficient quantities, the activity of superoxide dismutase is diminished, and the damage to cell membranes caused by superoxide radicals is increased. When functioning in this enzyme, copper works together with the mineral zinc, and it is actually the ratio of copper to zinc, rather than the absolute amount of copper or zinc alone, that helps the enzyme function properly.

**Development of Bone & Connective Tissue**

Copper is also a component of lysyl oxidase, an enzyme that participates in the synthesis of collagen and elastin, two important structural proteins found in bone and connective tissue. Tyrosinase, a copper-containing enzyme, converts tyrosine to melanin, which is the pigment that gives hair and skin its color.

**Melanin Production**

As a part of the enzymes cytochrome c oxidase, dopamine hydroxylase, and Factor IV, copper plays a role in energy production, the conversion of dopamine to norepinephrine and blood clotting, respectively. Copper is also important for the production of the thyroid hormone called thyroxine and is necessary for the synthesis of phospholipids found in myelin sheaths that cover and protect nerves.

Copper is a vital element of the natural dark pigment, melanin, which imparts coloration to skin, hair, and eyes. Melanin can be produced by melanocytes only in the presence of the cuproenzyme called tyrosinase. Intake of copper supplements helps in protecting the graying hair.

## 4.6. MANGANESE

### 4.6.1. Properties & Formulation

Manganese, a trace mineral that participates in many enzyme systems in the body, was first considered an essential nutrient in 1931. Researchers discovered that experimental animals fed a diet deficient in manganese demonstrated poor growth and impaired reproduction. Manganese is found widely in nature, but occurs only in trace amounts in human tissues. The human body contains a total of 15-20 milligrams of manganese, most of which is located in the bones, with the remainder found in the kidneys, liver, pancreas, pituitary glands, and adrenal glands.

| | |
|---|---|
| **Atomic number** | 25 |
| **Atomic mass** | 54.9380 $g.mol^{-1}$ |
| **Electronegativity according to Pauling** | 1.5 |
| **Density** | 7.43 $g.cm^{-3}$ at 20°C |
| **Melting point** | 1247 °C |
| **Boiling point** | 2061 °C |
| **Vanderwaals radius** | 0.126 nm |
| **Ionic radius** | 0.08 nm (+2) ; 0.046 nm (+7) |
| **Isotopes** | 7 |
| **Electronic shell** | [ Ar ] $3d^5$ $4s^2$ |
| **Energy of first ionisation** | 716 $kJ.mol^{-1}$ |
| **Energy of second ionisation** | 1489 $kJ.mol^{-1}$ |
| **Standard potential** | - 1.05 V ( $Mn^{2+}$/ Mn ) |
| **Discovered** | Johann Gahn in 1774 |

### 4.6.2. Mechanisms of action

In the human body, manganese functions as an enzyme activator and as a component of metalloenzymes (an enzyme that contains a metal ion in its structure).

**Enzyme activator**

Manganese activates the enzymes responsible for the utilization of several key nutrients including biotin, thiamin, ascorbic acid, and choline. It is a catalyst in the synthesis of fatty acids and cholesterol, facilitates protein and carbohydrate metabolism, and may also participate in the production of sex hormones and maintaining reproductive health.

In addition, manganese activates the enzymes known as glycolsyltranserferases and xylosyltransferases, which are important in the formation of bone. It has also been theorized that

manganese is involved in the production of the thyroid hormone known as thyroxine and in maintaining the health of nerve tissue.

**A component of metalloenzymes**

Manganese has additional functions as a constituent of the following metalloenzymes:

- Arginase, the enzyme in the liver responsible for creating urea, a component of urine
- Glutamine synthetase, an enzyme involved in the synthesis of glutamine
- Phosphoenolpyruvate decarboxylase, an enzyme that participates in the metabolism of blood sugar
- Manganese-dependent superoxide dismutase, an enzyme with antioxidant activity that protects tissues from the damaging effects of free radicals.This enzyme is found exclusively inside the body's mitochondria (oxygen-based energy factories inside most of our cells).

Deficiency Symptoms

*What are deficiency symptoms for manganese?*

Because manganese plays a role in a variety of enzyme systems, dietary deficiency of manganese can impact many physiological processes. In experimental animals, manganese deficiency causes impaired growth, skeletal abnormalities, and defects in carbohydrate and fat metabolism.

In addition, offspring of experimental animals fed manganese-deficient diets develop ataxia, a movement disorder characterized by lack of muscle coordination and balance. This condition is caused by poor development of the otoliths, the structures in the inner ear that are responsible for equilibrium.

In humans, manganese deficiency is associated with nausea, vomiting, poor glucose tolerance (high blood sugar levels), skin rash, loss of hair color, excessive bone loss, low cholesterol levels, dizziness, hearing loss, and compromised function of the reproductive system. Severe manganese deficiency in infants can cause paralysis, convulsions, blindness, and deafness.

It is important to emphasize, however, that manganese deficiency is very rare in humans, and does not usually develop unless manganese is deliberately eliminated from the diet. In addition, it has been suggested that magnesium substitutes for manganese in certain enzyme systems if manganese is deficient, thereby allowing the body to function normally despite the deficiency.

Manganese plays a crucial role in many of the physiologic processes that our bodies go through. It acts as an activator and constituent of many enzymes.

**Antioxidant Properties**

MnSOD, or manganese superoxide dismutase is one of the primary antioxidant enzymes inside of mitochondria. Due to the fact that mitochondria consume over 90% of the oxygen that our cells produce, they are particularly vulnerable to oxidative stress. During the ATP synthesis, a reactive oxygen species known as superoxide radical is produced in mitochondria. MnSOD converts these superoxide radicals to hydrone peroxide, which is then in turn reduced further to water by other antioxidant enzymes present in our bodies.

**Metabolism**

Enzymes that require to be activated by manganese play a necessary role in the metabolism of several substances, including cholesterol, amino acids, and carbohydrates. An enzyme that contains manganese called pyruvate carboxylase, as well as a manganese activated enzyme called PEPCK are required in the process of gluconeogenesis. This is the process in which glucose is produced by non-carbohydrate precursors.

Another enzyme that contains manganese known as arginase is used by liver during the urea cycle, a process in which the liver detoxifies ammonia generated during the metabolism of amino acids.

**Development of Bones**

If our bodies do not receive enough manganese and become deficient, it may result in abnormal skeletal development. Glycosyltransferases is a group of enzymes that requires manganese to act as a preferred cofactor – these enzymes are needed for the synthesizing of proteogylcans, which are beneficial to the formation and creation of healthy bone and cartilage.

## 4.7. IRON

### 4.7.1. Properties & Formulation

Iron is a ductile, malleable, silver-gray metal (group VIII of the periodic table).

| | |
|---|---|
| **Atomic number** | 26 |
| **Atomic mass** | 55.85 $g.mol^{-1}$ |
| **Electronegativity according to Pauling** | 1.8 |
| **Density** | 7.8 $g.cm^{-3}$ at 20°C |
| **Melting point** | 1536 °C |
| **Boiling point** | 2861 °C |
| **Vanderwaalsradius** | 0.126 nm |
| **Ionic radius** | 0.076 nm (+2) ; 0.064 nm (+3) |
| **Isotopes** | 8 |
| **Electronic shell** | [ Ar ] $3d^6$ $4s^2$ |
| **Energy of first ionisation** | 761 $kJ.mol^{-1}$ |
| **Energy of second ionisation** | 1556.5 $kJ.mol^{-1}$ |
| **Energy of third ionisation** | 2951 $kJ.mol^{-1}$ |
| **Standard potential** | - o.44 V ($Fe^{2+}$/ Fe ) ; 0.77 V ( $Fe^{3+}$/ $Fe^{2+}$ ) |
| **Discovered by** | The ancients |

Iron can be found in meat, whole meal products, potatoes and vegetables. The human body absorbs iron in animal products faster than iron in plant products. Iron is an essential part of hemoglobin; the red colouring agent of the blood that transports oxygen through our bodies.

The body needs iron to make the proteins hemoglobin and myoglobin. Hemoglobin is found in red blood cells and myoglobin is found in muscles. They help carry and store oxygen in the body. Iron is also part of many other proteins and enzymes in the body.

Your body needs the right amount of iron. If you have too little iron, you may develop iron deficiency anemia. Causes of low iron levels include blood loss, poor diet, or an inability to absorb enough iron from foods.

### 4.7.2. Mechanisms of action

Iron is essential for the proper growth and development of the human body. It helps metabolize proteins and plays a role in the production of hemoglobin and red blood cells. Iron deficiency can lead to conditions like iron deficiency anemia, chronic anemia, cough, and pre-dialysis anemia.

The health benefits of iron include the eradication of different causes of fatigue. Iron also plays a key role in strengthening the immune system by making it strong enough to fight off infections. Iron builds concentration, treats insomnia, and regulates body temperature.

**Hemoglobin formation**

The main health benefit of iron is the formation of hemoglobin.

Hemoglobin is the principal carrier of oxygen throughout the body and gives the dark red color to blood. Since iron is a part of hemoglobin, it gives the dark shade of red to the blood and also aids in transporting oxygen to the body cells. Additional hemoglobin is vitally important because human beings tend to lose blood in various ways, through injuries, both internal and external. Most notably, women lose considerable amounts of blood every month during their menstruation years, which is one of the major reasons why women are more likely to suffer from anemia than men.

**Oxygen carrier**

One of the most important health benefits of iron is that it acts as a carrier of oxygen and helps transfer oxygen from one body cell to another. This is a critical function of iron as oxygen is required by each and every body part to perform routine body functions.

**Muscle function**

Iron is a vital element for muscle health and is found in myoglobin, a muscle protein. Myoglobin carries oxygen from hemoglobin and diffuses it throughout muscle cells. This is required for contraction of muscles.

Iron is a vital element for muscle health. It is present in the muscle tissues and helps to provide the supply of oxygen required for contraction of muscles. Without iron, muscles lose their tone and elasticity; muscle weakness is one of the most obvious signs of anemia.

**Brain function**

Our brain uses approximately 20% of the oxygen in our bloodstream. Iron helps supply oxygen to blood making it very important for brain health.

Increased development of the brain is also one of the many benefits of iron. Since oxygen supply in the blood is aided by iron and the brain uses approximately 20% of the blood oxygen, iron is directly related to brain health and its functions. Proper flow of blood in the brain can stimulate

cognitive activity and help to create new neural pathways to prevent cognitive disorders like dementia and Alzheimer's disease, so proper iron intake and its subsequent brain oxygenation is essential.

# 5. INNER7® USES

## 5.1. TRANSDERMAL MAGNESIUM THERAPY

The advantages of transdermal absorption are varied including the direct application of medicine over an area of pain, the more rapid entry into the bloodstream, bypassing the digestive tract where it can be reduced in its effect by enzyme and acid actions, and increased absorption and bioavailability. In the case of magnesium it can reduce the common side effect of diarrhea that is the single-most limiting factor to increasing our magnesium levels sufficiently.

For topical applications, avoid direct contact with eyes and mucus membranes. INNER7® passes directly into the tissues via the skin. After 20 minutes, the majority of the magnesium will have been absorbed.

It is advisable to begin the first few days of application with modest use, and gradually work up to larger amounts.

## 5.2. SOAKING FEET AND BATH

Another potential way to administrate magnesium is via the method of soaking in a bath of magnesium. It will help draw toxins from the tissues and restores cellular minerals to an optimal level.

Sitting in a magnesium foot bath for 30 minutes once every week is very beneficial as it soaks out the toxins that we accumulate in our body while and at the same time soaking. The foot work as a convenient part of your body to accumulate the magnesium, as it is easily accessible for the circulatory system.

The other natural minerals and vitamins in INNER7® help clean and open our skin pours.

## 5.3. FACE CARE AND ACNE TREATMENT

INNER7® is ideal for dry skin conditions: The magnesium solution improves skin barrier function, enhances skin hydration, and reduces inflammation in atopic dry skin. INNER7® also helps the body absorb the nutrients needed to nourish dry skin

For the face

- Acne: The magnesium helps to stimulate and cleanse the skin, while removing old skin cells, stimulating blood flow and inducing the lymphatic system.

Magnesium is also an important mineral for stabilizing excessive hormone production, a major contributing cause to acne.

- Also good for skin problem: Psoriasis, Eczema, Dermatitis

**Magnesium and acne**

Taking one portion of magnesium citrate with two portions of calcium might clear a person's acne. Adding Vitamin C the mix can help your body absorb the magnesium. This can help because magnesium plays a role in hormone balance, which can affect acne. Magnesium also may help to combat acne by reducing stress. Having more dietary magnesium intake also has an association with reduced markers of systematic inflammation, according to an April, 2007 study published in the "American Journal of Clinical Nutrition."

## 5.4. HAIR TREATMENT

INNER7® can be used with dry, lacking shine hairs. The Magnesium into INNER7® works to promote healthy hair and helps keep hair silky smooth and moisturized.

INNER7® also helps reducing hair loss, caused by magnesium-deficient cells.

Recommended dosage: Add 2mL into your shampoo bottle.

**Magnesium and hair loss**

- There are many factors that affect hair loss. Hair loss is usually a subtle disorder that increases over a period of time. Often hair loss is associated with disease. Furthermore, there are many people who suffer from hair loss because of conditions such as stress, dandruff or diabetes. Sometimes other diseases or chemotherapy can also cause hair loss. Hair loss is also caused by a deficiency or overabundance in certain minerals or nutrients. Calcium and magnesium imbalance is a common contributor.
- A deficiency in the minerals magnesium, zinc, or calcium affects how the hair is nourished. A lack in these important minerals may eventually cause the hair to fall out. Furthermore, too much calcium in relation to the amount of magnesium in the body can also negatively affect the hair.

- Lot of times females have too much calcium in their body in relation to magnesium. This calcium and magnesium imbalance stems from eating too much sugar. Eating too much sugar can cause the body to become insulin resistant. When a lot of sugar is eaten, insulin levels become very high, thus taking the calcium from bones. Then the calcium gets deposited into the body's soft tissues, and as a result, calcium and magnesium levels in the body become unbalanced.

## 5.5. ORAL AND DENTAL CARE

For healing and maintaining oral health, apply to tooth brush, teeth, gums and under the tongue.
The magnesium plays a key role in the strength and formation of teeth.
Recommended dosage: Mix 1 mL in a cup of water and gargle for 20 seconds.

## 5.6. COOKING

The magnesium in INNER7® has great permeability, allowing to squeeze drops neither you are cooking meat, fish or vegetables.

Example1: cooking with fish. Just add INNER7® on the fish and leave it for 30 minutes. The saltiness penetrates to the core while impurities are removed, allowing making lovely, crisp grilled fish.

Example2: Stewed vegetables. Stewing them for a long time causes the vegetables to lose shape. This is because the pectin, which holds the vegetables together, is destroyed during the cooking process. The magnesium in INNER7® will prevent the pectin from being destroyed and also preserve vegetable flavours.

# 6. SAFETY

## S P E C I F I C A T I O N S

## INNER7®

***Organoleptic characteristics:***

| | | |
|---|---|---|
| Aspect: | Liquid | In conformity |
| Colour: | Transparent | In conformity |
| Odour: | Characteristic | In conformity |
| Solubility in water: | Soluble | In conformity |

***Microbiological analysis***

| | | |
|---|---|---|
| Aerobic plate count | Not more than 30/ml | In conformity |
| Coliform bacteria (MPN) | Negative/100ml | In conformity |
| Viable molds count | Negative/1ml | In conformity |
| Viable yeasts count | Negative/1ml | In conformity |
| Salmonella | Negative/25ml | In conformity |

***Minerals***

| | | | |
|---|---|---|---|
| Magnesium | 55,000 mg/L | Copper | 0.06 mg/L |
| Sodium | 44,000 mg/L | Manganese | 0.03 mg/L |
| Potassium | 17,000 mg/L | Iron | <1 mg/L |
| Calcium | 110 mg/L | Phosphorus | <1 mg/L |
| Zinc | 0.12 mg/L | Selenium | <0.05 mg/L |

***Heavy metals***

| | | |
|---|---|---|
| Lead | Not detected (QL0.01mg/L) | In conformity |
| Chromium | Not detected (QL0.05mg/L) | In conformity |
| Cadmium | Not detected (QL0.01mg/L) | In conformity |
| Arsenic | 0.03 mg/L (QL0.05mg/L) | In conformity |

***Storage conditions***

Keep in the original sealed packaging, at room temperature (not under 10°C).
Crystal precipitation will occur below 10°C.

# 7. CLINICAL TRIALS

## 7.1. BIOAVAILABILITY AND EFFICACY OF INNER7® ON WEIGHT MANAGEMENT, GLYCEMIX INDEX AND LIPID BALANCE

1. **TITLE CLINICAL TRIAL**

   Bioavailability and efficacy of INNER7® on weight management, glycemic index and lipid balance.

2. **TYPE OF STUDY**

   Placebo-controlled study comparing INNER7® vs. Placebo.

3. **SPONSOR**

   Innovation Labo - Kanaya Bldg 5F, 4-11-3 Hatchobori, Chuo-ku, Tokyo 104-0032 JAPAN

4. **LABORATORY**

   MEDICA TOKYO Co.LTD - 20-1, 3Chome Nishi-Shinjuku, Shinjuku-ku Tokyo JAPAN

   Managed by Dr Taro Hirata - clinical@medica-tokyo.jp

5. **PRODUCT INFORMATION**

   Product name: INNER7®

   Appearance: Liquid

   Colour: Transparent

   Taste: Characteristic

6. **STUDY OBJECTIVES**

   Evaluate the bioavailability of magnesium in INNER7® and its efficacy on several biological and anthropometric functions to assess the probable effect on weight management, glycemic index, and lipid balance.

7. **VARIABLE VALUED**

   Magnesium bioavailability, glycemia, insulemia, HOMA, triglycerides, total cholesterol, HDL-c, LDL-c, weight, BMI, waist circumference, hip circumference.

8. **TOTAL NUMBER OF PATIENTS**

   30 participants (22 women, 8 men) were enrolled in the study (15 subjects in INNER7® group and 15 subjects in placebo group).

9. **DURATION OF TREATMENT AND DOSAGE**

   - Oral glucose tolerance test (OGTT) was carried out with an auto test every 15 minutes for 120 minutes. Patients received either one dose of 2ml of INNER7® or placebo forehand.

- Effect of INNER7® on anthropometric and biological function: the participants were randomized to receive twice-daily of INNER7® (2ml with breakfast and 2ml in the evening with dinner) or placebo.

**Abstract**

**Objective:** The high prevalence of lifestyle diseases has contributed towards morbidity and mortality in diabetes, obesity, and cardiovascular disorders. Being abundant in minerals, various marine natural products have been reported in recent years to possess potent biological and medicinal potential. The present study evaluated the bioavailability of magnesium in INNER7®, a natural marine product and its efficacy on several biological and anthropometric functions to assess the probable effect on weight management, glycemic index, and lipid balance.

**Material and Methods:** A total of 30 participants were enrolled in the study (22 women and 8 men). In part 1, the effect of INNER7® on glycemia was assessed in a cross over design. Part 2 evaluated the effect of INNER7® on anthropometric (age, height, weight, body mass index [BMI], waist circumference and hip circumference) and biological function (glycemia, insulinemia, triglycerides, cholesterol, high density lipoprotein-cholesterol, and low density lipoprotein-cholesterol) of the participants in a 30 day study. Bioavailability of magnesium in INNER7® was also evaluated in comparison to conventional magnesium.

**Result:** INNER7® exhibited a statistically significant lowering in mean glycemia with test meal than the placebo. There was a reduction in the weight, BMI, hip circumference, and insulinemia in the INNER7® group. The study product also demonstrated a positive effect on lipid balance. The bioavailability of magnesium in was also found to be higher than that with conventional magnesium. The study product was well-tolerated.

**Conclusion:** INNER7® was found to have a positive effect on weight management, glycemia control and lipid balance of the body. The bioavailability of magnesium was also higher in INNER7® than conventional magnesium.

**Introduction**

The number of people with various lifestyle diseases has witnessed an uphill in recent years owing majorly to sedentary lifestyle and dietary changes. Increased prevalence of obesity and physical inactivity is a major risk factor for developing metabolic disorders like diabetes and other cardiovascular disorders (CVDs).[1]

The worldwide prevalence of diabetes for all age-groups was 2.8% in the year 2000, and is estimated to be 4.4% in year 2030. The total number of people with diabetes is expected to rise from 171 million in year 2000 to 366 million in year 2030 posing it as a global health burden.[2] Various studies have demonstrated that high glycemic index (HGI) leads to insulin resistance. In diabetic subjects, the chronic consumption of a low glycemic index (LGI) diet is generally found to improve plasma glucose and lipid profiles.[3] CVD, a leading cause of death among adults in the United States, includes abnormal lipid levels and elevated body mass index (BMI) as major risk factors.[4] Changes in dietary habits and lifestyle, exercise and use of alternative treatments are considered important for combating lifestyle diseases.

Owing to diverse biological activities and potential medicinal properties, biomaterials from marine origin have gained interest in recent years. Various polysaccharides, proteins, glycosaminoglycans and ceramics have been isolated and characterized from marine raw materials which have been found promising for range of biomedical applications.[5] Several marine natural products have also been utilized as leads for synthesis of bioactive molecules.[6-8] The presence of wide range of minerals like magnesium, calcium, potassium, selenium, zinc, copper, manganese, iodine, iron etc in the marine sources renders them as a source of pharmacologically active molecules.[7-9]

Magnesium is the fourth most abundant mineral in the body. Found abundantly in natural sources like green vegetables, legumes, nuts and seeds, magnesium is known as a magic mineral for diabetes.[10] Hypomagnesemia (low blood levels of magnesium) is frequently seen in individuals with type 2 diabetes due to increased urinary loss of magnesium associated with hyperglycemia.[11] Various studies have demonstrated the potential benefit of supplemental magnesium in controlling type 2 diabetes and lowering risk of coronary heart diseases.[12-14]

The present study was aimed to assess the bioavailability of magnesium in INNER7®, a natural marine product and its efficacy on several biological and anthropometric functions to assess the probable effect on weight management, glycemic index, and lipid balance.

**Material and Methods**

***Study Design***

As the study product contains magnesium, the bioavailability of magnesium from INNER7® was compared with that obtained from conventional. The initial level of magnesium in blood serum was identical in all participants of the study. The study analyzed the quantity of magnesium in blood serum after morning single oral dose of INNER7® compared to conventional magnesium.

The study was conducted in two parts. To assess the efficacy of INNER7® on glycemia, in part 1, 30 subjects were divided in 2 groups to receive test meal in a crossover design. Along with test meal, in group A, participants received placebo and in group B, participants received INNER7®. The participants were randomized to receive daily 2ml of placebo or INNER7® with breakfast.

The test meal used in part 1 was composed of 80 g of white bread, 15 g of butter, 100 g of 'fromage blanc' (fresh cheese like yogurt but with more protein and no bacteria), and 250 mL of coffee or tea. The test meal represented 357.1 kcal, with 13.6 g protein (15.23%), 12.3 g lipids (31%), and 48 g carbohydrates (53.77%). The test meal was taken by 15 persons after an overnight fast for 2 times with at least one week delay between the 2 mornings.

After the completion of part 1 of the study, in part 2, the 30 participants were divided into 2 groups to receive either placebo or INNER7® for one month in parallel design. The participants were on their usual diet and received placebo or INNER7® twice-daily (2ml with breakfast and 2ml in the evening with dinner).

***Patient Population***

A total of 30 patients (aged 29 to 71 years) were included in the study. Out of 15 participants in each group, 4 were men and 11 women in group A (on placebo) and 4 participants were men and 11 women in group B (on INNER7®). The anthropometric profile of the study population is presented. All the baseline demographics (age, height, weight, BMI, waist circumference and hip circumference) were comparable between the INNER7® group and the placebo group. The average BMI of the study population was 27.40 in group A and 27.82 in group B. The participants included in the study were overweight with high waist and hip circumference indicating a higher risk for developing diabetes and cardiovascular disorders.

*Table 1. Anthropometric profile of study population*

| **Parameter** | **Placebo** | **INNER7®** |
|---|---|---|
| Age (years) | 54.7 | 55.4 |
| Height (m) | 1.63 | 1.63 |
| Weight (kg) | 81.33 | 82.41 |
| BMI(kg/m²) | 27.40 | 27.82 |
| WC (cm) | 98.73 | 100.77 |
| HC (cm) | 109.67 | 109.00 |
| WC/HC (ratio) | 0.90 | 0.93 |

As the major aim of this study was to assess the effect of INNER7® on glucidic metabolism, the study population was chosen having high post-prandial glycemia (>1.15 g/L =6.325 mmol/L) which is a characteristic of beginning of intolerance to glucose and usually leads to type 2 diabetes. The fasting insulinemia with values higher than the limit (2.6 µmol/L-11.1 µmol/L) also indicates beginning of insulin resistance.

*Table 2. Biological profile of study population*

| **Parameter** | **Placebo** | **INNER7®** |
|---|---|---|
| Glycemia | 6.05 | 5.78 |
| Insulinemia (µmol/L) | 15.73 | 12.86 |
| HOMA | 4.53 | 3.34 |
| Triglycerides | 1.42 | 1.97 |
| Cholesterol | 5.76 | 6.03 |
| HDL-c | 1.48 | 1.42 |
| LDL-c | 3.64 | 3.73 |

***Measurements***

In part 1 of the study, oral glucose tolerance test (OGTT) was carried out as a measure of glycemia with an auto test every 15 minutes for 120 minutes after a test meal with the study product, INNER7® or placebo.

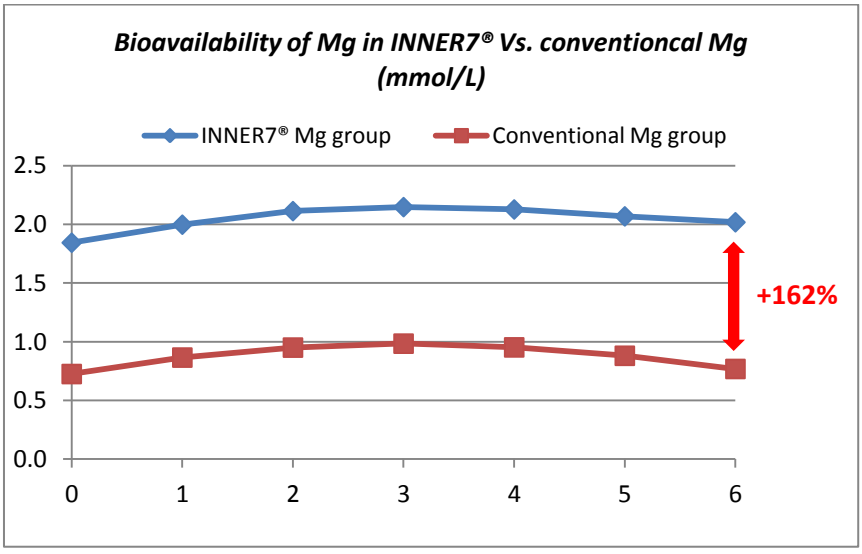
In part 2 of the study, one month protocol was followed and the participants underwent anthropometric measurements (weight, BMI, waist circumference, hip circumference, waist/hip ratio) and biological measurements for insulinemia, glycemia, HOMA index (insulinemia*glycemia/22.5=Insulin Resistance Index) cholesterol, high-density lipoprotein cholestrol (HDL-c), low-density lipoprotein cholesterol (LDL-C), and triglycerides at day 0 and day 30 of the study.

The participants were also assessed for product satisfaction while using the product and occurrence of side effects during the course of the study.

**Results**

In participants who received single dose of INNER7® product, the total magnesium level 6 hours after oral administration was higher (2.02 mmol/L) compared to conventional magnesium (0.77 mmol/L).

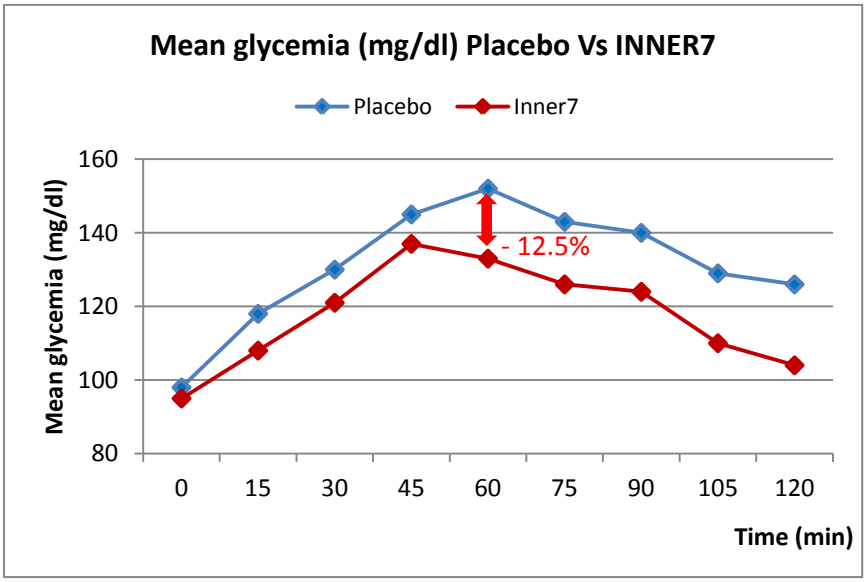
*Figure 1. Bioavailability of magnesium in INNER7® vs conventional magnesium (mmol/L)*




In this study, 30 eligible patients were included having similar baseline characteristics.

To evaluate the effect of INNER7® with test meal on the glycemic response, the level of glycemia (mg/dL) was compared at the start and end of the test. The OGTT carried out in part 1 of the study revealed a statistically significant lowering in mean glycemia (mg/dL) in INNER7® group as compared to placebo group. The glycemia peak obtained with INNER7® was bigger and occurred earlier with that obtained for placebo. The decrease in glycemia was also found to be quicker with INNER7® than with the placebo.

*Figure 2. Mean glycemia with INNER7® and placebo*




***One Month Protocol***

During one month protocol in part 2 of the study, participants in both the groups lost weight. A reduction of 2.25% in weight was observed in the INNER7® group against 0.39% in the placebo group. BMI was also found to be reduced following 30-day treatment. The reduction in BMI was 0.40% in the placebo group and 1.80% in the INNER7® group .

*Table 3. Weight and body mass index*

| Parameter | Placebo | INNER7® |
|---|---|---|
| $Weight_0$ (Kg) | 81.33 | 82.41 |
| $Weight_{30}$ (Kg) | 81.01 | 80.56 |
| $W_{30}$-$W_0$ (Kg) | -0.31 | -1.85 |
| $BMI_0$ (kg/m²) | 27.40 | 27.82 |
| $BMI_{30}$ (kg/m²) | 27.29 | 27.32 |
| $BMI_{30}$-$BMI_0$ (kg/m²) | -0.11 | -0.50 |

*Figure 3. Weight in placebo and INNER7® group at Day 0 and Day 30*

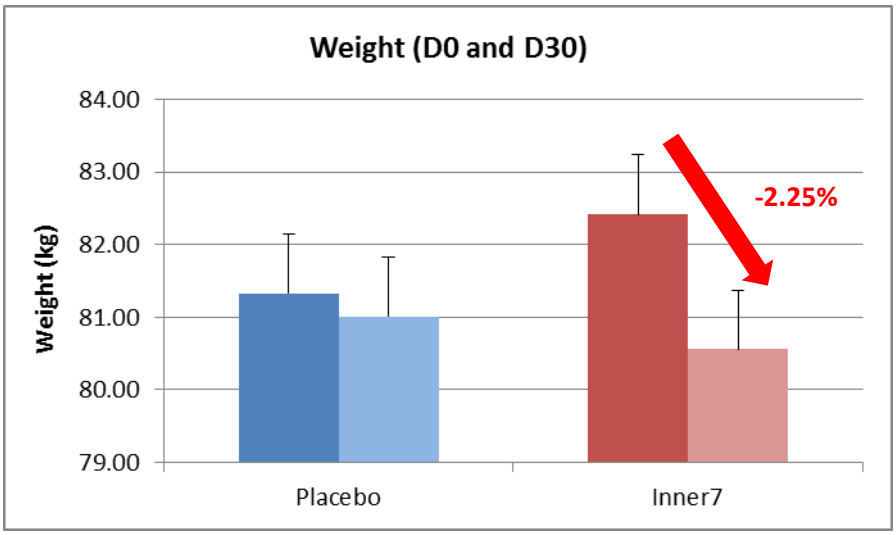
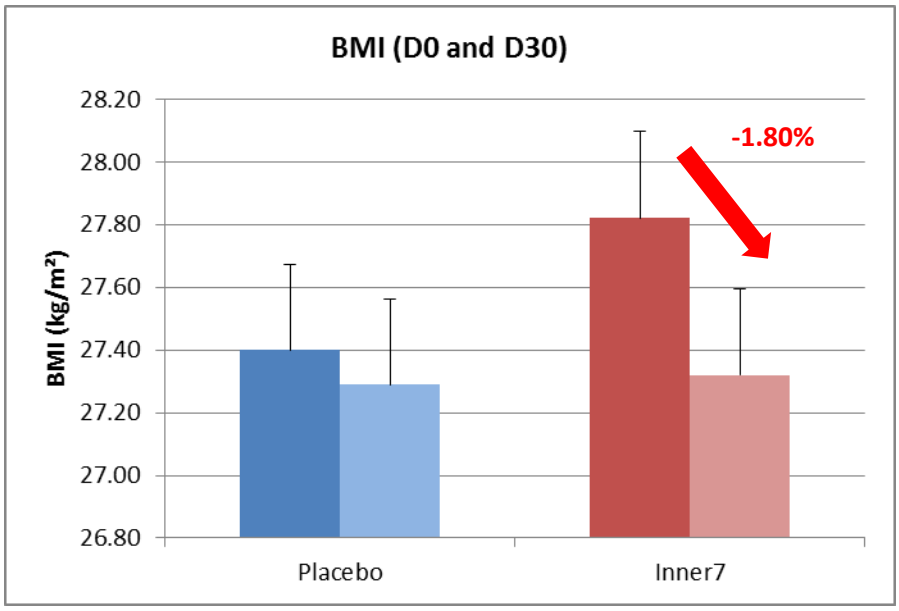



*Figure 4. BMI in placebo and INNER7® group at Day 0 and Day 30*




Following one month protocol, participants in the INNER7® group reported a reduction in the hip circumference (4.50%) and in the waist circumference (5.66%). However, participants in the placebo group observed a gain in both the waist circumference (0.55%) as well as in the hip circumference (0.34%).

*Table 4. Waist and hip circumference*

| Parameter | Placebo | INNER7® |
|---|---|---|
| $WC_0$ | 98.73 | 100.77 |
| $WC_{30}$ | 99.28 | 95.07 |
| $WC_{30}$-$WC_0$ | +0.55 | -5.70 |
| | | |
| $HC_0$ | 109.67 | 109.00 |
| $HC_{30}$ | 110.04 | 104.10 |
| $HC_{30}$-$HC_0$ | +0.37 | -4.90 |
| | | |
| $WC/HC_0$ (ratio) | 0.90 | 0.92 |
| $WC/HC_{30}$ (ratio) | 0.90 | 0.88 |
| $WC/HC_{30}$-$WC/HC_0$ | 0.00 | -0.04 |

*Figure 5. Waist circumference in placebo and INNER7® group at Day 0 and Day 30*

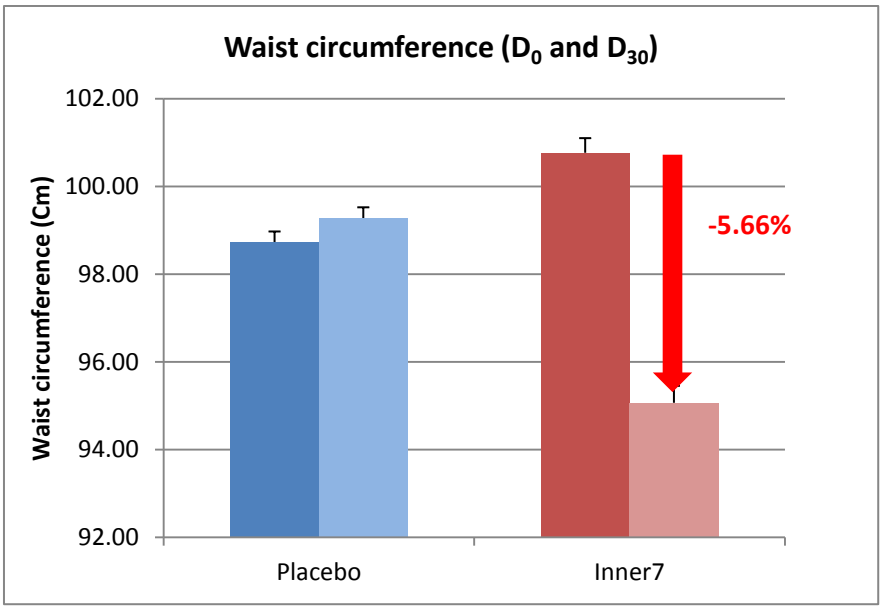
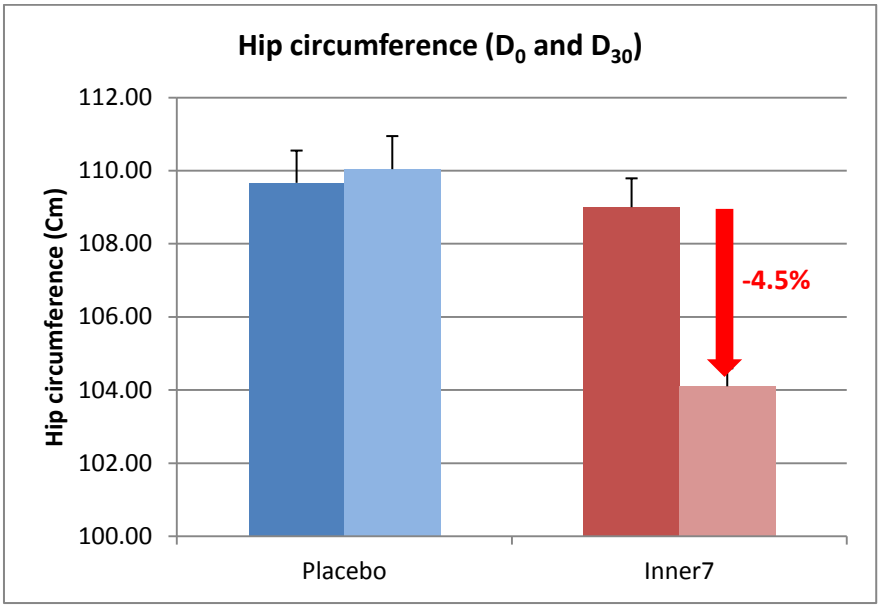


*Figure 6. Hip circumference in placebo and INNER7® group at Day 0 and Day 30*




In participants following one month protocol, the glycemia level was found to increase by 2.63% in the placebo group and decrease by 3.29% in the INNER7® group. The insulinemia level was reduced by 9.32% in the placebo and by 17.26% in the study product group after 30-day treatment. As a consequence of the changes in glycemia and insulinemia level, the homeostasis model assessment (HOMA) index reduced by 19.98% in the placebo group and by 6.94% in the INNER7® group.

*Table 5. Glycemic metabolism*

| Parameter | Placebo | INNER7® |
|---|---|---|
| $Gl_0$ (mmol/L) | 6.05 | 5.78 |
| $Gl_{30}$ (mmol/L) | 6.21 | 5.59 |
| $Gl_{30}$-$Gl_0$ (mmol/L) | 0.16 | -0.19 |
| | | |
| $I_0$ (µmol/L) | 15.73 | 12.86 |
| $I_{30}$ (µmol/L) | 14.27 | 10.64 |
| $I_{30}$-$I_0$ (µmol/L) | -1.47 | -2.22 |
| | | |
| $HOMA_0$ | 4.23 | 3.30 |
| $HOMA_{30}$ | 3.94 | 2.64 |
| $HOMA_{30}$-$HOMA_0$ | -0.29 | -0.66 |

*Figure 8. Glycemia (GI) in placebo and INNER7® group at Day 0 and Day 30*

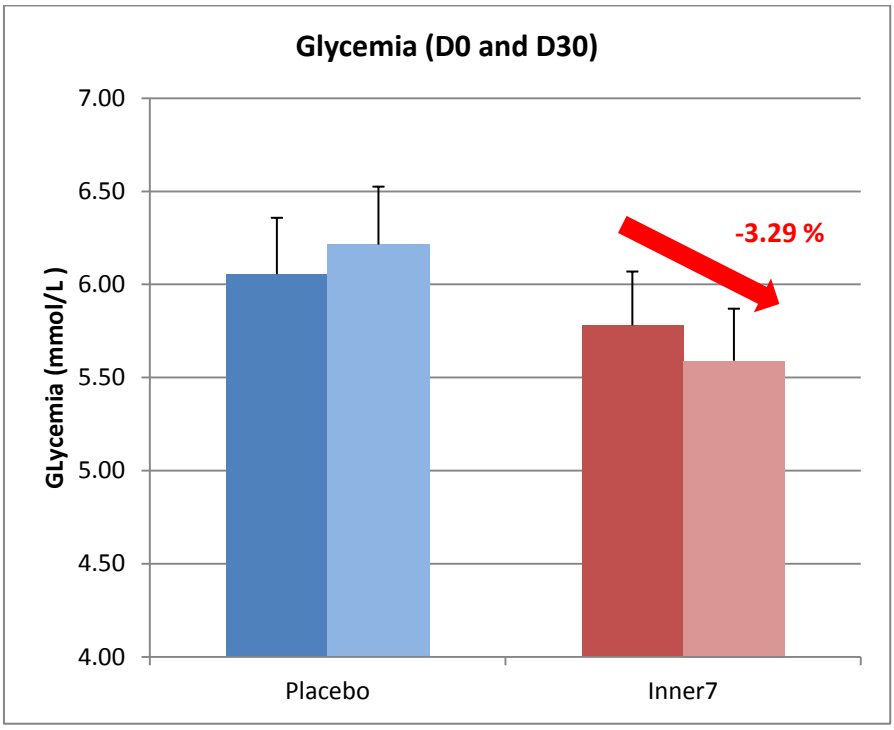


*Figure 9. Insulinemia (I) in placebo and INNER7® group at Day 0 and Day 30*

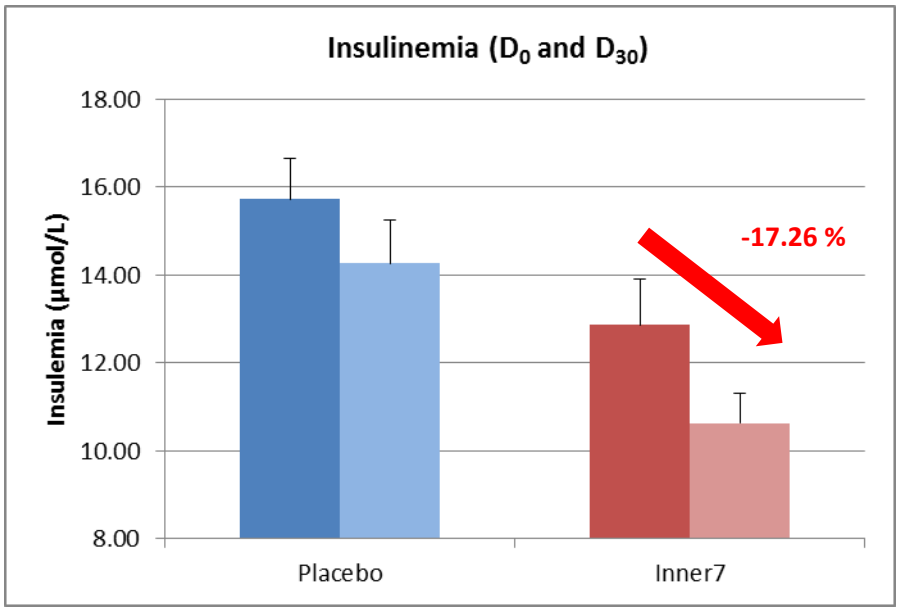
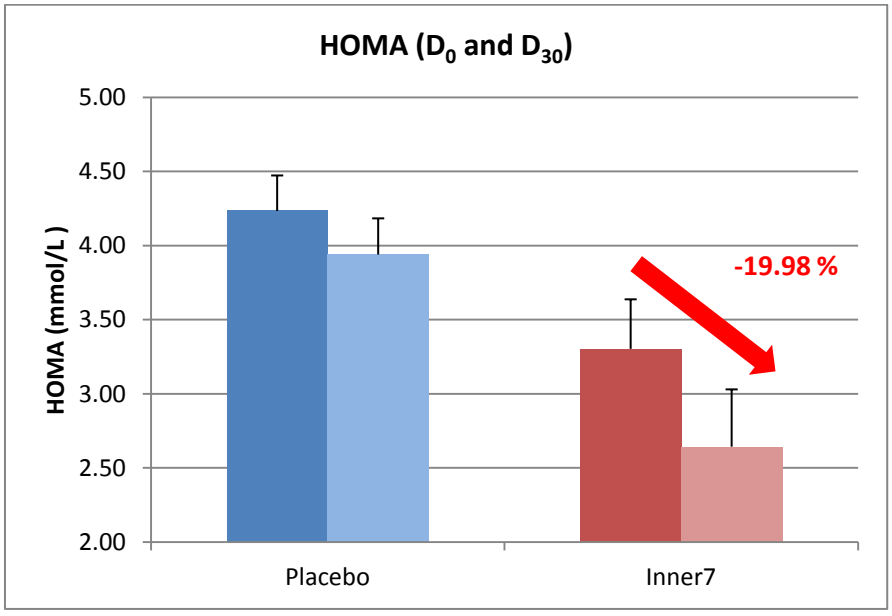



*Figure 10. Homeostasis model assessment (HOMA) in placebo and INNER7® group at Day 0 and Day 30*




The participants in the study were also assessed for their lipid metabolism. Lipid profiling revealed a higher reduction in the level of triglycerides on day 30 (18.86%) in the INNER7® group as compared to placebo group (5.01%). The reduction in total cholesterol was also higher in the INNER7® group (2.42%) than the placebo group (1.76%). The level of HDL-c which is considered to be beneficial for the body was found to increase by 2.39% in the INNER7® group. No change in the

HDL-c levels was reported in the placebo group. A reduction of 5.67% was observed in LDL-c level in the INNER7® group and 2.09% in the placebo group.

*Table 6. Lipid metabolism*

| Parameter | Placebo | INNER7® |
|---|---|---|
| $TG_0$ | 1.42 | 1.97 |
| $TG_{30}$ | 1.35 | 1.60 |
| $TG_{30} – TG_0$ | -0.07 | -0.37 |
| | | |
| TCh | 5.76 | 6.03 |
| $TCh_{30}$ | 5.66 | 5.89 |
| $TCh_{30}$- $TCh_0$ | -0.10 | -0.15 |
| | | |
| HDL-$c_0$ | 1.48 | 1.42 |
| HDL-$c_{30}$ | 1.48 | 1.46 |
| HDL-$c_{30}$-HDL-$c_0$ | 0 | +0.03 |
| | | |
| LDL-$c_0$ | 3.64 | 3.73 |
| LDL-$c_{30}$ | 3.56 | 3.52 |
| LDL-$c_{30}$-LDL-$c_0$ | -0.08 | -0.21 |

*Figure 12. Triglycerides (TG) in placebo and INNER7® group at Day 0 and Day 30*

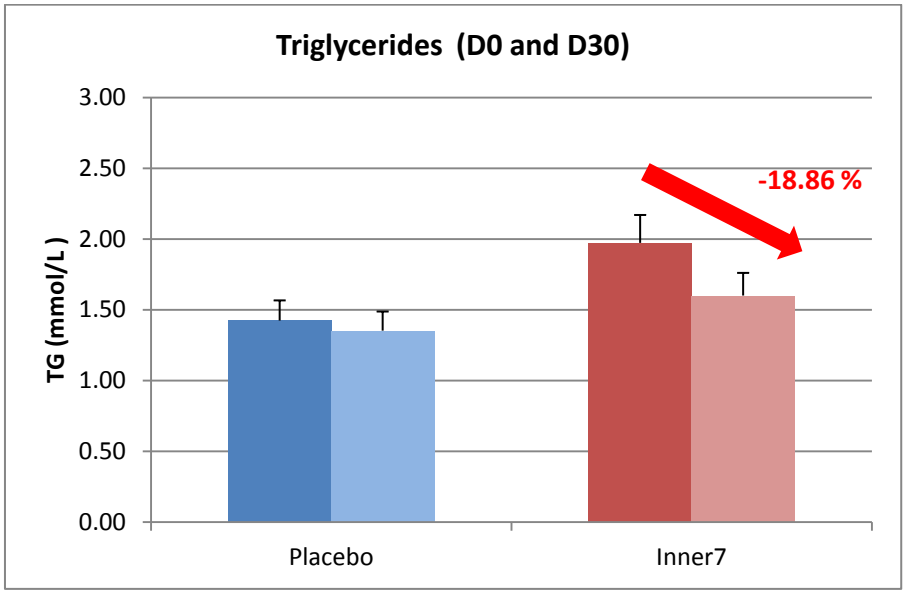


*Figure 13. Total cholesterol (TCh) in placebo and INNER7® group at Day 0 and Day 30*

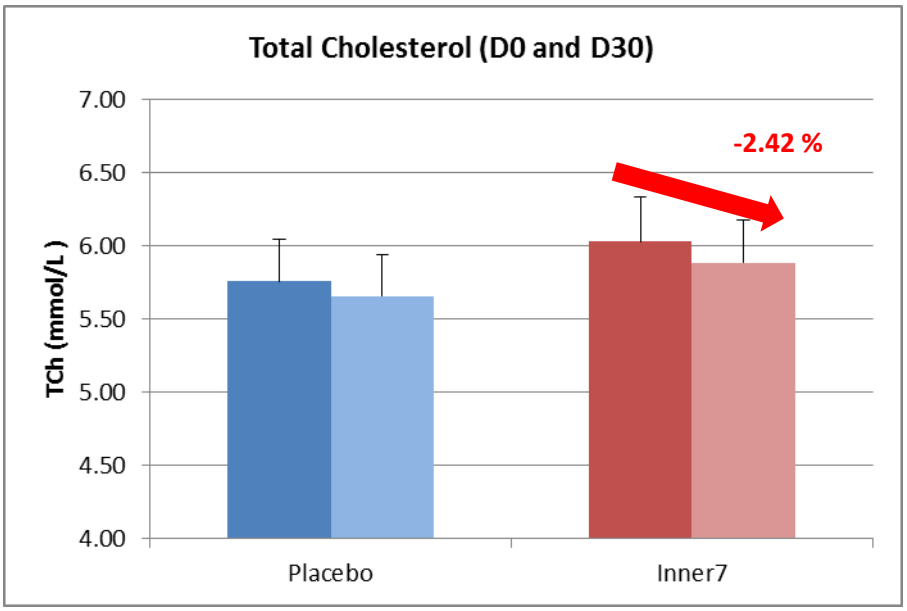



*Figure 14. High-density lipoprotein cholesterol (HDL-c) in placebo and INNER7® group at Day 0 and Day 30*

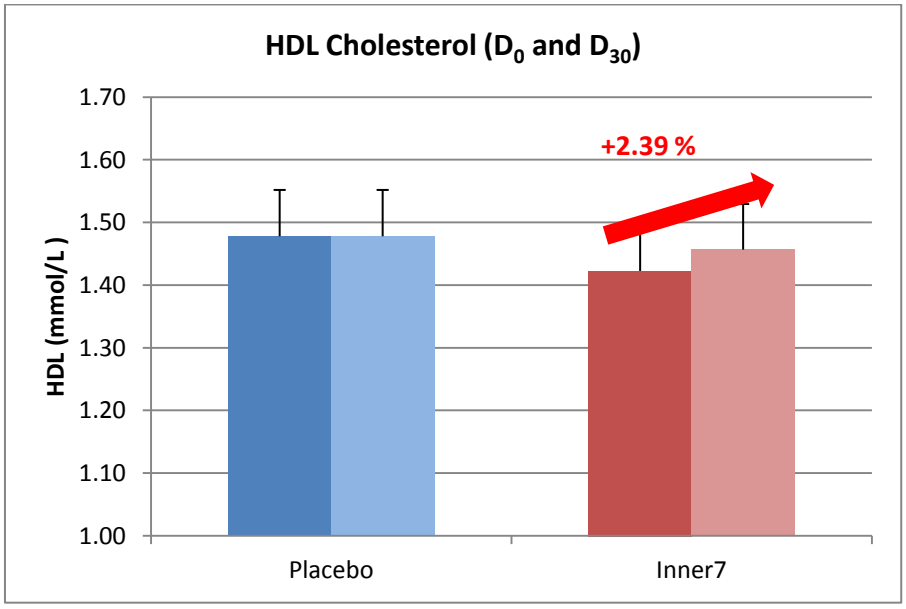
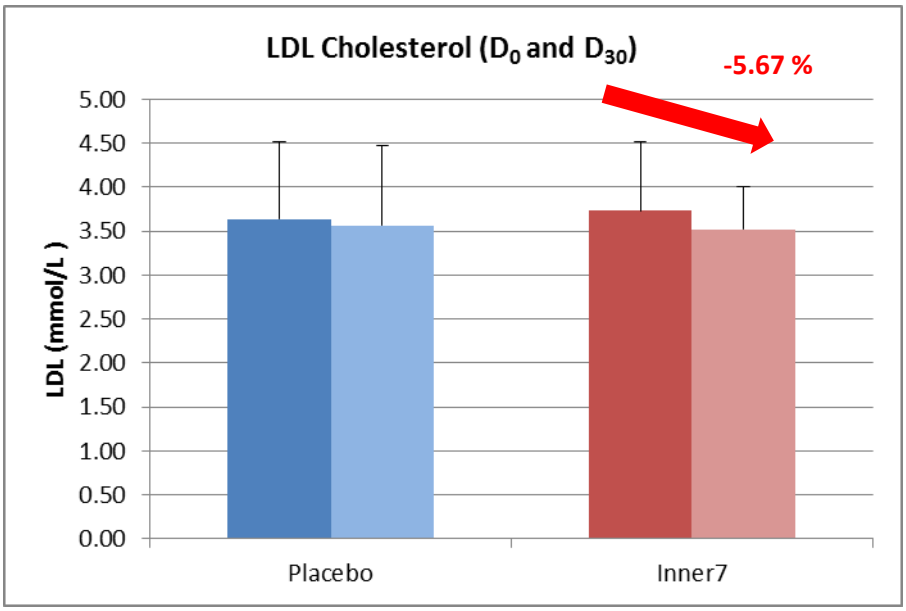



*Figure 15. Low-density lipoprotein cholesterol (LDL-c)in placebo and INNER7® group at Day 0 and Day 30*




**Tolerance and Impression**

One participant in each group reported side effects at the end of study; however, the use of the products was not withdrawn due to the events. The product was found to be compliant and easy to use.

In the INNER7® group, 4 participants reported lowering effect of the product on appetite, 2 participants reported slimming effect and 2 reported positive effect on stress. In the placebo group, one participant reported slimming effect.

**Discussion and Conclusion**

Various active compounds from marine sources are known to possess potent biological and medicinal activities. Along with presence of minerals like calcium, potassium, selenium, zinc, copper, manganese, iodine and iron in INNER7®, this marine product is particularly abundant in magnesium which has been reported to possess role in preventing and managing diabetes, insulin resistance and CVDs. However, the effectiveness of the magnesium supplement is influenced by the bioavailability of the magnesium present in that supplement. In this study, the bioavailability of magnesium in INNER7® was higher than the conventional magnesium.

INNER7® was found to demonstrate efficacy against glycemic response with a test meal having tendency towards quicker lowering of glycemia peak. The waist and hip circumference which relates closely to the BMI are determinant of the waist: hip ratio and act as an indicator of obesity. INNER7® was found to reduce weight, BMI, waist and hip circumference indicating positive effect

on weight management and obesity after one month treatment. The study also provides evidence of the efficacy of INNER7® on insulin resistance and lipid component of the body. INNER7® was well-tolerated in the study population. A total of 6 subjects in the INNER7® group reported positive impact of the product (lowering of appetite, slimming effect and stress relieving).

In conclusion, the present study provided evidence for the positive effect of INNER7® on weight management, glycemia control and lipid balance of the body and higher bioavailability of magnesium in this natural marine product.

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## 7.2. EFFICACY OF INNER7® ON PREVENTION OF WATER STORAGE

**Customer:**

INNOVATION LABO TOKYO
Kanaya Bldg 5F, 4-11-3 Hatchobori, Chuo-ku, Tokyo 104-0032 JAPAN

**Scientific supervisor:**

Dr. Taro HIRATA

**Setting**:

MEDICA TOKYO LABORATORIES
20-1, 3Chome Nishi-Shinjuku, Shinjuku-ku Tokyo JAPAN

**Study protocol number:**

Study MM/BP 4503-02

**Objective**:

Investigate the efficacy of INNER7® on the prevention of water storage.

**Design**:

Controlled clinical trial.

**Subjects:**

30 healthy female subjects aged 20–65.

**Interventions:**

On the study day, subjects self-administered a dose of 1mL of INNER7® at 8:00 am, and a dose of 1mL of INNER7® at 4:00 pm.

**Main outcome measures:**

Subjects monitored their urination frequency and fluid output for 3 consecutive days.

**Results:**

The results of this study show a significant increase in urination frequency and volume after administration of INNER7®.

**Introduction**

INNER7® is a balanced complex of minerals from deep sea water from more than 300 meters. INNER7® contains more than 60 bio-minerals (mainly Magnesium, Calcium, Potassium, Selenium, Zinc and Copper) which tend to be deficient in our modern-day diet but essential for proper functioning of the body and prevention of water storage.

**Design**

This study was conducted over a period of 3 days, with a group of healthy female subjects aged 20–65. The mean age of participants was 35.3 years. The study was performed according to the guidelines of the Declaration of Helsinki and Tokyo for humans, and subjects were asked to abstain from consumption of alcohol and medications.

**Procedure**

In this study, INNER7® was ingested by volunteers to investigate whether an increased urinary frequency and volume would result.

Subjects monitored their urination frequency and fluid output for 3 consecutive days starting at day -1. On the trial day (day 0), subjects self-administered a 1mL dose of INNER7® at 8:00 am, and 1mL dose of INNER7® at 4:00 pm. At the end of follow-up day (day 1), subjects returned the data sheets for analysis.

Day −1: Record of urine output
Day 0: Record of urine output, INNER7® intake
Day 1: Record of urine output

Participants were given with a graduated disposable beaker for measuring their urine volume.

**Statistics**

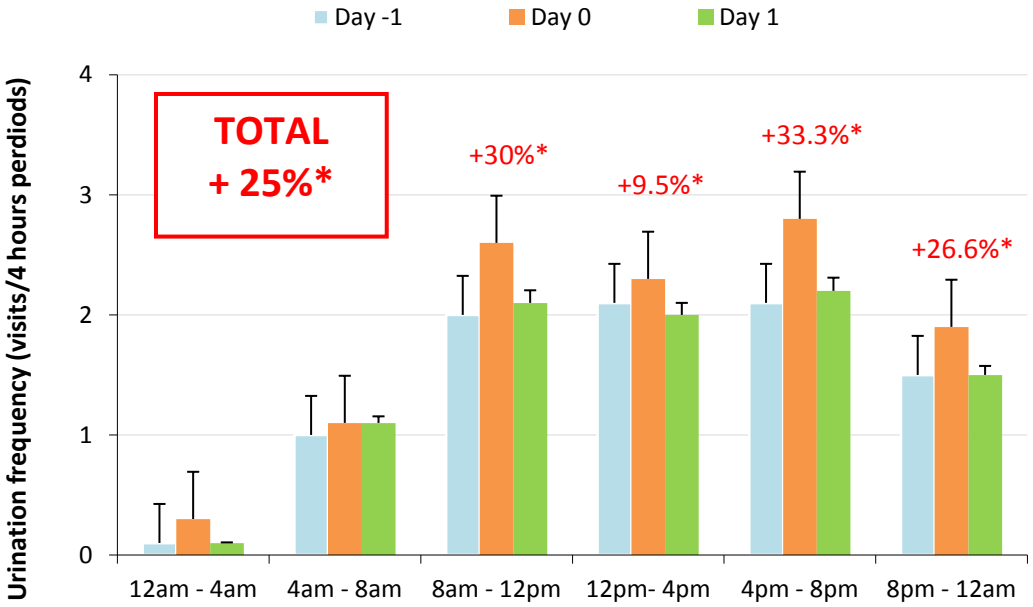
All data were recorded and data were analysed in 4-hours ranges on both the frequency of urination and volume of fluid output. The first day of data collection (day −1) was used as the control day, and the subsequent treatment day (day 0) and the follow-up day (day 1) were analysed in comparison to that day.

**Results**

**Frequency of urination**

The mean daily frequency of urination on the control day (day −1) was 8.8. This increased to 11.0 on the day of INNER7® intake (and decreased on the day after the trial to 9.0 (day 1).

**Figure 1: Urination frequency of Inner7**



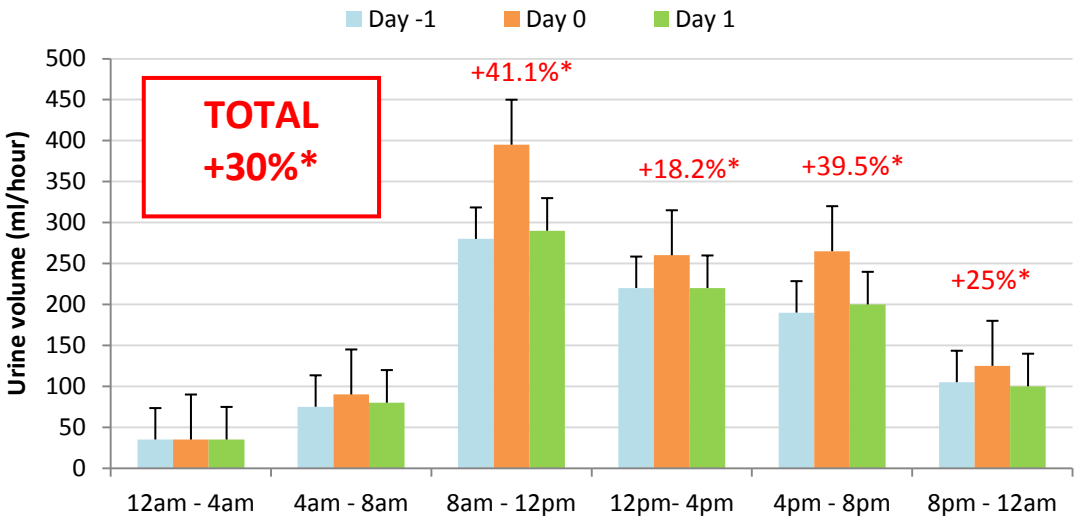

*Day0 compared to Day -1**

Figure 1 shows a significant increase in the frequency on the day of the trial from 8:00 am to 12pm, and from 4pm to 8pm, coinciding with the first and second intakes. A smaller increase was observed during 4 to 8 hours after the intakes.

**Urinary Output Volume**

The mean fluid output per subject at day -1 was 870 ml. This value increased to 1135 ml during trial day (day0) and decreased to 900 ml on last day (day 1). We can see from these statistics a significant difference in the volume of fluids output during the trial.

## Figure 2: Diuretic effect of Inner7




*Day0 compared to Day-1**

Figure 2 shows a significant increase in urinary volume for all subjects between 8pm and 12pm, as well as between 4pm to 8pm, coinciding with the first and second intakes of INNER7®. We can also observe smaller increases during 4 to 8 hours after the intakes.

**Discussion**

The data presented in this study show clearly the diuretic activity of a INNER7®. There was a significant increase in urination frequency (+25%) demonstrated by subjects after administration of INNER7®. The urine volume also increased significantly (+30%). In particular, the 4-hours perios after the first and the second intakes have shown the most important changes, suggesting that INNER7® has fast acting in the body. INNER7® was well tolerated with no side effects.

**Conclusion**

The data from this human trial demonstrate that magnesium and other mineral complex in INNER7® increases the frequency and volume of fluids output in healthy human subjects.

## 7.3. NET ACID EXCRETION AND URINE pH MEASUREMENT

**Customer:**

Innovation Labo - Kanaya Bldg 5F, 4-11-3 Hatchobori, Chuo-ku, Tokyo 104-0032 JAPAN

**Scientific supervisor:**

Dr. Taro HIRATA

**Setting**:

MEDICA TOKYO LABORATORIES
20-1, 3Chome Nishi-Shinjuku, Shinjuku-ku Tokyo JAPAN

**Study protocol number:**

Study MM/ST-0634-09

**Objective**:

Investigate the effect of INNER7® on net acid excretion and urine pH.

**Design**:

Controlled clinical trial.

**Subjects:**

Participants: 40 healthy 5-15kgs overweight people between 20 and 55 years old (25 females and 15 males).

**Interventions:**

Subjects were given INNER7® 2 ml per day for 30 days and counselled to eat normally.

**Main outcome measures:**

Daily net acid excretion measurement to predict acid-base balance precisely. 24h urine sample measurements at D0 and D30.

**Results:**

The tests showed a decrease on net acid excretion from D0 to D30. They also show a more alkaline urine pH at D30 than D0.

**Introduction**

***The acid-base balance concept and its metabolic implications***

The biology of the body's ph balance

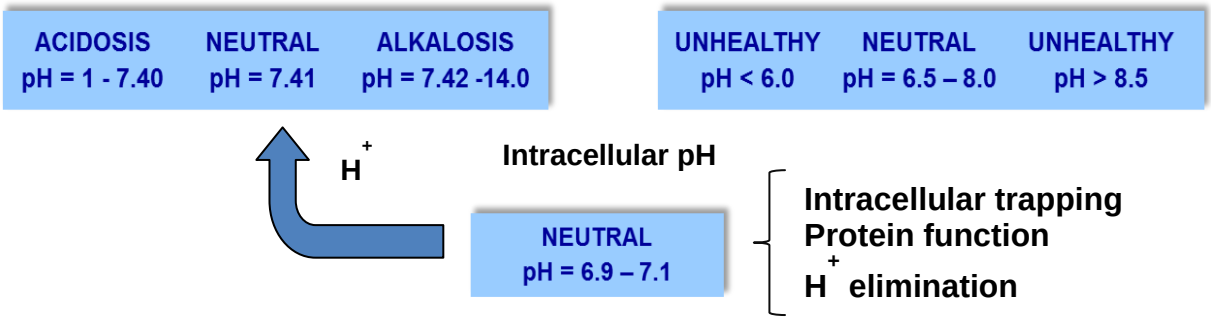
- pH $\Rightarrow$ acid/alkaline range from 0 - 14 with 7 as neutral. It derives from the concentration of free hydrogen ions (H+ or protons). This concentration is expressed in logarithmic terms with one pH unit changing the acid concentration by the factor of 10.
- Acid/alkaline balance is extremely important for normal physiology. Blood maintains a slightly alkaline range of 7.35- 7.45 to maintain a net gradient of H+ which derives from cells metabolism (7.1-7.4).
- Cell metabolism generates $H^+$. Diet.
- Physiological buffers: blood ($HCO_3^-$, Hb), lungs ($CO_2$ $\Uparrow$), bone alkaline minerals [Ca, $(CO_3)^{2-}$, $(PO_4)^{3-}$], conective tissue and fat cells.


The bloodstream is the most critically buffered system of the entire body, far more sensitive than any other. Arterial and venous blood must maintain a slightly alkaline pH: arterial blood pH = 7.41 and venous blood pH = 7.36. Because the normal pH of arterial blood is 7.41, a person is considered to have acidosis when the pH of blood falls below this value and to have alkalosis when the pH rises above 7.41.

A normal pH in these areas is 7.34 and 7.40, a slightly more acid profile, because body cells dump as much free hydrogen (H+) as possible, buffering the blood as much as possible. However, pH in these areas can dangerously drop to concentrations of pH = 5.0.

Urine pH values: In a pH balanced body, urine is slightly acid in the morning, (pH = 6.5 - 7.0) generally becoming more alkaline (pH = 7.5 - 8.0) by evening in healthy people primarily because no food or beverages are consumed while sleeping. Whereas, during the day the body buffers the pH of the food and beverages consumed by releasing electrolytes and the pH level goes up. This process allows the kidneys to begin the elimination process slowly.

Outside the range implies that cells are being burdened with caustic pH fluids within and without surroundings. Long term experience outside this range is unhealthy. However, the pH of urine can range from an extremely unhealthy low of 4.5 to a high if 8.5, which it tolerates a little easier, depending on the acid/base status of the extracellular fluids. A high pH value may indicate the body is over buffering to compensate for a physiological system that is too acidic.




Certain diets are associated with acidic urine. The impact of protein is due to the amino acids met and cys which are metabolized to sulphuric acid. In addition, normal metabolism of cells releases protons to the extracellular space.

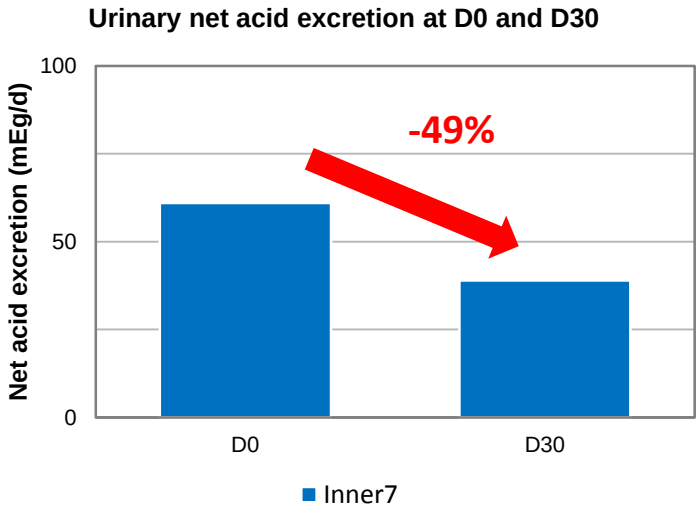
In contrast, plant based diets rich in organic acids like citric acid yield bicarbonate (alkalinizing effect). These anions are associated to alkaline metals such as potassium. In fact, the ratio protein/K+ is a tool to estimate acid-base balance.

Although it might seem that citrus fruits would have an acidifying effect on the body, the citric acid they contain actually has an alkalinizing effect in the system.

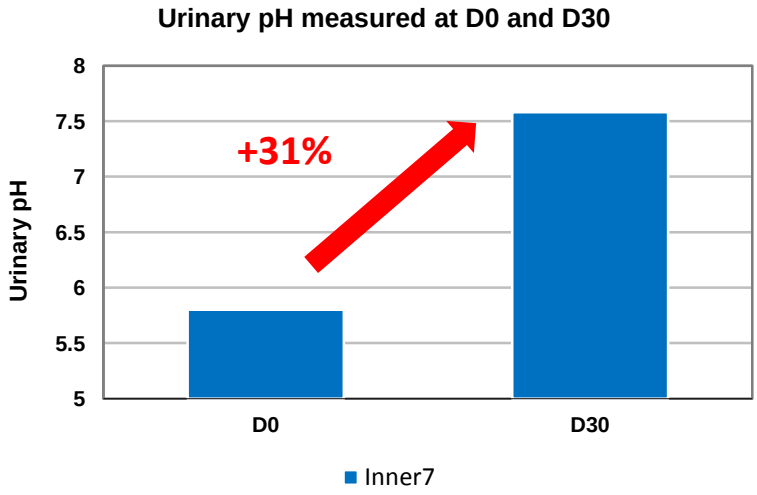
**Protocol & Method**

- Daily net acid excretion measurement to predict acid-base balance precisely
- Urine collected within a 24 hour interval is analyzed for all excreted components needed to assess acid-base balance and daily net acid excretion is therefore calculated.
- 24h urine sample measurements at D0 and D30.

**Study findings**




The tests showed a decrease on net acid excretion from 61 mEq/d at D0 to 39 mEq/d at D30.




For each person, the mean pH was compared with D0 and D30. pH of urine samples was around 5.91-6.03 for D0 for INNER7®. There was some variability in pH with time of day.

INNER7® showed a more alkaline pH at D30 than D0 (the average was a pH of 7.58 ± 0.11 at D30). INNER7® showed an alkalizing profile on urine pH (+1.78 pH) at D30.

# 8. CONCLUSION

INNER7® is a new generation of balanced mineral complex, with a focus on magnesium, which is involved in over 300 enzymatic reactions within human body.

INNER7® plays many crucial roles:

- Supports optimal hormone balance
- Supports a balanced PH in the body
- Reduce the size of belly by improving transit
- Supports body's fat burning system

INNER7® contains the magnesium as core ingredient for immediate efficacy that customers can feel from the first intake. Its minerals come from deep see water which offer an unmatched quality and purity and form a natural complex that human body can recognize without side effect.

The results of clinical trials performed on INNER7® have demonstrated significant results:

| | |
|---|---|
| **Magnesium Bioavailability** | **Glycemia peak (OGTT test)** |
| **+162%** | **-12.5%** |

| | | |
|---|---|---|
| **Mean glycemia** | **Insulinemia** | **HOMA** |
| **-3.29%** | **-17.26%** | **-19.98%** |

| | | | |
|---|---|---|---|
| **Weight** | **BMI** | **Waist Circumference** | **Hip Circumference** |
| **-2.25%** | **-1.80%** | **-5.66%** | **-4.50%** |

| | | | |
|---|---|---|---|
| **Triglycerides** | **Total Cholesterol** | **HDL-cholesterol** | **LDL-cholesterol** |
| **-18.86%** | **-2.42%** | **+2.39%** | **-5.67%** |

| | | |
|---|---|---|
| **Urination Frequency** | **Diuretic Effect** | **Alkaline urine pH** |
| **+25%** | **30%** | **31%** |

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# Therapeutic Uses of Magnesium

Mary P. Guerrera, MD[1], Stella Lucia Volpe, PhD[2], Jun James Mao, MD[3]

[1]*University of Connecticut School of Medicine, Farmington, Connecticut*
[2]*University of Pennsylvania School of Nursing, Philadelphia, Pennsylvania*
[3]*University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania*

Magnesium is an essential mineral for optimal metabolic function. Research has shown that the mineral content of magnesium in food sources is declining, and that magnesium depletion has been detected in persons with some chronic diseases. This has led to an increased awareness of proper magnesium intake and its potential therapeutic role in a number of medical conditions. Studies have shown the effectiveness of magnesium in eclampsia and preeclampsia, arrhythmia, severe asthma, and migraine. Other areas that have shown promising results include lowering the risk of metabolic syndrome, improving glucose and insulin metabolism, relieving symptoms of dysmenorrhea, and alleviating leg cramps in women who are pregnant. The use of magnesium for constipation and dyspepsia are accepted as standard care despite limited evidence. Although it is safe in selected patients at appropriate dosages, magnesium may cause adverse effects or death at high dosages. Because magnesium is excreted renally, it should be used with caution in patients with kidney disease. Food sources of magnesium include green leafy vegetables, nuts, legumes, and whole grains.

Magnesium is the fourth most abundant essential mineral in the body. It is distributed approximately one half in the bone and one half in the muscle and other soft tissues; less than one percent is in the blood.1 Studies estimate that 75 percent of Americans do not meet the recommended dietary allowance of magneisum,2 which has raised concern about the health effects of magnesium deficiency. Lifestyle factors (e.g., poor nutrition, excess alcohol intake), some medications (e.g., diuretics), and lower mineral content in commonly eaten foods (e.g., fruit, vegetables) have led to an increase in studies evaluating the potential link of magnesium deficiency to a number of diverse medical conditions, and magnesium's possible effectiveness in supplementation.3–5

SORT: KEY RECOMMENDATIONS FOR PRACTICE

| Clinical recommendation | Evidence rating | References |
|---|---|---|
| Magnesium is effective for treating eclampsia and preeclampsia. | A | 20–25 |
| Intravenous magnesium is effective for treating torsade de pointes and managing rapid atrial fibrillation. | A | 26,27 |
| In severe acute asthma, parenteral magnesium supplementation improves peak expiratory flow rate and forced expiratory volume in one second, and reduces hospital admissions. | B | 29,32 |
| Oral and parenteral magnesium is possibly effective in improving symptoms of migraine. | B | 33–37 |
| Magnesium is a widely accepted and effective approach to treat dyspepsia. | B | 38 |
| Magnesium is accepted as a standard treatment for constipation, but there are few rigorous studies to prove its effectiveness. | B | 40 |

A = consistent, good-quality patient-oriented evidence; B = inconsistent or limited-quality patient-oriented evidence; C = consensus, disease-oriented evidence, usual practice, expert opinion, or case series. For information about the SORT evidence rating system, go to http://www.aafp.org/afpsort.xml.

Early signs of magnesium deficiency include loss of appetite, nausea, vomiting, fatigue, and weakness. Persons may experience numbness, tingling, muscle contractions and cramps, seizures, personality changes, abnormal heart rhythms, and coronary spasms as magnesium levels decrease. Severe deficiency may lead to hypocalcemia and hypokalemia.1 Conditions that may lead to hypomagnesemia include poorly-controlled diabetes mellitus; chronic malabsorptive problems(e.g., Crohndisease, gluten-sensitive enteropathy, regional enteritis); medication use (e.g., diuretics, antibiotics); alcoholism; and older age (e.g., decreased absorption of magnesium, increased renal exertion).1

There are challenges in diagnosing magnesium deficiency because of its distribution in the body. Magnesium is an intracellular cation and its blood concentrations may not accurately mirror magnesium status.6 However, reductions in normal serum magnesium concentrations (1.8 to 2.3 mg per dL [0.74 to 0.95 mmol per L]) signify deficiency. Therefore, serum magnesium concentrations are specific, but not sensitive, to magnesium deficiency.7 Magnesium homeostasis is related to calcium and potassium status, and should be evaluated in combination with these two cations.7 There are other methods to assess magnesium status, but the serum level is the most common and practical test in the clinical setting.8

Pharmacology

Magnesium is the second most abundant intracellular divalent cation and is a cofactor for more than 300 metabolic reactions in the body.9,10 These processes include protein synthesis, cellular energy production and storage, cell growth and reproduction, DNA and RNA synthesis, and stabilization of mitochondrial membranes.11–14 Magnesium is one of the minerals responsible for managing bone metabolism, nerve transmission, cardiac excitability, neuromuscular conduction, muscular contraction, vasomotor tone, and blood pressure.11–14 Magnesium also plays a significant role in glucose and insulin metabolism, mainly through its impact on tyrosine kinase activity, phosphorylase b kinase activity, and glucose transporter protein activity.15–19 Because of these vital roles, magnesium levels may be affected by stressors to the body, such as in certain disease states. Supplementation with magnesium may have therapeutic effects in these situations.

Uses and Effectiveness

Magnesium has been used for numerous conditions. The most common indications are discussed here in order of most supported to least supported in the literature.

ECLAMPSIA AND PREECLAMPSIA

Magnesium sulfate (intravenous and intramuscular) has been shown to be relatively effective for treating eclampsia and preeclampsia, although it has been considered the standard of care for decades.20 In 2003, two Cochrane reviews showed that magnesium use in patients with eclampsia was superior to that of phenytoin (Dilantin) and lytic cocktail,21,22 with another study showing magnesium to be more effective than nimodipine (Nimotop).23 A different 2003 Cochrane review showed that 1 to 2 g of intravenous magnesium sulfate per hour reduced the risk of eclampsia in patients with preeclampsia by more than one half.24 The use of magnesium does not appear to have harmful effects on the mother or infant in the short term.25

## ARRHYTHMIA

A well-known use of intravenous magnesium is for correcting the uncommon ventricular tachycardia of torsade de pointes.26 Results of a meta-analysis suggest that 1.2 to 10 g of intravenous magnesium sulfate is also a safe and effective strategy for the acute management of rapid atrial fibrillation.27 A six-week randomized, double-blind crossover trial showed that oral magnesium supplementation reduced the frequency of asymptomatic ventricular arrhythmia in patients with stable congestive heart failure secondary to coronary artery disease.28

## ASTHMA

A 2000 Cochrane review of magnesium sulfate for exacerbations of acute asthma in the emergency department found that evidence does not support routine use of intravenous magnesium in all patients with acute asthma; however, it appears safe and beneficial for severe acute asthma by improving peak expiratory flow rate and forced expiratory volume in one second.29 In a meta-analysis of acute asthma in children, intravenous magnesium demonstrated probable benefit in moderate to severe asthma in conjunction with standard bronchodilators and steroids30; however, a randomized controlled trial showed that oral magnesium added no clinical benefit to standard outpatient therapy for chronic stable asthma in adults.31 In a 2005 Cochrane review of inhaled magnesium sulfate in acute asthma, nebulized magnesium in addition to a beta2 agonist were shown to improve pulmonary function and trend toward benefit in fewer hospital admissions.32

## HEADACHE

Studies have found that patients with cluster headaches and classic or common migraine, especially menstrual migraine, have low levels of magnesium.33,34 A prospective, multicenter, double-blind randomized study conducted in Germany showed that a single daily dosage of 600 mg oral trimagnesium dicitrate significantly reduced the frequency of migraine compared with placebo, whereas a lower twice daily dosage was found ineffective.35,36 For acute treatment of migraine, intravenous magnesium sulfate showed a statistically significant improvement in the treatment of all symptoms in patients with aura, or as an adjuvant therapy for associated symptoms in patients without aura.37

## DYSPEPSIA

Another common condition with several self-treatment options is dyspepsia, a key symptom of gastroesophageal reflux disease (GERD). Antacids are widely used for dyspepsia; however, studies comparing antacids with histamine H2 receptor antagonists (H2 blockers) have been limited. A randomized, double-blind, crossover study showed that on-demand treatment with the antacid hydrotalcite (aluminum hydroxide, magnesium hydroxide, carbonate, and water) was more effective than famotidine (Pepcid) or placebo.38 An editorial on these findings questioned the standard use of H2 blockers, and recommended shifting to more individualized treatment of mild or intermittent GERD.39

## CONSTIPATION

Patients often self-treat constipation with over-the-counter products, such as magnesium hydroxide (Milk of Magnesia) or magnesium citrate. However, there are few studies demonstrating effectiveness, as shown in a systematic review of chronic constipation.40 Despite this, many physicians and patients have found these treatments helpful, which indicates that a lack of evidence is not necessarily synonymous with a lack of effect.41

OTHER

Magnesium is associated with maintaining or improving bone mineral density as a dietary component in combination with potassium, fruits, and vegetables, or as an oral supplement.42,43 One study suggested that adults 18 to 30 years of age with higher magnesium intake have a lower risk of developing metabolic syndrome.44 Another study demonstrated a positive association between hypomagnesemia and metabolic syndrome in adults.45 A 2002 Cochrane review showed that magnesium lactate or citrate twice a day was effective for leg cramps in pregnant women.46 A 2001 Cochrane review of three small trials showed that in patients with dysmenorrhea, magnesium was more effective than placebo for pain relief and the need for additional medication was less.47 Studies have linked magnesium deficiency to myocardial infarction, congestive heart failure, primary hypertension, and angina pectoris,48 but evidence is still limited to recommend its use for these conditions.

Contraindications, Adverse Effects, and Interactions

Although oral magnesium supplementation is well-tolerated, magnesium can cause gastrointestinal symptoms, including nausea, vomiting, and diarrhea.49 Overdose of magnesium may cause thirst, hypotension, drowsiness, muscle weakness, respiratory depression, cardiac arrhythmia, coma, and death.49

Concomitant use of magnesium and urinary excretion–reducing drugs, such as calcitonin, glucagon (Glucagen), and potassium-sparing diuretics, may increase serum magnesium levels, as may doxercalciferol (Hectorol).50 Concomitant oral intake of magnesium may influence the absorption of fluoroquinolones, aminoglycosides, bisphosphonates, calcium channel blockers, tetracyclines, and skeletal muscle relaxants. Because of this, concomitant use should be monitored or avoided when possible.51

Additionally, because magnesium is cleared renally, patients with renal insufficiency (creatinine clearance of less than 30 mL per minute [0.50 mL per second]) may be at increased risk of heart block or hypermagnesemia; therefore, magnesium levels should be monitored. As with any dietary supplement, the quality of the product is important. Some magnesium products were found to contain lead.52

Dosages

Oral magnesium supplementation is safe in adults when used in dosages below the upper intake level of 350 mg per day (elemental magnesium).51 However, higher dosages have been studied and may be used for specific medical problems. Table 1 provides selected food sources of magnesium and the amount of magnesium per serving1; there is no upper intake level for dietary magnesium. Magnesium is safe in children when used in dosages below the tolerable upper intake level of 65 mg per day for children one to three years of age, 110 mg per day for children four to eight years of age, and 350 mg per day for children older than eight years.8 Table 2 lists some common forms and dosages of magnesium.

Table 1
Selected Food Sources of Magnesium

| **Food** | **Magnesium (in mg)** |
|---|---|
| Halibut, cooked, 3 oz | 90 |
| Almonds, dry roasted, 1 oz | 80 |

| Food | Magnesium (in mg) |
|---|---|
| Cashews, dry roasted, 1 oz | 75 |
| Spinach, frozen or cooked, one half cup | 75 |
| Cereal, shredded wheat, two rectangular biscuits | 55 |
| Oatmeal, instant, fortified, prepared with water, 1 cup | 55 |
| Potato, baked with skin, one medium | 50 |
| Peanuts, dry roasted, 1 oz | 50 |
| Wheat bran, crude, 2 tablespoons | 45 |
| Yogurt, plain, skim milk, 8 fl oz | 45 |
| Bran flakes, three fourths cup | 40 |
| Rice, brown, long-grained, cooked, one half cup | 40 |
| Avocado, California, one half cup pureed | 35 |
| Kidney beans, canned, one half cup | 35 |
| Banana, raw, one medium | 30 |
| Milk, reduced fat (2%) or fat free, 1 cup | 27 |
| Bread, whole wheat, commercially prepared, one slice | 25 |
| Raisins, seedless, one fourth cup packed | 25 |
| Whole milk, 1 cup | 24 |

note: There is no upper intake level for dietary magnesium.

Adapted from the National Institutes of Health Office of Dietary Supplements. Magnesium. Accessed January 12, 2009.

Table 2
Common Magnesium Formulations and Dosages

| Supplement | Elemental magnesium content | Dosage schedule for adults* |
|---|---|---|
| Magnesium oxide (MagOx) | 61% elemental magnesium<br>242 mg in 400-mg tablet | Two tablets per day with food |
| Magnesium hydroxide (Milk of Magnesia) | 42% elemental magnesium<br>167 mg in 400 mg per 5 mL oral suspension | 5 to 15 mL as needed up to four times per day |
| Magnesium citrate | 16% elemental magnesium<br>48 mg elemental magnesium and 13 mg potassium in 290 mg per 5 mL oral solution | One half to one full bottle (120 to 300 mL) |
| Magnesium gluconate (Mag-G) | 5% elemental magnesium<br>27 mg in 500-mg tablet | One or two divided tablets per day |
| Magnesium chloride (Mag-SR) | 12% elemental magnesium<br>64 mg in 535-mg tablet | Two tablets once per day |
| Magnesium sulfate | 10% elemental magnesium<br>1 g per 100 mL solution for injection | Atrial fibrillation: IV 1.2 to 5 g initial dose over one to 30 minutes<br>Asthma: IV 25 to 75 mg per kg single dose (study of children younger than 18 years)<br>Eclampsia, preeclampsia: IV 4 to 6 g over 15 to 20 minutes, then 1 to 2 g per hour |

| Supplement | Elemental magnesium content | Dosage schedule for adults* |
|---|---|---|
| Magnesium sulfate (Epsom salts) | 10% elemental magnesium 98.6 mg in 1 g salts | Cathartic: mix 2 to 4 teaspoons in 8 oz water; take up to twice per day |
| Magnesium lactate (Mag-Tab SR) | 12% elemental magnesium 84 mg in 84 mg tablet | One to two tablets every 12 hours |
| Magnesium aspartate hydrochloride (Maginex DS) | 10% elemental magnesium 122 mg in 1,230 mg dietary supplement granules | Mix in 4 oz water; take up to three times per day |

IV = intravenous.

*— Recommended dietary allowance for adults 19 to 30 years of age: 310 mg per day for women and 400 mg per day for men; for adults older than 30 years: 320 mg per day for women and 420 mg per day for men.

Bottom Line

Magnesium is an essential mineral with evidence of effectiveness in treating eclampsia and preeclampsia, arrhythmia, severe asthma, and migraine (Table 3). The National Center for Complementary and Alternative Medicine is currently investigating the role of magnesium supplementation in mild to moderate persistent asthma.53 There are few studies to support wide use of magnesium for treating constipation and dyspepsia. Some of the potential indications that require further investigation include lowering the risk of metabolic syndrome, treating leg cramps in pregnant women, preventing osteoporosis, and alleviating dysmenorrhea. Diagnosis of mild to moderate magnesium deficiency is challenging because patients may be asymptomatic, and usual diagnostic testing is specific but not sensitive. Magnesium testing and supplementation should be considered in at-risk patients, especially those on diuretics, with poor nutritional intake, or with malabsorptive states. Supplementation of magnesium should generally not exceed the age-adjusted tolerable upper intake level and should be used with caution in patients with kidney dysfunction or in those taking certain medications.

Table 3
Key Points About Magnesium

| | |
|---|---|
| Effectiveness | Effective: eclampsia and preeclampsia, arrhythmia, severe asthma, migraine, dyspepsia, constipation<br>Possibly effective: lowering risk of metabolic syndrome, improving glucose and insulin metabolism, preventing osteoporosis, improving symptoms of leg cramps in pregnant women, dysmenorrhea |
| Adverse effects | Oral supplementation generally is safe and well-tolerated; some reports of nausea, vomiting, diarrhea; overdose may lead to hypotension, muscle weakness, and coma |
| Contraindications | Patients with renal impairment (creatinine clearance of less than 30 mL per minute [0.5 mL per second]) may be at risk of heart block or hypermagnesemia |
| Oral dosage and tolerable upper intake level | Adults: 350 mg per day of elemental magnesium<br>Children: 65 mg per day for children one to three years of age; 110 mg per day for children four to eight years of age; 350 mg per day for children older than eight years |
| Cost | Less than $20 for 30 tablets |

| | |
|---|---|
| Food sources | Green leafy vegetables, fish, almonds, legumes, whole grains (see Table 1) |

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# Serum magnesium concentration in children with functional constipation treated with magnesium oxide

Maiko Tatsuki[1], Reiko Miyazawa[1], Takeshi Tomomasa[2], Takashi Ishige[1], Tomoko Nakazawa[1], and Hirokazu Arakawa[1]

*[1]Department of Pediatrics, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan*
*[1]PAL Clinic, 694 Tanakajima-machi, Isesaki, Gunma 372-0802, Japan*

**Abstract**

AIM: To determine whether hypermagnesemia recently reported in adult patients possibly develops in children with functional constipation taking daily magnesium oxide.

METHODS: We enrolled 120 patients (57 male and 63 female) aged 1-14 years old (median: 4.7 years) with functional constipation from 13 hospitals and two private clinics. All patients fulfilled the Rome III criteria for functional constipation and were treated with daily oral magnesium oxide for at least 1 mo. The median treatment dose was 600 (500-800) mg/d. Patients were assessed by an interview and laboratory examination to determine possible hypermagnesemia. Serum magnesium concentration was also measured in sex- and age-matched control subjects (*n* = 38).

RESULTS: In the constipation group, serum magnesium concentration [2.4 (2.3-2.5) mg/dL, median and interquartile range] was significantly greater than that of the control group [2.2 (2.0-2.2) mg/dL] ($P < 0.001$). The highest value was 3.2 mg/dL. Renal magnesium clearance was significantly increased in the constipation group. Serum magnesium concentration in the constipation group decreased significantly with age ($P < 0.01$). There was no significant correlation between the serum level of magnesium and the duration of treatment with magnesium oxide or the daily dose. None of the patients had side effects associated with hypermagnesemia.

CONCLUSION: Serum magnesium concentration increased significantly, but not critically, after daily treatment with magnesium oxide in constipated children with normal renal function.

**Keywords:** Children, Constipation, Hypermagnesemia, Magnesium oxide, Renal dysfunction

## INTRODUCTION

Magnesium-containing cathartics are used worldwide to treat chronic constipation[1-3]. Approximately 45 million Japanese patients are estimated to undergo treatment with magnesium oxide as an antacid or cathartic annually[4]. Many children with functional constipation are taking these drugs for long periods of time; sometimes over several years.

Hypermagnesemia is a rare clinical condition[5,6]. Most cases are iatrogenic and due to increased intake of magnesium, which occurs after intravenous administration of magnesium[7,8] or oral

ingestion of high doses of magnesium-containing antacids or cathartics[9-12]. Magnesium homeostasis is dependent mainly on gastrointestinal absorption and renal excretion. The kidney is the principal organ involved in magnesium regulation. Renal magnesium excretion is very efficient, because the thick ascending limb of Henle has the capacity to reject completely magnesium reabsorption under conditions of hypermagnesemia[5,6], and therefore, hypermagnesemia commonly arises in patients with renal dysfunction.

In 2008, the Ministry of Health, Labour and Welfare (MHLW) of Japan reported 15 adult patients with hypermagnesemia, including two cases of death due to oral ingestion of magnesium oxide from April 2005 to August 2008[4]. Although most of these elderly patients with constipation had dementia, schizophrenia, or renal dysfunction, the MHLW has recommended measuring the serum level of magnesium in patients who regularly use magnesium oxide.

It is now important for pediatricians to know whether hypermagnesemia can develop in children without abnormal renal function after administration of a common or high dose of magnesium oxide. The purpose of this study was to determine serum magnesium concentration in children with functional constipation treated with daily magnesium oxide.

## MATERIALS AND METHODS

### Patients

We enrolled 120 patients (57 male and 63 female) aged 1-14 years with functional constipation from 13 hospitals and two private clinics in Japan. At entry, all patients fulfilled the Rome III criteria for functional constipation, which meant that they had at least two of the following characteristics: fewer than three bowel movements weekly; more than one episode of fecal incontinence weekly; large stools in the rectum shown by digital rectal examination or palpable on abdominal examination; occasional passage of large stools; retentive posturing and withholding behavior; and painful defecation. All patients had been treated for at least 1 mo with daily magnesium oxide as an oral laxative. The medication was given once daily or in split doses. The dose was dependent on the patient's condition.

Children with known organic causes of constipation, including Hirschsprung disease, spinal and anal congenital abnormalities, previous colon surgery, inflammatory bowel disease, allergy, metabolic or endocrine diseases, renal dysfunction, and severe neurological disability were excluded from the study. Patients with poor drug compliance were also excluded.

In each patient, we recorded the date of initiation of constipation, daily dose of magnesium oxide, and duration of treatment. We also determined whether the patient had symptoms that could be side effects of hypermagnesemia, such as vomiting, nausea, thirst, blushing, feeling of exhaustion, or somnolence. The laboratory examinations carried out were as follows: serum level of magnesium, calcium, phosphorus, blood urea nitrogen (BUN), and creatinine concentration. Urinary concentrations of magnesium and creatinine were also measured. Magnesium clearance and fractional excretion of magnesium (FEMg) were calculated as follows: Magnesium clearance = urine magnesium (mg/dL)/urine creatinine (mg/dL); FEMg = urine magnesium (mg/dL)/serum magnesium (mg/dL) × serum creatinine (mg/dL)/urine creatinine (mg/dL).

### Control group

Serum magnesium concentrations were also measured in the control group which comprised 38 children (24 male and 14 female) aged 1-15 years who visited the Department of Pediatrics at

Gunma University Hospital, and were without any history of hematological disease, tumor, heart failure, metabolic or endocrine diseases, renal dysfunction, or severe neurological disability. None of these children were treated with magnesium oxide.

**Statistical analysis**

Laboratory values, duration of treatment, and daily dose of magnesium oxide are shown as the median and interquartile ranges. Statistical significance of differences was tested by $\chi^2$ test or Mann-Whitney *U* test, as appropriate. Spearman's rank correlation coefficients were calculated for the correlation between the serum level of magnesium and age, duration of treatment, and drug dose. $P < 0.05$ was regarded as significant. All analyses were carried out using SPSS for Windows (SPSS statistics 17.0).

**RESULTS**

Subject characteristics and serum magnesium concentrations are shown in Table Table1.1. In the constipation group, the median treatment duration with magnesium oxide was 1.3 (0.4-2.6) years and median daily dose was 600 (500-800) mg/d; 33 (25-45) mg/kg per day. After administration of magnesium oxide, the outcome of constipation was investigated in 83 patients. Bowel habits in all patients were improved, and 75% of patients were stable. However, 11% of children had fewer than three bowel movements weekly, 28% of them had withholding behavior, and 34% had painful defecation during the follow-up period. None of the patients still had overflow-incontinence.

Table 1

Subject characteristics and serum magnesium concentrations

| | Control group ($n$ = 38) | Constipation group ($n$ = 120) | *P* value |
|---|---|---|---|
| Age (yr) | 5.5 (2.0-10.8) | 4.7 (3.0-6.8) | NS[a] |
| Gender, male (%) | 63.2 | 47.5 | NS[b] |
| Serum magnesium concentration (mg/dL) | 2.2 (2.0-2.3) | 2.4 (2.3-2.5) | < 0.001[a] |

[a]Mann-Whitney *U* test;
[b]$\chi^2$ test. NS: Not significant.

The median serum magnesium concentration in the constipation group was significantly greater than that in the control group (Table (Table1).1). The highest magnesium concentration was 3.2 mg/dL in a 3.5-year-old patient treated with 1320 mg/d; 88 mg/kg per day magnesium oxide for 2.8 years.

The median urinary magnesium to creatinine ratio in the constipation group was significantly elevated compared with that reported previously [0.23 (0.15-0.37) ($n$ = 76) *vs* 0.15 (0.12-0.20) ($n$ = 16), $P < 0.05$)[13]. The median FEMg in the constipation group was 0.03 (0.02-0.05).

Serum magnesium concentration in the constipation group decreased significantly with age ($P < 0.01$) (Figure (Figure1A).1A). There was no significant correlation between the serum level of magnesium and duration of treatment (Figure (Figure1B).1B). The treatment dose had no effect on serum magnesium level (Figure (Figure1C1C).

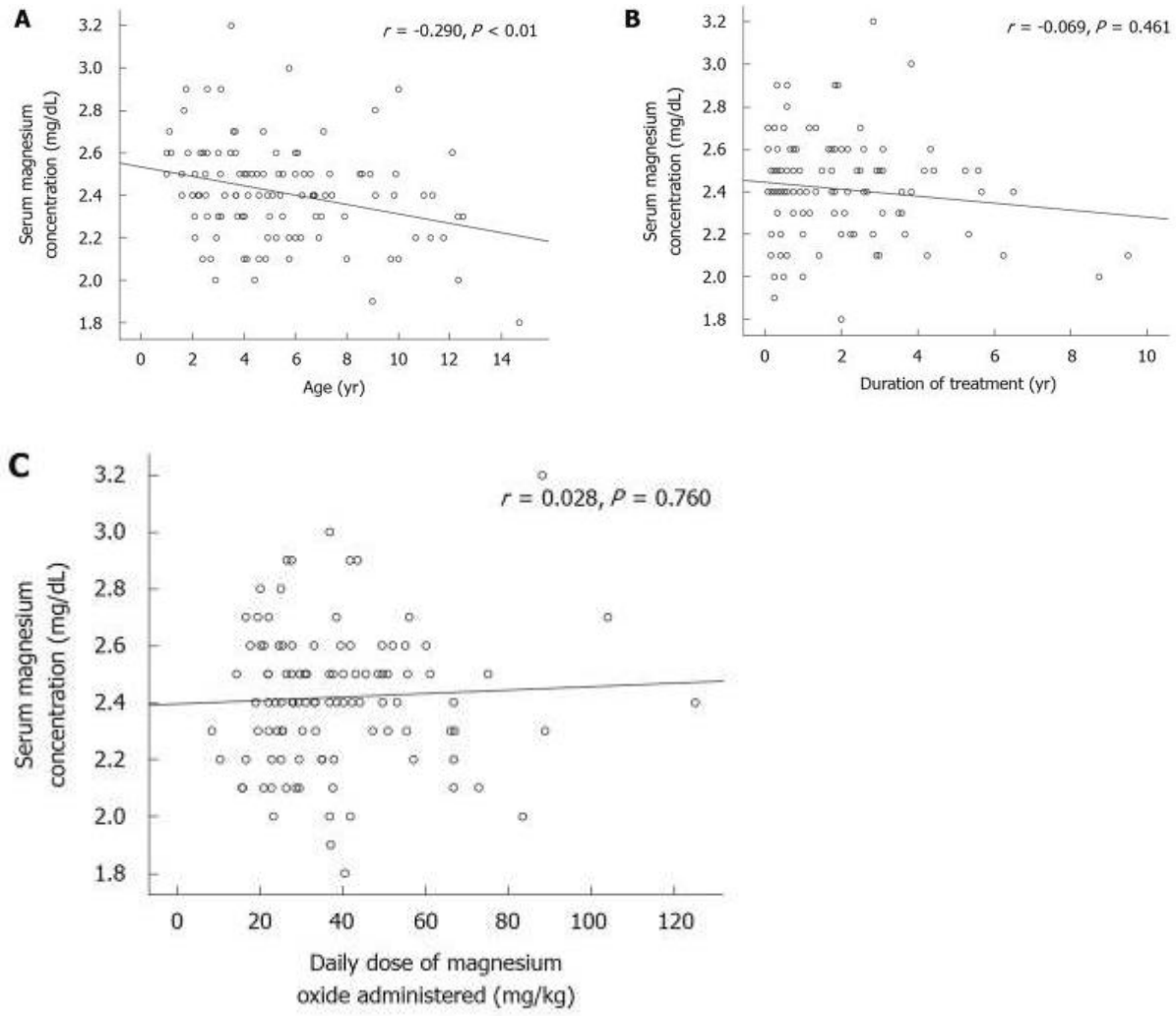


Figure 1
Correlation between serum level of magnesium and age (A), and duration of treatment with magnesium oxide (B), and dose of magnesium oxide (C).

Serum level of calcium [9.9 (9.5-10.2) mg/dL], phosphorus [5.2 (4.8-5.6) mg/dL], creatinine [0.3 (0.3-0.4) mg/dL], and BUN [13.0 (10.8-15.5) mg/dL] were not abnormal in any of the patients. None of the patients had side effects associated with hypermagnesemia.

**DISCUSSION**

In 2008, the MHLW of Japan reported that 15 patients aged 32-98 years (median, 71 years) who had been treated with magnesium oxide developed severe side effects of magnesium toxicity, such as hypotension, bradycardia, electrocardiographic changes (atrial fibrillation), loss of consciousness, coma, respiratory depression, and cardiac arrest. The serum magnesium concentration in two fatal cases was 20.0 mg/dL and 17.0 mg/dL. As a result of these reported cases, the MHLW has recommended that the serum concentration of magnesium in subjects on continuous magnesium therapy should be determined[4].

Most cases of hypermagnesemia in adults result from large intravenous doses of magnesium[7,8] or from excessive enteral intake of magnesium-containing cathartics[9-12]. Symptomatic hypermagnesemia is likely to occur in patients with renal dysfunction. In fact, 10 of the 15 Japanese patients reported as cases of hypermagnesemia by the MHLW had renal dysfunction.

Several cases of hypermagnesemia from enteral magnesium intake in patients with normal renal function have been reported previously[14-17]. It is known that non-renal risk factors for hypermagnesemia are age, gastrointestinal tract disease, and administration of concomitant medications, particularly those with anticholinergic and narcotic effects[18]. Five elderly Japanese patients without renal dysfunction had intestinal necrosis, severe constipation, and abnormal abdominal distention with intestinal expansion, and these abdominal risk factors could have increased the serum concentration of magnesium.

Hypermagnesemia has also been reported in pediatric practice[14,19]. These case reports include a 14-year-old girl without renal dysfunction who was taking magnesium hydroxide because of severe constipation[14], and a 2-year-old boy with neurological impairments who was taking 2400 mg/d of magnesium oxide administered as part of a regimen of megavitamin and megamineral therapy[19]. These cases developed increased serum magnesium levels as high as 14.9 mg/dL and 20.3 mg/dL, respectively.

Magnesium oxide is commonly used in patients with chronic constipation; however, not only has the optimum dose for children not been established, but there has been no study to evaluate the concentration of serum magnesium after oral administration of magnesium oxide. The aim of the present study was to determine the serum magnesium concentration in pediatric cases receiving magnesium cathartics for chronic constipation.

In our study, the median serum magnesium concentration was 2.4 mg/dL in the constipation group, which was significantly greater than that in the control group (2.2 mg/dL). Thirty patients (25%) in the constipation group and none in the control group had a serum magnesium concentration greater than the maximum value of the normal range in healthy Japanese children (2.6 mg/dL). The high critical limit of serum magnesium concentration has been reported as 4.9 ± 2.0 mg/dL in adults and 4.3 ± 1.1 mg/dL in children[20]. The highest value in our study was 3.2 mg/dL, and none of our patients reached the critical limit of serum magnesium concentration or developed symptoms due to hypermagnesemia. The median urinary magnesium to creatinine ratio in the constipation group was significantly elevated compared with that in healthy subjects, which suggests that serum magnesium level is regulated by an increase in renal excretion in those children with normal renal function, and is maintained within its appropriate range.

In our study, serum magnesium level in constipated children treated with magnesium oxide, but not in the control children, decreased significantly with age. No correlation was found between duration of treatment or daily dose of magnesium oxide and serum magnesium concentration. These data are consistent with those reported by Woodard et al[21] They reported that the increase in serum magnesium concentration in 102 adults who received multiple doses of magnesium citrate did not correlate with the quantity of magnesium administered[21]. Elderly patients are at risk of magnesium toxicity as kidney function declines with age, but it is not clear whether young children have a higher risk of hypermagnesemia. Alison et al[22] reported on a 6-week-old infant who had increased serum magnesium level (14.2 mg/dL) and life-threatening apnea due to 733 mg/d magnesium hydroxide that was used to treat constipation. Brand et al[23] and Humphrey et al[24] reported on premature infants with hypermagnesemia following antacid administration in order to decrease the risk of gastrointestinal hemorrhage. One infant had an increased serum magnesium level of 13.3 mg/dL and developed intestinal perforation. These reports indicate that infancy, prematurity or young age might be a possible risk factor for hypermagnesemia.

According to our results, we conclude that serum magnesium concentrations increase significantly after daily magnesium oxide intake, but the magnitude of the increase appears modest. Younger age, but not prolonged use of daily magnesium oxide might be a relative risk factor, and it should

be determined by further studies whether serum magnesium concentration should be assessed in these subjects.

## COMMENTS

### Background

Magnesium-containing cathartics are commonly used to treat chronic constipation. Although hypermagnesemia is a rare clinical condition, it can occur as a side effect of increased intake of magnesium salts.

### Research frontiers

The Japanese government has recently reported fatal cases of hypermagnesemia in adults treated with magnesium oxide. In our study, serum magnesium concentrations increased significantly after daily magnesium oxide intake, but the magnitude of the increase appeared modest. Serum magnesium levels in constipated children treated with magnesium oxide, but not in the control children, decreased significantly with age. No correlation was found between duration of treatment or daily dose of magnesium oxide and serum magnesium concentration.

### Innovations and breakthroughs

Recent reports have highlighted that serum magnesium concentration increases significantly, but not critically, after daily treatment with magnesium oxide in children with normal renal function.

### Applications

The present study indicated the safety of daily magnesium oxide treatment for children with chronic constipation.

### Peer review

The authors are to be congratulated for providing evidence of the apparent safety of a commonly used and effective therapy to treat an important health issue seen commonly in children.

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# Magnesium Deficiency in Patients on Long-Term Diuretic Therapy for Heart Failure

Pin Lim and Edward Jacob

**Abstract**

Magnesium levels in serum, erythrocytes, skeletal muscle, and bone were measured in 10 patients with valvular heart disease who had received diuretic therapy for heart failure for an average of 3·3 years. Five patients were found to have diminished values for skeletal muscle, indicating significant magnesium deficit. Values for erythrocytes were low in only two of the five patients, and none had low values for serum ultrafiltrate and bone: Magnesium replacement therapy restored skeletal muscle values to normal. Clinical features consistent with the presence of magnesium deficiency were found in all five magnesium-deficient patients. These features were, with few exceptions, corrected by magnesium replacement. The latter also corrected low skeletal muscle potassium values present in all five patients with low skeletal muscle magnesium, four of whom showed clinical features of digoxin poisoning before magnesium therapy was given. Concomitant secondary aldosteronism, inadequate dietary intake, and digoxin therapy had probably augmented the magnesium loss due to diuretic therapy.

Introduction

Administration of diuretics may lead to significant urinary loss of magnesium (Martin et al., 1952; Barker et al., 1959; Smith et al., 1959; Wacker, 1961; Demartini et al., 1967; Parfitt, 1969). Patients with chronic valvular heart disease are often given long-term diuretic therapy for heart failure. The latter is itself associated with aldosteronism (Singer and Wener, 1953; Wolff et al., 1957), which could augment the urinary excretion of magnesium (Horton and Biglieri, 1962). It was therefore thought to be of interest to assess the magnesium status of patients receiving long-term diuretic therapy for heart failure in view of the clinical importance of magnesium deficiency, particularly with regard to digoxin toxicity and cardiac arrhythmias (Wacker, 1961; Demartini et al., 1967). In this study magnesium status was assessed on the basis of the concentration of magnesium in all accessible tissues; they included skeletal muscle and bone, which together contain over 80% of the total body magnesium (Widdowson and Dickerson, 1964).

## Materials and Methods

Ten patients who had been admitted to hospital in congestive heart failure due to chronic valvular disease were studied. They had all been taking one or more diuretic drugs without break in the preceding six months at least, and had no history of having taken magnesium-containing drugs-for example, antacids-during the previous 12 months or more. There were no concomitant conditions likely to have caused magnesium loss, such as alcoholism, chronic vomiting, or diarrhoea.

The subjects were studied when they were not in overt heart failure and were clinically stable. Clinical features known to be associated with magnesium deficiency and those of digoxin toxicity were looked for. Blood was drawn without venous stasis for the estimation of serum magnesium (total and ultrafiltrable), erythrocyte magnesium, and serum calcium (total and ionized), sodium, potassium, and creatinine. Biopsy of skeletal muscle and bone was carried out within a day of clinical examination. Specimens of bone were taken from the iliac crest (Williams and Nicholson, 1963). The skin incision was extended over the tensor fasciae latae and a small specimen (about 0-5 g) was taken from this muscle. The biopsy specimens were analysed for magnesium and, in the

case of skeletal muscle, potassium. Magnesium deficiency was deemed to be present if the magnesium level in skeletal muscle or bone
(or both) was below the range found in specimens of normal tissues (see below).

Magnesium replacement was started as soon as magnesium deficiency was established provided that there was no clinical or biochemical evidence of renal insufficiency. Such patients were treated initially as inpatients for about two weeks, each receiving an infusion of 84 mEq of magnesium sulphate (with 1 litre of 5% dextrose) over six hours every other day. They were assessed clinically just before the start of replacement therapy and daily while receiving infusions. Two patients were taken off diuretics and they continued to receive intravenous magnesium replacement therapy until their magnesium deficit had been made good, as indicated by the excretion of more than 90% of the infused magnesium in the urine within 48 hours of starting the infusion (Fourman and Morgan, 1962). After receiving their initial infusions three other subjects continued replacement therapy as outpatients, and during this period they were assessed clinically only once a week. During outpatient treatment they were each given 15 ml of magnesium hydroxide mixture by mouth twice a day (equivalent to 96 mEq magnesium/day) and magnesium replacement was arbitrarily assumed to have been achieved after a total of six weeks' treatment. Serum magnesium, calcium, sodium, and potassium and erythrocyte magnesium were estimated every fourth day on inpatients but only once a week during outpatient follow-up.

Serum total magnesium was estimated with the atomic absorption spectrophotometer as described previously (Lim et al., 1969a). Serum calcium was similarly estimated (with wavelength set at 4,227 A). In the estimation of serum ultra-filtrable magnesium the method of preparing serum ultra-filtrate previously described (Lim et al., 1969b) was used. An aliquot of the ultrafiltrate prepared was used for the determination of ionized calcium spectrophotometrically (Unicam SP 600) by means of the pH-independent complexiметric indicator tetramethylmurexide (Nordin and Smith, 1965).
Skeletal muscle and erythrocyte magnesium were estimated as previously described (Lim et al., 1969a). Skeletal muscle potassium was measured on an aliquot of the skeletal muscle powder prepared as for magnesium estimation. The residue after incineration was dissolved in 0.1 N hydrochloric acid and the potassium content estimated with the E.E.L. flame photometer. Bone magnesium was estimated in the same way as skeletal muscle magnesium. Specimens of skeletal muscle for the estimation of normal magnesium and potassium values were obtained during surgical operations from consenting patients who were free of metabolic disorders-for example, during herniorrhaphy and orthopaedic operations (Lim et al., 1969a). Specimens of bone for estimation of normal magnesium values were obtained at necropsy from victims of road traffic accidents with no evidence of concomitant disease. Serum and urine sodium and potassium were estimated with the E.E.L. flame photometer, and serum and urine creatinine by the method of Bonsnes and Taussky (1945).

**Results**

The duration of diuretic therapy varied from seven months to nine years (mean 3-3 years). All patients had received chlorothiazide, and frusemide and mersalyl had also been administered in some cases (Table I). There was evidence of cardiac cirrhosis in Cases 1, 7, and 8. All patients except Case 6 had either a poor diet or a poor appetite, ingesting not more than 30 g of protein daily. All patients were receiving maintenance doses of digoxin and potassium supplements.

***Skeletal Muscle Magnesium***
-Of the 10 subjects studied three (Cases 2, 5, and 8) had skeletal muscle magnesium values well below normal and two (Cases 1 and 10) had values just below the normal range (Table I). The mean

for all 10 patients was significantly lower (P <0 01) than that for normal controls (Table II). After magnesium replacement therapy in Cases 1, 2, and 5, skeletal muscle biopsy was repeated and gave normal magnesium values (Table I.). There appeared to be no correlation between the duration of diuretic therapy and the severity of magnesium depletion as indicated by the skeletal muscle magnesium level.

***Bone Magnesium***

-All the patients, including those with reduced skeletal muscle magnesium levels, had normal bone magnesium levels. The mean value for the five patients with low skeletal muscle magnesium was not significantly different from that for normal controls (Table II).

***Clinical Features***

-Nausea and vomiting or ventricular ectopic beats, or both, the common manifestations of digoxin toxicity, were present in Cases 1, 2, 5, 7, and 10, in four of which there was evidence of magnesium deficiency (Table III). These features were promptly relieved after one magnesium infusion except in the patient who was not magnesium-deficient (Case 7).

This patient, who was normokalaemic, was given a trial course of magnesium infusions, but her symptoms persisted for three days. Digoxin was withdrawn from all five patients immediately after the appearance of features of toxicity. Clinical features consistent with the presence of magnesium deficiency were seen in all five magnesium-deficient patients. The commonest were muscle cramps, exaggerated tendon reflexes, and paraesthesiae, and these were noticeably relieved in the course of magnesium therapy (Table III). Some of these clinical features were also present in Cases 7 and 9, but they were not relieved by the magnesium infusion in Case 7.

***Serum Magnesium***

-Of the five patients with low skeletal muscle magnesium two (Cases 1 and 8) had low serum total magnesium (1-60 and 1-62 mg/100 ml respectively), while all those with normal skeletal muscle magnesium had normal serum total magnesium. The mean value for the five magnesium-deficient patients was significantly lower (P <0 001) than that for normal controls (Table II). Magnesium replacement in the five deficient patients, however, did not result in any significant change in their mean value. In all 10 patients, including the two with hypomagnesaemia, the serum untrafiltrable magnesium values were within the normal range. Magnesium replacement did not significantly change the mean value for the deficient patients. Low serum albumin levels due to liver cirrhosis in Cases 1 and 8 (2-8 and 2-5 g/100 ml respectively) would account for their low serum total magnesium values.

***Erythrocyte Magnesium***

-Two (Cases 2 and 8) of the five patients with low skeletal muscle magnesium had low erythrocyte magnesium values (3-68 and 4-20 mg/100 ml cells respectively). The mean value for the five patients was significantly lower (P <0-001) than that for the normal controls (Table II).

With magnesium therapy the low values rose.

***Calcium***

-All 10 patients were normocalcaemic, and the level of ionized calcium, the biologically important moiety of serum calcium, was just below the lower limit of normal in only one patient (Case 2).

***Potassium***

-Only one of the 10 patients (Case 1) had hypokalaemia, which was mild and was corrected with magnesium repletion alone. Skeletal muscle potassium, however, was noticeably reduced in all the magnesium-deficient patients (Table I). There was a statistically significant difference (P <0-001)

between the mean for the magnesium-deficient patients and that for normal controls (Table II). Magnesium replacement brought the values back to normal in all three patients in whom it was possible to repeat muscle biopsy (Table I).

TABLE I—*Clinical and Biochemical Data on 10 Patients on Prolonged Diuretic Therapy for Heart Failure*

| Case No.: | 1* | 2* | 3 | 4 | 5* | 6 | 7 | 8* | 9 | 10* |
|---|---|---|---|---|---|---|---|---|---|---|
| Sex and age | F. 65 | F. 35 | M. 34 | M. 51 | F. 47 | M. 27 | F. 39 | M. 56 | M. 55 | M. 34 |
| Duration (years) of chlorothiazide therapy | 9 | 3 | 3 | 7 months | 4 | 3 | 2½ | 2 | 3½ | 3½ |
| Supplementary diuretics (frusemide or mersalyl) | + | + | − | + | + | − | − | − | − | − |
| Clinical features suggesting digoxin toxicity | + | + | − | − | + | − | + | − | − | + |
| Skeletal muscle magnesium (mEq/kg dry fat-free solids; normal range 59·4-82·2) | 58·8 (67)† | 48·8 (71·5) | 61·3 | 67·3 | 53·1 (64·2) | 62·4 | 61·5 | 50·7 | 59·5 | 58·5 |
| Skeletal muscle potassium (mEq/kg dry fat-free solids; normal range 294-386) | 250 (320)† | 218 (341) | 334 | 370 | 292 (340) | 390 | 368 | 282 | 408 | 254 |

*Patient magnesium-deficient.
†Values in parentheses are those obtained after magnesium replacement therapy.

TABLE II—*Analysis of Biochemical Data (Mean ± S.D.) on Normal Controls and Patients on Prolonged Diuretic Therapy for Heart Failure*

| | Muscle Magnesium (mEq/kg Dry Fat-free Solids) | Bone Magnesium (mEq/kg Dry Fat-free Solids) | Serum Total Magnesium (mg/100 ml) | Serum Ultrafiltrable Magnesium (mg/100 ml Ultrafiltrate) | Erythrocyte Magnesium (mg/100 ml Cells) | Muscle Potassium (mEq/kg Dry Fat-free Solids) |
|---|---|---|---|---|---|---|
| A, Normal controls (No. of subjects) | 70·8 ± 5·7 (30) | 212·6 ± 23·1 (47) | 2·0 ± 0·17 (87) | 1·69 ± 0·17 (25) | 6·26 ± 0·88 (56) | 340·2 ± 23·0 (30) |
| B, Magnesium-deficient patients (n = 5) | 54·2 ± 4·3 | 205·4 ± 17·7 | 1·68 ± 0·08 | 1·40 ± 0·01 | 4·44 ± 0·51 | 259·2 ± 29·2 |
| C. All patients studied (n = 10) | 58·3 ± 5·5 | 200·6 ± 16·2 | 1·77 ± 0·13 | 1·50 ± 0·11 | 4·57 ± 0·45 | 316·6 ± 66·2 |
| Difference AB | P <0·001 | N.S. | P <0·001 | P <0·001 | P <0·001 | P <0·001 |
| Difference AC | P <0·01 | N.S. | P <0·01 | P <0·01 | P <0·001 | P <0·01 |

N.S. = Not significant.

TABLE III—*Clinical Features present before Replacement Therapy**

| Case No. | 1† | 2† | 3 | 4 | 5† | 6 | 7 | 8† | 9 | 10† |
|---|---|---|---|---|---|---|---|---|---|---|
| Anorexia | 2 | | | | 1 | | | 2 | | 2 |
| Nausea | 2 | 2 | | | 1 | | N.R. | | | 2 |
| Vomiting | 1 | | | | 1 | | | | | 2 |
| Tremor | 3 | | | | 5 | | | | | |
| Cramps | | 10 | | | P.R. | | P.R. | 4 | N.C. | 4 |
| Paraesthesiae | 10 | | | | | | P.R. | 4 | N.C. | 6 |
| General weakness and fatigue | | | | | 5 | | N.R. | | | 10 |
| Ventricular extrasystoles | 1 | | | | | | | | | |
| Exaggerated tendon reflexes | N.R. | 14 | | | | | N.R. | | N.C. | 10 |

*Figures indicate number of days from start of replacement therapy when symptom or sign disappeared. Blank space denotes absence of symptom or sign.
†Patient magnesium-deficient.
N.R. = No response at conclusion of magnesium therapy.
P.R. = Partial response at conclusion of magnesium therapy (frequency of cramps reduced).
N.C. = No change (no magnesium therapy).

## Discussion

The prevalence of magnesium deficiency, as shown by low skeletal muscle magnesium, among patients with heart failure on long-term diuretic therapy seems to be of the order of 50%. There appears to have been no previous investigation into the frequency and severity of magnesium deficiency in similar circumstances, although the clinical importance of such a deficiency has been emphasized (Wacker, 1961; Seller et al., 1966; Wacker and Parisi, 1968).

Skeletal muscle magnesium accounts for about 20% of the total body magnesium (Widdowson and Dickerson, 1964) and is a reliable index of total body magnesium (MacIntyre and Davidsson, 1958; MacIntyre et al., 1958). Moreover, if the magnesium status of this tissue accurately reflects that of metabolically active cells in general, the skeletal muscle magnesium level is a logical index of clinical magnesium status, since magnesium in intimately involved in a wide range of cellular enzyme reactions. Bone, erythrocyte, and serum magnesium levels, in contrast, appear to be unreliable in the diagnosis of magnesium deficiency.

In the present series of cases intracellular potassium was invariably low when intracellular magnesium was low, and replacement with magnesium alone led to the restoration to normal of the intracellular levels of both cations. Each of the patients received a constant potassium supplement of 40 mEq/day before and throughout the study. The intimate relation between intracellular potassium and magnesium found in experimental work on rats (Whang and Welt, 1963) is thus confirmed in man. The high incidence of digoxin toxicity among magnesium-deficient patients (four out of five) is worth noting. The effect of magnesium replacement is difficult to assess as digoxin was withdrawn once toxicity was suspected. Potassium deficiency has long been known to predispose to digoxin toxicity. It is not clear, however, whether the increased liability to

digoxin toxicity in these deficient patients is related to magnesium deficiency per se or to concomitant secondary intracellular potassium depletion. In any case, magnesium supplements alone would correct both abnormalities and might be expected to reduce the liability to digoxin toxicity.

All the patients were in a stable clinical state when they were studied. Practically all the clinical features suggestive of magnesium deficiency in the five deficient patients were abolished by magnesium replacement therapy. In Cases 7 and 9, however, there was no biochemical evidence of magnesium deficiency although their clinical resemblance to the five deficient patients was noticeable. These two subjects serve as a useful reminder that clinical features alone can be misleading.

In view of the well-documented magnesuric property of diuretics (Martin et al., 1952; Barker et al., 1959; Smith et al., 1959; Wacker, 1961; Demartini et al., 1967; Parfitt, 1969) the present patients, who had been taking diuretics on average for 3-3 years, had probably lost quite large quantities of magnesium in the urine. One of the major factors in magnesium conservation is reduction of urinary magnesium excretion, so that if excretion is not stimulated by diuretics the obligatory urinary loss may be reduced to less than 1 mEq/day when dietary magnesium is severely restricted (Barnes et al., 1958). All the magnesium-deficient patients had a poor dietary intake, and with the impaired alimentary absorption expected in heart failure and the persistent renal magnesium loss the presence of magnesium deficiency is not surprising.

It may be relevant that intracellular magnesium depletion is known to occur in patients with protein calorie malnutrition (Montgomery, 1960). Excessive secretion of aldosterone in patients with cardiac oedema is well established (Singer and Wener, 1953; Wolff et al., 1957), and aldosterone augments the urinary excretion of magnesium (Horton and Biglieri, 1962).

Furthermore, digoxin inhibits renal tubular transport of magnesium (Kupfer and Kosovsky, 1965). It would be interesting to define the relative contributions of hyperaldosteronism, digoxin therapy, inadequate dietary intake, and diuretic therapy to the state of magnesium deficiency met with in patients such as these.

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# Magnesium and outcomes in patients with chronic kidney disease: focus on vascular calcification, atherosclerosis and survival

Ziad A. Massy[1,2] and Tilman B. Drüeke[2]

[1]*Departments of Clinical Pharmacology and Nephrology, Amiens University Hospital, CHU-Amiens South*
[2]*INSERM Unit 1088, UFR de Médicine et de Pharmacie, Université de Picardie Jules Verne, Amiens, France*

**Abstract**

Patients with chronic kidney disease (CKD) have a high prevalence of vascular calcification, and cardiovascular disease is the leading cause of death in this population. However, the molecular mechanisms of vascular calcification, which are multifactorial, cell-mediated and dynamic, are not yet fully understood. We need to address ways to improve outcomes in CKD patients, both in terms of vascular calcification and cardiovascular morbidity and mortality—and to these ends, we investigate the role of magnesium. Magnesium's role in the pathogenesis of vascular calcification has not been extensively studied. Nonetheless, several *in vitro* and animal studies point towards a protective role of magnesium through multiple molecular mechanisms. Magnesium is a natural calcium antagonist and both human and animal studies have shown that low circulating magnesium levels are associated with vascular calcification. Clinical evidence from observational studies of dialysis patients has shown that low-magnesium levels occur concurrently with mitral annular calcification, peripheral arterial calcification and increased carotid intima–media thickness. Few interventional studies have been performed. Two interventional studies suggest that there may be benefits such as retardation of arterial calcification and/or reductions in carotid intima–media thickness in response to magnesium supplementation in CKD patients, though both studies have limitations. Finally, observational studies have shown that low serum magnesium may be an independent risk factor for premature death in CKD patients, and patients with mildly elevated serum magnesium levels could have a survival advantage over those with lower magnesium levels.

**Introduction**

Cardiovascular disease is the leading cause of death in both chronic kidney disease (CKD) and peritoneal dialysis/haemodialysis patients. In fact, the risk of dying because of cardiovascular disease in adults with CKD is about an order of magnitude higher than for the general population, even after adjusting for age and diabetic status [1]. It is also notable that patients with CKD undergoing dialysis have 2- to 5-fold more coronary artery calcification (CAC) than age-matched individuals with angiographically proven coronary artery disease [2]. The high incidence of cardiovascular mortality appears, at least partially, attributable to increased medial calcification of the large arteries, including the aorta, which in turn can result in increased arterial wall stiffness and pulse pressure and decreased myocardial perfusion during diastole [3, 4]. However, intimal calcification associated with atherosclerosis is even more frequent than medial calcification, especially in patients with CKD Stages 3–5 before dialysis therapy is started [5]. Both intimal and medial calcification are probably major direct or indirect contributors to cardiovascular disease and excess cardiovascular mortality of CKD patients [6, 7]. Figure 1 shows typical x-ray aspects of intimal calcification, medial calcification and mixed intimal and medial calcifications in pelvic and femoral arteries. Vascular disease prevention is therefore important, with the aim to reduce the

incidence of cardiovascular morbidity and mortality. While at least some traditional coronary risk factors (e.g. increased age, dyslipidaemia, diabetes and smoking) play a role in haemodialysis patients, several non-traditional factors associated with CKD are also likely to be involved [8, 9]. These include anaemia, uraemic toxins, oxidative stress, protein glycation and carbamylation and the disorder of mineral and bone metabolism (CKD–MBD) [4, 8, 10, 11].

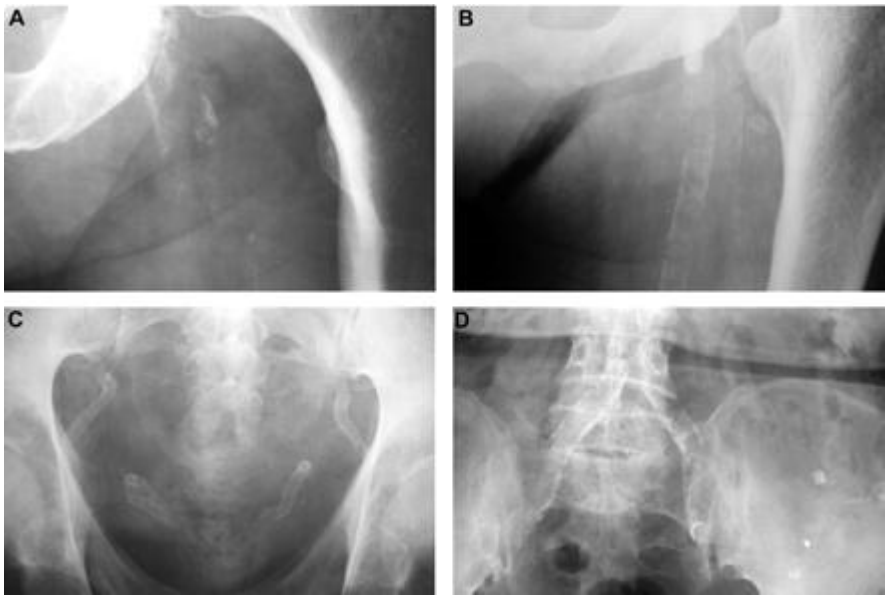


**Figure 1 -** Intima and media calcification in CKD patients. Arterial calcifications can be classified as intima calcification, present as discrete plaques with irregular and patchy distribution (**A**) or as media calcification, present as uniform linear railroad track-type (angiogram-like; **B** and **C**). The presence of both, intima and media calcification is reflected as discordances (**D**). Shown are soft tissue posteroanterior fine-detail native (unenhanced) radiographs of the pelvis and the thigh taken in CKD patients in the recumbent position. A and B, femoral artery; C and D, pelvic artery. With permission from Oxford University Press, London *et al.* [6].

Although being part of CKD–MBD, magnesium's role in CKD–MBD has been underestimated and generally neglected. Here, we review the role of magnesium in vascular calcification with particular focus on CKD and look towards potential interventions to improve outcomes for this group of patients.

## Magnesium and the pathogenesis of vascular calcification

### Vascular calcification: in vitro evidence and potential pathogenic mechanisms

The process of vascular calcification may start early during the course of CKD, prior to the start of dialysis, and worsens progressively, often in an accelerated fashion compared with the general population [12, 13]. Disturbances in mineral and bone metabolism appear to play a major role in the pathogenesis and rapid progression of vascular calcification [11, 14–16] (see Figure 2 for details). However, it is notable that compared with calcium and phosphate, the role of magnesium in this pathologic process has been the subject of few studies.

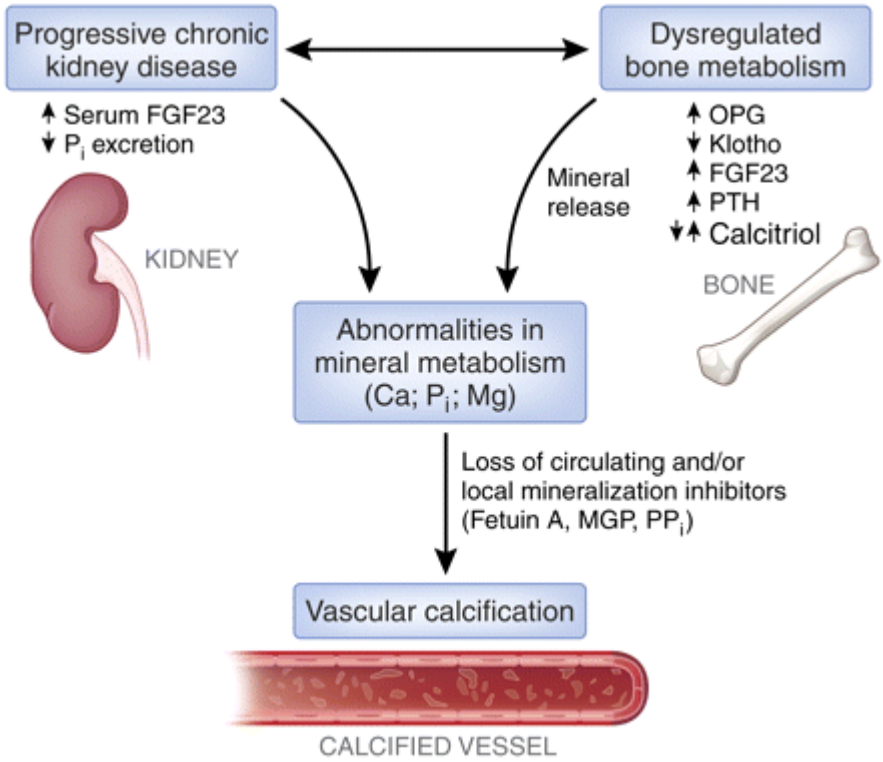


**Figure 2 -** Mechanisms of vascular calcification in CKD patients. Disturbances of mineral and bone metabolism are common in patients with CKD. The progressive loss of kidney function is accompanied—among other changes—by elevated serum FGF23 levels, a decrease in inorganic phosphate excretion and a dysregulation of bone metabolism. These anomalies are intimately interrelated. Indicators of this disturbed state are pathological changes of various biomarkers such as OPG, Klotho, FGF23, PTH and calcitriol. Whether their altered serum levels are the cause or the consequence of the skeletal abnormalities requires further study. The resulting derangements in mineral metabolism, as reflected by altered serum and vascular tissue levels of Ca, $P_i$ and Mg are accompanied by additional metabolic changes and inflammation. This leads to loss of circulating and/or local mineralization inhibitors such as fetuin A, $PP_i$ and MGP, further supporting the development of vascular calcification. Ca, calcium; FGF23, fibroblast growth factor 23; Mg, magnesium; MGP, matrix Gla protein; OPG, osteoprotegerin; $P_i$ , inorganic phosphate; $PP_i$, inorganic pyrophosphate; PTH, parathyroid hormone. (modified after Schoppet, Shroff *et al.* [17]).

The pathogenesis of vascular calcification is not well understood, but it is likely to be multifactorial [9]. It appears to be a cell-mediated, dynamic and actively regulated process that closely resembles the formation of normal bone tissue [4, 17–20]. Several non-mutually exclusive theories or mechanisms have been advanced to explain the onset and progression of vascular calcification, during which a central role is played by the vascular smooth muscle cells (VSMCs) that compose the medial layer of the vessel wall. Figure 3 gives a comprehensive overview of the mechanisms and factors that act upon VSMCs, influencing their conversion into osteoblast-like cells—a phenotype that is commonly found in calcified vessels. Initially, a soluble amorphous calcium–phosphate complex is deposited in presence of excessive calcium phosphate mineral. It is unlikely to cause harm if stabilized effectively by inhibitory proteins, such as fetuin A, carboxylated matrix Gla protein (MGP) and osteopontin, and by the inorganic inhibitory compound pyrophosphate [17, 21–24]. According to three recent reports, however, the starting point could be the formation of nanocrystals that could directly stimulate calcification and vascular cell differentiation [25, 26, 27]. Subsequently, when there is an imbalance between calcification inhibitors and promoters,

amorphous calcium phosphate and/or nanocrystals may be transformed into the stable hydroxyapatite crystal. Alterations in calcium and phosphate balance, as observed in patients with CKD, clearly promote vascular calcification and may be considered as non-traditional risk factors for cardiovascular disease in these patients [9, 28] (Figure 2).

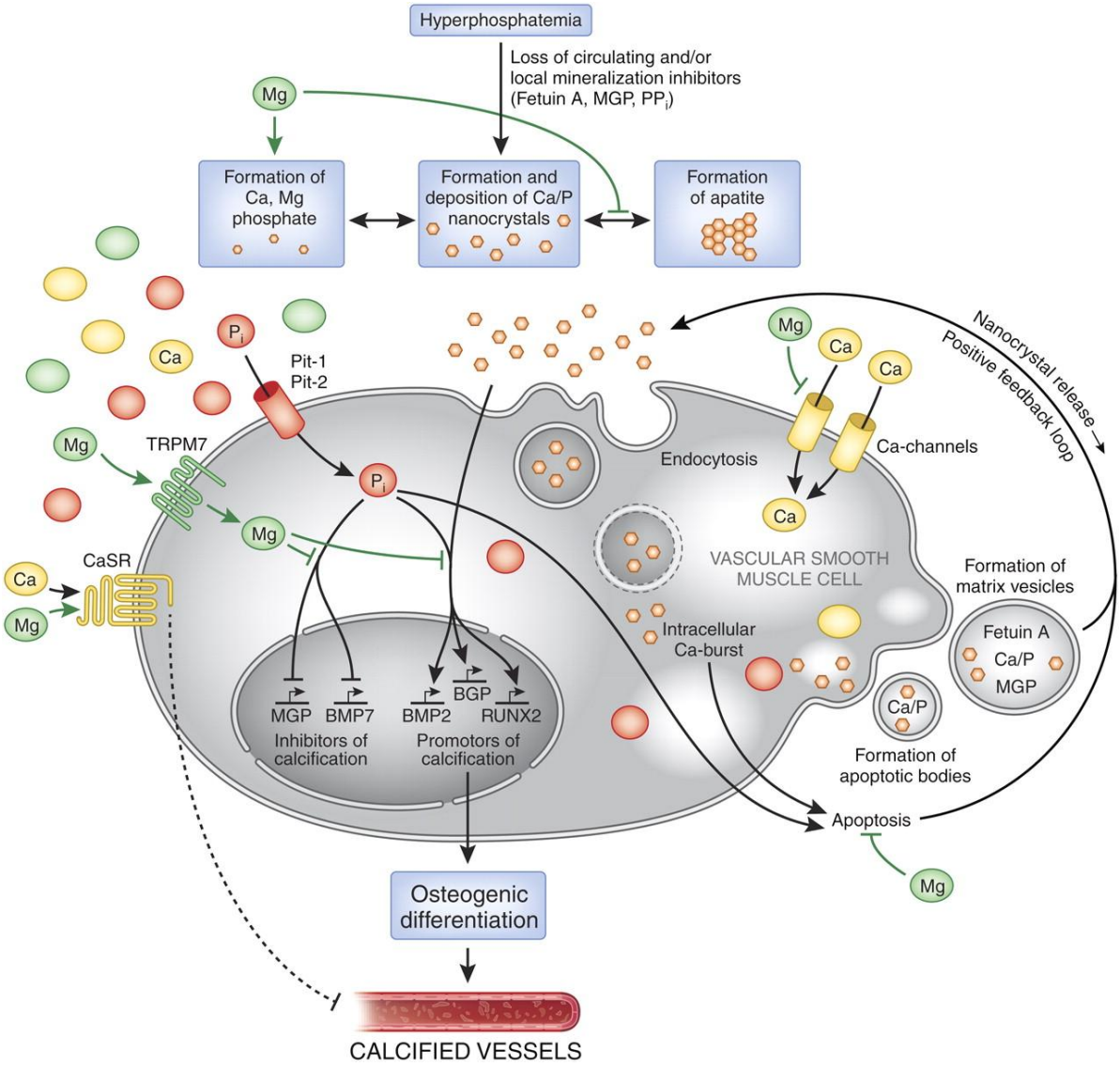


**Figure 3 -** The putative protective roles of magnesium in the course of vascular calcification. Abnormalities in mineral metabolism, particularly hyperphosphataemia as well as the loss of circulating and/or local mineralization inhibitors such as fetuin A, MGP or $PP_i$, initially lead to the formation and deposition of Ca/P nanocrystals [25, 26]. These nanocrystals are taken up by VSMCs, most likely via endocytosis [29]. The lysosomal degradation of the endocytosed crystals results in an intracellular release of calcium and phosphate. Inorganic phosphate additionally accumulates in the cell via uptake through the sodium-dependent phosphate transporter Pit-1 (and Pit-2) [30, 31]. In an attempt to compensate for excess Ca/P, VSMCs form matrix vesicles loaded with Ca/P products as well as the mineralization inhibitors fetuin A and MGP [32]. The intracellular Ca-burst induced by endocytosed nanocrystals [33] as well as the phosphate uptake [34] trigger VSMC apoptosis, resulting in the formation of Ca/P containing apoptotic bodies [35]. Both apoptotic bodies and matrix vesicles are ultimately causing a positive feedback loop through nanocrystal release into the surrounding milieu, thus amplifying the calcification process. Furthermore, Ca/P nanocrystals as well as $P_i$ induce the expression of genes that promote the

calcification/mineralization process such as RUNX2, BMP2 and BGP, while at the same time repressing the expression of MGP or BMP7, factors that are known to inhibit the progression of calcification. This causes a transdifferentiation of VSMCs to osteoblast-like cells, ultimately resulting in vessel calcification. Magnesium interferes with this process of vascular calcification on different levels: firstly, Mg inhibits the transformation from amorphous Ca/P to any apatite (carbonatohydroxyapatite) [36, 37] and forms Mg-substituted tricalcium (whitlockite) under certain conditions, which is more soluble than apatite [37], resulting in smaller, more soluble deposits [37, 38]. Secondly, magnesium functions as a Ca-channel antagonist [39] and thus inhibits the entry of Ca into the cells. Thirdly, within the cell, via TRPM7, Mg restores the balance between the expression of calcification promotors and inhibitors by neutralizing the inhibition of MGP and BMP7 induced by phosphate [40]. Furthermore, it regresses the phosphate- and Ca/P nanocrystal-induced enhanced expression of RUNX2 and BMP2 [41] preventing the VSMCs from osteoblastic conversion and calcification. In addition, magnesium acts on the CaSR [42]; activation of this receptor by calcimimetics has been shown to inhibit VSMC calcification [43]. The underlying molecular mechanisms have not been identified so far. BGP, bone GLA protein, osteocalcin; BMP, bone morphogenetic protein; Ca, calcium; CaSR, calcium-sensing receptor; Mg, magnesium; MGP, matrix Gla protein; $P_i$, inorganic phosphate; Pit, inorganic phosphate transporter; $PP_i$, inorganic pyrophosphate; RUNX2, runt-related transcription factor 2, cbfa1, core-binding factor subunit alpha-1; TRPM, transient receptor potential melastatin; VSMC, vascular smooth muscle cell.

Calcium phosphate deposition, mainly in the form of carbonate and hydroxyapatite, respectively [$(Ca,Na)_{10}(PO_4,CO_3)_6(OH)_2$, $Ca_{10}(PO_4)_6CO_3$ and $Ca_{10}(PO_4)_6(OH)_2$], which also are the mineral compounds of bone, is the hallmark of vascular calcification and can occur in the blood vessels, myocardium and cardiac valves [36, 27, 28, 44]. (This issue has been discussed in greater detail in the review by Jahnen-Dechent and Ketteler [45] in this supplement.) A recent *in vitro* investigation of the role of calcium phosphate deposition in VSMC calcification suggests that calcium phosphate deposition is initially a passive phenomenon, which then triggers the aforementioned osteogenic changes, resulting in the formation of more organized apatite crystal ultrastructures [26].

An analysis of the crystalline composition of soft tissue calcification in uraemic patients identified non-visceral and arterial calcification to be hydroxyapatite, while heart, lung and skeletal muscle calcification was identified as an amorphous or microcrystalline compound composed of calcium, magnesium and phosphorus [46]. More recently, synchrotron X-ray-μ-fluorescence and diffraction data were used to examine vascular calcification. The aortic vessel wall mineral deposits in calcitriol- and non-calcitriol-treated rodent models of uraemia-induced vascular calcification [44] were composed of amorphous calcium phosphate precipitate, apatite and—in animals treated with calcitriol—also whitlockite (magnesium-substituted tricalcium phosphate or calcium magnesium orthophosphate: $[(Ca,Mg)_3(PO_4)_2]$). In contrast to this animal work, a very recent detailed investigation of tissue samples taken from iliac arteries of uraemic patients revealed the colocalization of hydroxyapatite with whitlockite in three of six patients, indicating that this type of calcium phosphate crystals is not only found in soft tissue calcifications but also in the vascular space [27]. The presence of magnesium in calcification is not unexpected as it inhibits the formation of apatite and stabilizes amorphous calcium phosphate [36, 22, 47]. In addition, other inhibitors of calcifications, namely calcium binding or calcification-inhibitory proteins such as fetuin A, osteopontin and MGP, were found in close association with microcalcifications [27].

Several *in vitro* studies have shown that magnesium can have an inhibitory effect on hydroxyapatite formation and precipitation, as well as on the calcification process. Posner's group showed in the 1970s and 1980s that magnesium stabilized amorphous calcium phosphate and inhibited the formation of calcium-acidic phospholipid–phosphate complexes in metastable calcium phosphate solutions [48, 49]. Of interest, Bennett *et al.* [50] found that magnesium was

also able to inhibit calcium pyrophosphate dihydrate crystal formation *in vitro*. More recently, the effect of magnesium was examined on *in vitro* VSMC transformation into osteoblast-like cells and calcification [40]. The addition of 2.0–3.0 mM magnesium to a high-phosphate medium prevented osteogenic differentiation and calcification, in part via the restoration of the activity of the cation channel known as transient receptor potential melastatin 7 (TRPM7). Magnesium also increased the expression of anti-calcification proteins, including osteopontin and MGP [40]. Furthermore, it was shown that magnesium can stimulate the calcium-sensing receptor (CaSR), which is expressed on VSMCs [51, 52]. Stimulation of the CaSR by calcimimetics reduced mineral deposition in VSMCs and delayed the progression of both aortic calcification and atherosclerosis in uraemic apoE$^{(-/-)}$ mice [43]. The exact underlying mechanisms have not been resolved so far but this suggests that one of the mechanisms of how magnesium influences VSMC calcification might be via the CaSR. Thus, magnesium could protect against vascular calcification via multiple molecular mechanisms.

Potential mechanisms of the inhibitory effects of magnesium in the calcification process are shown in Figure 3.

**Evidence from animal experiments**

Low serum levels of magnesium are associated with vascular calcification, both in humans and in a number of experimental animal studies [53–56]. Several of the animal studies have demonstrated that changes in dietary magnesium levels can cause or prevent vascular calcification [53–55]. A rat model consisting of aortic transplantation associated with medial calcification of the grafted vessel was used to show that dietary supplementation with a combination of magnesium, alkali citrate and bases was capable of preventing aorta transplant-induced calcification [54]. The effect of dietary magnesium and calcium has also been examined in the *Abcc6*$^{-/-}$ mouse, a pseudoxanthoma elasticum (PXE) mouse model which mimics the clinical features of PXE (a genetic disorder characterized by calcification of connective tissue in skin, Bruch's membrane of the eye and blood vessel walls) [53]. Disease severity was measured by quantifying calcification after up to 12 months dietary treatment. An increase in dietary intake of calcium and magnesium resulted in significantly fewer calcifications of kidney blood vessels than mice given an unsupplemented diet or fed a calcium-enriched diet alone (both $P < 0.05$) [53] (Figure 4). In the same mouse model, it was shown that treatment with the phosphate binder magnesium carbonate prevented the onset as well as the progression of calcification, whereas treatment with lanthanum carbonate had no effect [57].

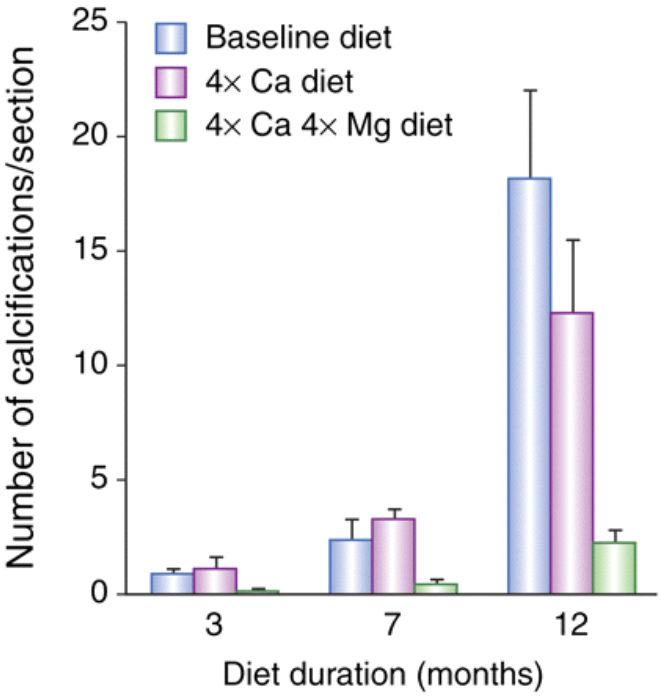


**Figure 4 -** Effect of diet on the number of calcifications in blood vessels in the kidney cortex of *Abcc6*$^{-/-}$ mice. Histograms represent the average number of calcifications per kidney section as a function of diet and diet duration. Diets supplemented with calcium plus magnesium ('4 × Ca, 4 × Mg' diet) slowed down calcification significantly [compared with baseline unsupplemented diet or diet supplemented with calcium alone (4 × Ca diet)] after 3, 7 and 12 months (Kruskal–Wallis test, $P < 0.05$ for all comparisons)] [53]. With kind permission from Springer Science + Business Media, Gorgels *et al.* [53] (Figure 2).

The effect of phosphate and magnesium intake has been investigated in a mouse model (DBA/2) associated with dystrophic cardiac calcification [55]. DBA/2 mice with either a low-magnesium or a high-phosphate intake developed marked cardiac calcifications; moreover, a combination of low-magnesium and high-phosphate intake caused severe calcification of cardiac and renal tissues. However, when increasing dietary magnesium content and reducing dietary phosphate, cardiac calcification could be partially prevented.

Magnesium is also considered to be 'a natural calcium antagonist' as one of its major functions in biological systems is to modulate the neuromuscular activity of calcium ions [39, 58]. Thus, contractility of all types of muscle is dependent upon the actions and interactions of these two divalent cations. The magnesium ion can block calcium movement across VSMC membranes and lower peripheral and cerebral vascular resistance [39]. More generally speaking, magnesium deficiency appears to enhance the activity of calcium in the body, while an excess of magnesium may block it, and as such magnesium may help to control cardiovascular function [58].

- Mechanisms of vascular calcification are poorly understood but are likely to be multifactorial, cell-mediated and dynamic processes.
- Low serum magnesium levels are associated with vascular calcification in human and in animal studies. Animal studies show that dietary magnesium can prevent, or help mitigate, vascular calcification.
- Magnesium has potential to protect against vascular calcification via multiple molecular mechanisms.

**Clinical evidence for the role of magnesium in calcification, atherosclerosis and survival**

**Vascular calcification, atherogenesis and magnesium: observational studies**

The influence of serum magnesium levels on vascular calcification has been suspected for a considerable time, as shown by a number of observational studies in patients with CKD (Table 1). In an early observational study, 44 end-stage renal disease (ESRD) patients receiving peritoneal dialysis therapy were followed up for a mean duration of 27 months [59]. Half of the patients (*n* = 22) developed peripheral arterial calcifications (detected in the hands, ankles or feet), while the remainder either did not develop any calcifications or had calcifications that regressed. The arterial calcification group had significantly lower mean serum magnesium levels (±SD) than the group without calcifications [1.11 ± 0.21 mmol/L (2.69 ± 0.52 mg/dL) and 1.24 ± 0.21 mmol/L (3.02 ± 0.51 mg/dL), respectively; P < 0.001]. Moreover, there were no significant between-group differences in parameters such as serum concentrations of calcium, phosphorus, calcium × phosphorus product, total alkaline phosphatases or intact parathyroid hormone (iPTH). Although the difference between serum magnesium levels in the calcification and no-calcification/calcification regression groups was striking, the study was limited by the semi-quantitative nature of vascular calcification assessment. Nevertheless, these results suggested that there may be a role for modest hypermagnesaemia as a preventative strategy for arterial calcification in patients with ESRD.

| Authors (year) | Patients | Study design | Parameter | Assessment technique | P-value[b] |
|---|---|---|---|---|---|
| Observational studies | | | | | |
| Ishimura *et al.* (2007) [56] | 390 (non-diabetic haemodialysis) | Prospective single blind follow-up over 4 months | Calcification of the hand arteries | Radiographic findings of the hands | 0.036 |
| Tzanakis *et al.* (2004) [62] | 93 (haemodialysis) and 182 age- and sex-matched healthy controls | Cross-sectional analysis | Carotid intima–media thickness | B-mode ultrasound | 0.001 |
| Tzanakis *et al.* (1997) [60] | 56 (haemodialysis) | Retrospective analysis of 8 years | Mitral annular calcification | Doppler echocardiography | 0.008 |
| Meema *et al.* (1987) [59] | 44 (CAPD) | Prospective follow-up | Progression/regression of arterial calcification | Radiographic surveys | 0.001 |
| Interventional studies | | | | | |
| Spiegel *et al.* (2009) [71] | 7 (haemodialysis) | Prospective interventional follow-up over 18 months (Mg carbonate) | CAC | Electron beam tomography | 0.0737[c] |
| Turgut *et al.* | 47 (haemodialysis) | Prospective interventional | Intima–media thickness of | Ultrasound | 0.014[d] |

| | | | | | |
|---|---|---|---|---|---|
| (2008) [75] | | follow-up over 2 months (Mg citrate) | the carotid artery | | |

- *a Results indicate that higher serum magnesium correlates with reduced vascular calcification and reduced intima–media thickness.*
- *b P-values indicate the significance level related to lower and higher serum magnesium levels or*
- *c progression vs baseline and*
- *d intervention versus no intervention, respectively. CAPD, continuous ambulatory peritoneal dialysis; CAC, coronary artery calcification.*

**Table 1 -** Observational and interventional studies investigating the influence of serum magnesium levels on vascular calcification[a]

Another observational study has been conducted in 390 patients undergoing maintenance haemodialysis that excluded patients with diabetes, and which used hand radiography to detect visible calcification of hand arteries in an examiner blinded manner [56]. Phalangeal vessel calcification was detected in 52 patients (13%). Mean serum magnesium levels (±SD) measured over a 4-month period were significantly lower in patients with vascular calcification [1.11 ± 0.12 mmol/L (2.69 ± 0.28 mg/dL)] than in those without [1.14 ± 0.14 mmol/L (2.78 ± 0.33 mg/dL); $P < 0.05$]. In addition, multivariate analysis showed that serum magnesium concentration was a significant independent factor associated with vascular calcification [odds ratio 0.28; 95% confidence interval (CI) 0.09–0.92 per 0.41 mmol/L (1 mg/dL) increase in serum magnesium levels; $P = 0.036$] after adjustment for age, sex, duration of haemodialysis and serum calcium, phosphate and iPTH concentrations. However, as in the study by Meema *et al.*, the authors only used a semi-quantitative assessment of the small hand arteries by X-ray examination [56, 59].

Mitral valve calcification is common in patients undergoing haemodialysis, and magnesium may exert a protective role against this type of cardiovascular calcification as well [60, 61]. This question has been investigated in a cross-sectional observational study of chronic haemodialysis patients ($n = 56$) in which 23 patients (41%) had mitral annular calcification [60]. There were no significant differences between patients with or without mitral annular calcification with regard to serum phosphate, calcium, calcium × phosphate product or iPTH, but magnesium levels were significantly lower in patients with calcification ($P < 0.05$). Further statistical analysis showed that patients with serum magnesium levels <1.23 mmol/L (3.0 mg/dL) were twice as likely to develop mitral valve calcification as those with magnesium levels >1.23 mmol/L (3.0 mg/dL) ($\chi^2 = 6.98$; $P = 0.008$). Moreover, multiple logistical regression showed that serum magnesium levels could predict the occurrence of mitral annular calcification with 86% accuracy when controlling for patients' age and biochemical factors other than magnesium levels [60].

More recently, Tzanakis *et al.* [62] in a cross-sectional study reported a negative association of both serum and intracellular magnesium levels with carotid intima–media thickness in patients undergoing haemodialysis, using multivariate analysis. The authors compared 93 stable chronic haemodialysis patients with 182 age- and sex-matched healthy control subjects with normal renal function. Intima–media thickness of both common carotids was assessed by ultrasonography: it was found to be significantly larger in the haemodialysis patients than the healthy controls ($P < 0.001$). Thus, for a 0.5 mmol/L (1.0 mEq/L) change in serum magnesium concentrations, a 0.35-mm change in carotid intima–media thickness was observed ($P = 0.01$).

Results from an observational study conducted within the general population in Japan ($n = 728$) point to a similar direction. Lower serum magnesium levels were significantly and independently associated with greater mean intima–media thickness ($P = 0.004$) and the risk of at least two carotid plaques ($P = 0.03$) [63] (see also Geiger and Wanner [64] in this supplement). Magnesium

deficiency has also been reported to be related to the progression of atherosclerosis in several studies, including the observational Atherosclerosis Risk in Communities (ARIC) Study in middle-aged adults [65, 66].

Overall, these observational data suggest that magnesium may play an important protective role in the development and/or acceleration of arterial atherosclerosis in both patients with chronic kidney failure and in the general population since carotid intima–media thickness as measured by ultrasonography is thought to be a surrogate marker for increased risk of myocardial infarction and stroke [67].

Thus, it appears that magnesium deficiency, caused either by poor diet or impaired magnesium metabolism, may be the missing link between various cardiovascular risk factors and atherosclerosis [68]. This issue is discussed further in the review by Geiger and Wanner [64] in this supplement.

Evidence based on observational studies:

- ESRD patients with peripheral arterial calcification had lower mean serum magnesium levels than those without calcifications or whose calcifications had regressed.
- Mitral annular calcification in chronic haemodialysis patients was strongly associated with low serum magnesium levels.
- The above effects were independent of several other commonly involved factors such as serum phosphate, calcium, calcium × phosphate product or parathyroid hormone (PTH) levels.
- There was a strong association between lower serum magnesium levels and increased carotid intima–media thickness in patients undergoing long-term haemodialysis treatment.
- Similar associations were also observed in the general population.

**Vascular calcification, atherogenesis and magnesium: intervention studies**

Given the potential involvement of low-magnesium levels in vascular calcification, as shown by various observational studies, some interventional studies have investigated the use of magnesium, though as yet there is no hard evidence in patients with ESRD (Table 1). A case study has described the resolution of soft tissue calcification after treatment with a dialysate containing a high concentration of magnesium [69]. Interestingly, clinical symptoms of soft tissue calcification such as joint swelling or pain reappeared when magnesium concentration in dialysate was subsequently reduced and again improved when the high dialysate magnesium level was re-instituted.

A pilot study conducted in 30 stable haemodialysis patients suggested that oral magnesium carbonate was generally well tolerated and effective in controlling serum phosphorus while reducing elemental calcium ingestion [70]. Following-on from this study, magnesium carbonate was given as a long-term phosphate binder to seven haemodialysis patients, all of whom had baseline CAC scores >30 and were treated with a dialysate containing 1.25 mmol/L (2.5 mEq/L) calcium and 0.375 mmol/L (0.75 mEq/L) magnesium, three times weekly [71]. This open-label, prospective pilot study evaluated changes in CAC scores from baseline and at 6, 12 and 18 months using electron beam computed tomography. There was no significant CAC score progression throughout the study (median per cent change in CAC score from baseline was 8, 4 and 8% at 6, 12 and 18 months, respectively). Furthermore, a paired test for directional change of CAC score was not statistically significant from baseline to 18 months ($P = 0.0737$). However, this study did not have a control group. For comparison, in other studies that included comparator groups, CAC in

patients with CKD showed progression rates from baseline to month 12 in the range of 5–33% for sevelamer and 25–75% for calcium-containing phosphate binders. [72–74].

A larger study randomized 47 chronic haemodialysis patients to two groups: a magnesium group in which patients were given oral magnesium citrate at a dosage of 610 mg every other day in addition to daily oral calcium acetate and a control group in which patients received only calcium acetate as a phosphate binder [75]. The study lasted 2 months. Mean serum calcium and phosphorus concentrations did not change in either group. Serum magnesium concentration also did not change in the control group but increased in the magnesium group by the end of the study. Magnesium supplementation was generally well tolerated: none of the patients presented with signs of magnesium toxicity such as arrhythmia or neuromuscular manifestations, none developed severe hypermagnesaemia and only one discontinued treatment because of diarrhoea. Carotid intima–media thickness was measured by ultrasound. At baseline, both groups had similar carotid intima–media thickness values. After 2 months, mean carotid intima–media thickness was reduced significantly in the magnesium group (0.70 versus 0.97 mm for left carotid artery, P = 0.001; 0.78 versus 0.95 mm for right carotid artery, P = 0.002) but not in the control group. In addition, there was a significant inverse association between the absolute change in serum magnesium concentrations and in right (but not left) carotid intima–media thickness after 2 months of magnesium treatment ($R$ = −0.443; P = 0.014). Serum PTH levels were also reduced in the magnesium group, but not in the control group. This led the authors to suggest that the beneficial effect of magnesium supplementation on carotid intima–media thickness might, among other factors, also be linked to a better control of hyperparathyroidism. However, baseline serum PTH was two times higher in the magnesium group than the control group. The authors concluded that magnesium supplementation might be useful in reducing the progression of atherosclerosis in chronic dialysis patients [75].

Suggestive evidence from interventional studies:

- Long-term administration of oral magnesium supplements to CKD patients on intermittent haemodialysis therapy might retard arterial calcification (based on a pilot study).
- Oral magnesium supplementation over a 2-month period led to a significant reduction in carotid intima–media thickness.

**Magnesium and survival of haemodialysis patients: observational studies**

The potential relationship between serum magnesium levels and survival has been investigated in 515 ESRD patients undergoing intermittent haemodialysis treatment [76]. During a mean (±SD) follow-up of 51 (±17) months, 103 patients died (41 from cardiovascular causes). When analysing the results according to the patients' baseline serum magnesium levels, mortality rates were significantly higher in the group with lower baseline magnesium levels [<1.14 mmol/L (2.77 mg/dL), $n$ = 261] than in the group with higher baseline magnesium levels [≥1.14 mmol/L (2.77 mg/dL); $n$ = 254] (P < 0.001) (Figure 5). It is important to note, at this point, that serum magnesium concentration at baseline correlated strongly with concentrations 1 year later ($r$ = 0.835, P = 0.0001). Multivariate Cox proportional hazard analysis showed that serum magnesium levels were a significant and independent predictor of overall mortality {hazard ratio [per 0.41 mmol/L (1 mg/dL) increase in magnesium], 0.485 [95% CI, 0.241–0.975], P = 0.0424} after adjustment for confounding factors such as patients' age, sex, duration of haemodialysis and presence of diabetes. Lower serum magnesium concentration was also a significant and independent predictor of death owing to non-cardiovascular causes [hazard ratio 0.318 (95% CI, 0.132–0.769), P = 0.011], but not for death from cardiovascular causes [hazard ratio 0.983 (95% CI, 0.313–3.086), P = 0.976]. The

authors concluded that it might be worthwhile considering a higher dietary intake of magnesium and/or an adjustment of dialysate magnesium concentrations.

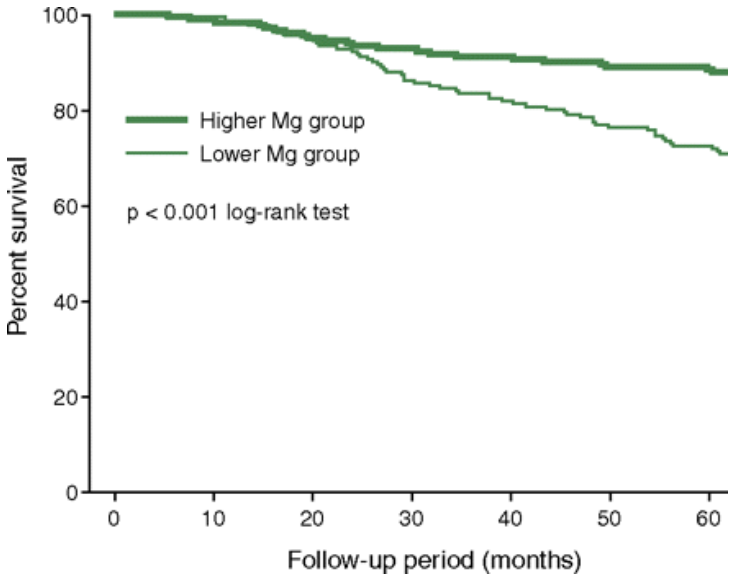


Figure 5 - Kaplan–Meier analysis of all-cause mortality rates during a 51-month follow-up of 515 chronic haemodialysis patients. The relative risk of mortality was significantly greater in the group with lower baseline serum magnesium levels (<1.14 mmol/L, *n* = 261) than in that whith the higher baseline serum magnesium levels (≥1.14 mmol/L; *n* = 254) [76]. All-cause mortality, P < 0.001 (log-rank test); after adjustment by Cox multivariate analysis, P < 0.05. Reprinted from Ishimura *et al.* [76], with permission.

Another observational, retrospective and as yet preliminary study showed an association between increased serum magnesium concentrations and reduced relative risk of mortality in a large haemodialysis patient cohort [77, 78]. The analysis was done in a subgroup of all chronic haemodialysis patients treated at Fresenius Medical Care North America facilities, *n* = 110 271 (total sample) who had at least one serum magnesium result between 1 October and 31 December 2007 (baseline) and who survived until 1 January 2008, *n* = 27 544 (subsample). The subgroup was considered to be representative of the entire patient cohort. Mortality was followed until the end of 2008 and Cox models were constructed. A quarter of the patients (27 544 of 110 271) had serum magnesium levels recorded at baseline. The mean (±SD) concentration was 0.93 ± 0.16 mmol/L (2.26 ± 0.38 mg/dL). Compared with magnesium levels of 0.80–0.95 mmol/L (1.94–2.31 mg/dL) (mid-normal levels, used as a reference), the unadjusted hazard ratio for mortality in the observation period decreased significantly with increasing magnesium concentrations [beginning at 0.95–1.05 mmol/L (2.31–2.55 mg/dL); P < 0.0001], to a hazard ratio of 0.68 [magnesium concentration >1.15 mmol/L (>2.80 mg/dL); P < 0.0001]. Results for Cox models were similar when adjusting for case mix or for five quality indicators at baseline (serum albumin and phosphorus, haemoglobin, e*Kt*/*V* and vascular access). More recently, similar results were found in a European database [79]. However, it must be pointed out that both these studies have not yet been published in a peer review journal.

What is clearly needed are prospective randomized trials examining the question whether increased serum or cytoplasmic magnesium levels or a magnesium intake in amounts such as those provided by magnesium containing phosphate binders is beneficial or not, in terms of hard outcomes in patients with CKD.

Magnesium and survival in haemodialysis patients: (partly based on data presented in abstract form only)

- Patients with slightly elevated serum magnesium concentrations may have a survival advantage.
- Low serum magnesium concentrations may be independent predictors of death.

**Conclusions**

A growing body of evidence from *in vitro* investigations, animal models and both observational as well as interventional clinical studies point to the possibility that low magnesium levels are associated with vascular calcification. Moreover, several observational studies suggest a relationship between increased serum magnesium concentrations and better survival rates for patients receiving long-term dialysis treatment. Preliminary results from an uncontrolled interventional trial suggest that long-term intervention with magnesium in dialysis patients may retard arterial calcification. However, many questions remain unanswered and hard evidence is as yet lacking. In order to conclusively show possible benefits and to demonstrate the absence of harm with the long-term intake of oral magnesium in patients with CKD, we still have to wait for the results of randomized controlled trials.

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# Consequences of Magnesium Deficiency on the Enhancement of Stress Reactions; Preventive and Therapeutic Implications (A Review)

Mildred S. Seelig, MD, MPH, Master ACN

*Department of Nutrition, Schools of Public Health and Medicine, University of North Carolina, Chapel Hill*

**ABSTRACT**

Stress intensifies release of catecholamines and corticosteroids, that increase survival of normal animals when their lives are threatened. When magnesium (Mg) deficiency exists, stress paradoxically increases risk of cardiovascular damage including hypertension, cerebrovascular and coronary constriction and occlusion, arrhythmias and sudden cardiac death (SCD). In affluent societies, severe dietary Mg deficiency is uncommon, but dietary imbalances such as high intakes of fat and/or calcium (Ca) can intensify Mg inadequacy, especially under conditions of stress. Adrenergic stimulation of lipolysis can intensify its deficiency by complexing Mg with liberated fatty acids (FA). A low Mg/Ca ratio increases release of catecholamines, which lowers tissue (i.e. myocardial) Mg levels. It also favors excess release or formation of factors (derived both from FA metabolism and the endothelium), that are vasoconstrictive and platelet aggregating; a high Ca/Mg ratio also directly favors blood coagulation, which is also favored by excess fat and its mobilization during adrenergic lipolysis. Auto-oxidation of catecholamines yields free radicals, which explains the enhancement of the protective effect of Mg by anti-oxidant nutrients against cardiac damage caused by beta-catecholamines. Thus, stress, whether physical (i.e. exertion, heat, cold, trauma - accidental or surgical, burns), or emotional (i.e. pain, anxiety, excitement or depression) and dyspnea as in asthma increases need for Mg. Genetic differences in Mg utilization may account for differences in vulnerability to Mg deficiency and differences in body responses to stress.

**Key Teaching Points:**

- Mg deficiency intensifies adverse reactions to stress that can be lifethreatening.
- Such reactions are mediated by excess release of the stress hormones: catecholamines and corticosteroids - which are increased by low Mg and high Ca levels, and which further lower tissue Mg.
- Low Mg/Ca levels favor excess release or formation of vasoconstrictive and platelet aggregating factors (derived from FA metabolism and endothelium).
- Low Mg/Ca levels also directly enhance intravascular blood coagulation - in microcirculation and in large blood vessels.
- Eclampsia is a coagulative microangiopathic disorder with high adrenergic activity; essential hypertension is also a stress-related disease.
- Low Mg levels are implicated in eclampsia, cardiac arrhythmias with and without AMI, and bronchial asthma, each of which is a stressful event that is favorably responsive to prompt (adjunctive) pharmacologic Mg treatment.

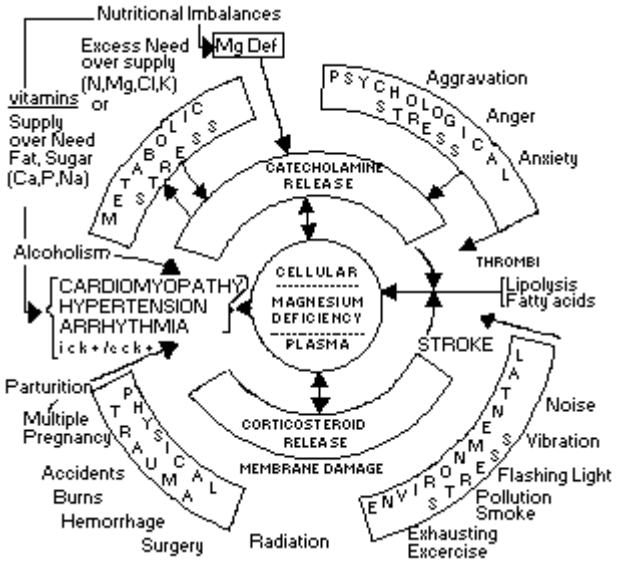
**Abbreviations**

AMI acute myocardial infarction; Ca calcium; CVD cardiovascular disease; CS corticosteroid; e.c. extracellular; FA fatty acids; FFA free fatty acids; GCS glucocorticoid; i.c. intracellular; IHD ischemic heart disease; i.v. intravenous; K potassium; MCS mineralcorticoid; Mg magnesium; RDS respiratory distress syndrome; SCD sudden cardiac death; SIDS sudden infant death syndrome; PGI2 prostacycline; TXA thromboxane

**INTRODUCTION**

Stress, both physical and emotional, evokes release of the stress hormones: catecholamines and corticosteroids, which mediate release and utilization of substrates for energy production and for improved skeletal and cardiac muscle performance. However, their excesses, which cause Mg loss and inactivation can be implicated in cardiovascular disorders - involving thrombotic events and arrhythmias, when Mg intakes from imbalanced diets, and serum and tissue levels are sub-optimal (Fig. 1).

**Fig. 1. Stress and magnesium**




Cardiac complications of stress often derive from the oxygen debt created by arterial constriction, usually of arteriosclerotic arteries, that reduces oxygen supply in the face of (stress-induced) increased energy consumption. There is suggestive evidence that Mg deficiency contributes to sudden cardiac death (SCD). Pain of acute myocardial infarction (AMI), angina pectoris, cancer, trauma, is stressful. In AMI especially when there is underlying Mg deficiency as is caused by diuretics, additional Mg loss induced by stress of pain and anxiety, might be a factor in its morbidity and mortality. Prompt intravenous (i.v.) pharmacologic treatment with Mg has improved AMI survival. Mg inadequacy of pregnancy might make additional Mg need during the stress of labor a factor in periand post-partum problems. Complications of bronchial asthma and of its drug treatment with Mg-wasters: beta-adrenergic agonists, corticosteroids (CS), and theophylline, resemble signs of Mg deficiency that can culminate in arrhythmia and sudden death. Providing i.v. Mg in doses comparable to that effective in eclampsia, is valuable adjunctive therapy in management of intractable bronchial asthma. Mg supplements have improved endurance and reduced cramps and fatigue in athletics; might they also protect against SCD of athletes?

New findings on interactions of prostanoids with Mg provide insight into how intravascular coagulation is involved in the pathogenesis of thrombotic arterial lesions that increase vulnerability to acute changes caused by stress. The mutual enhancement by the anti-oxidant, vitamin E and by Mg, of their protective effects against stress-induced myocardial damage, that is intensified by Mg deficiency, is interrelated with catecholamine-release of free radicals, as well as with loss of tissue Mg.

## MECHANISMS OF INTERACTIONS OF STRESS, STRESS HORMONES AND MAGNESIUM

### Stimulation by Stress of Secretion of Catecholamines and Corticosteroids

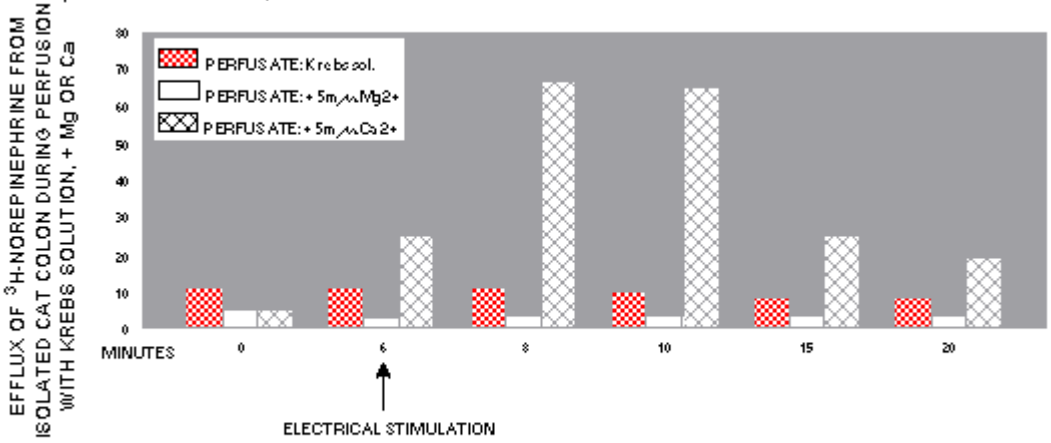
Classic studies of activation of the sympathetic system by emotional or sensory stimuli showed that pain, hunger, fear and rage increased epinephrine urinary excretion (1). During aggressive and violent action, norepinephrine release predominates (2). Isolation or overcrowding, forced exercise, cold or hot environments, noise, light flashes, electric shocks, or other anxiety-evoking stimuli, including frustration in access to food, or listening to recordings of fights involving the species under study - have increased secretion and/or release of catecholamines by the adrenal medulla, nerves and ganglia. The heart also synthesizes, stores and releases norepinephrine (2-5); almost immediately after a coronary occlusion, catecholamines are released from granules within the heart (5,6).

Elevation of plasma CS and increased urinary excretion of CS and its metabolites have been reported in monkeys subjected to anxiety or aggression (7), and in humans under emotional and physical stresses (2,8-10).

### Stress Reactions as Affected by Magnesium and Calcium

*Interrelations among Catecholamines, Magnesium and Calcium.* Catecholamine-secreting granules from adrenal medulla or nerve endings, suspended in low Mg/high Ca or high Mg/low Ca solutions, release more catecholamine in low Mg and less in high Mg media; Ca has reciprocal effects(11-15). (Figure 2) The effect of verapamil, a Ca channel-blocker, on catecholamine release has been compared to that of Mg, a physiologic Ca-blocker (16).

Figure 2. Catecholamine release of Norepinephrine from postganglionic nerve endings. Response to acetyl Choline release after electrical stimulation. Adapted from Bouillin, 1967.




Hypomagnesemia occurs in patients with elevated blood catecholamines: in AMI, cardiac surgery and insulin-induced hypoglycemia stress tests (17). Epinephrine infused into healthy volunteers, with and without prior treatment with Ca-blocking agents, lowered both serum Mg and K (18). Infusion of pathophysiologic amounts of epinephrine, or a therapeutic dose of salbutamol (a beta 2-catecholamine agonist) lowered plasma Mg levels in normal subjects. Epinephrine, but not norepinephrine, significantly reduced plasma Mg in healthy men (19). Infused beta-blockers had no significant effect on plasma Mg in 15 sedentary, healthy young men when maximally exercised (20).

Mg infusion (MgSO4, 60 mg/kg, i.v.) improved management of patients with pheochromocytoma prior to and during surgery to remove the catecholamine secreting tumor (21), and inhibited release of catecholamine produced by the stress of tracheal intubation (22). Experimental and clinical hyperadrenalemia caused elevated blood Mg levels, that fell to within normal limits after extirpation of an adrenal gland (23,24). The high erythrocyte (rbc) Mg (24) may be to the increased rbc Mg levels of most athletes, whose release of catecholamines increases during strenuous training (infra vide).

**Corticosteroids during Stress; Interrelations with Magnesium**

Prolonged isolation and other emotional, but not physical, stress has increased serum CS in rats (25). Runners exhibited increased excretion of both catecholamines and CS; Mg supplements significantly decreased their CS excretion (9). A marathon runner, whose CS levels gradually increased during a race, attained twice pre-race values at the end of the race (26). A mixed CS, with glucocorticosteroid (GCS) and mineralocorticosteroid (MCS) activity, caused negative Mg balance in normal volunteers, mostly by interfering with intestinal Mg absorption (27).

MCS hormones: aldosterone and desoxyycortosterone acetate (DOCA) affect Mg metabolism and are influenced by the Mg status. Each interferes with Mg absorption and increases urinary Mg excretion in experimental animals (28,29) and in humans with adrenal tumor-induced aldosteronism (30,31). Mg deficiency has increased MCS secretion in experimental animals, through induced juxtaglomerular hypertrophy (32,33). Anti-aldosterone agents reverse Mg deficiency of cardiac patients on long-term use of Mg-wasting diuretics, as reflected by restoration of depressed tissue Mg levels (34,35).

There is substantial experimental evidence (2,26,36-49) that cardiac damage - induced by stress or exogenous catecholaminesis intensified by CS, beta-adrenergic agonists, and by Mg deficiency. The myocardial lesions are characterized by necrosis and Ca deposition; Mg administration is protective.

**Catecholamines, Fatty Acid Release, Coagulation, and Prostanoids**

*Fatty Acid release of Stress; Interrelations with Mg.* Free fatty acids (FFA), an energy source during stress, are mobilized through lipolysis induced by beta-catecholamines (50,51). However, they bind and inactivate Mg in blood and heart, intensifying functional Mg deficiency. The stress of alcohol withdrawal increased serum FFA and lowered serum Mg (in dogs [52]). AMI increases catecholamine release and increases FFA levels (53,54), that is associated with a decline in serum Mg (55). Hyperexcitable (Type A) subjects, who are more vulnerable than Type B subjects to AMI, exhibit greater adrenergic release, have increased serum FFA and slight increase in plasma Mg and a small but significant decrease in rbc Mg (56).

Among five marathon racers, four were well trained; an untrained racer had been taking a Mg supplement (370 mg/day) for a week before the run (57). Blood samples drawn 6 times during the race showed steady increase in mean FFA that peaked at 26 miles, and that was associated with a steady fall in serum Mg in the trained runners, but not in the untrained, Mg supplemented man.

*Fat and Magnesium/Calcium Ratios in Coagulation.* Diets rich in saturated fats are implicated in hyperlipidemia and atherosclerosis, and increase thrombogenesis. Mg deficiency worsened both fat-induced intravascular hyper- coagulation (58-61) and atherogenesis; Mg was protective (42,46,59). It has prevented platelet aggregation on experimentally damaged endothelium (60,61), and has protected against spontaneous MI of rats, dogs and cocks on nutritionally imbalanced diets that caused Mg deficiency (42,62,63). Increased platelet aggregability increases myocardial vulnerability to ischemic injury in Mg deficient hamsters (64). The reciprocal effects of Ca and Mg on coagulation are considered elsewhere, re the need for Mg supplements of post-menopausal women taking both estrogen and Ca to slow osteoporosis (65), and in eclampsia, premenstrual syndrome, and migraine (66).

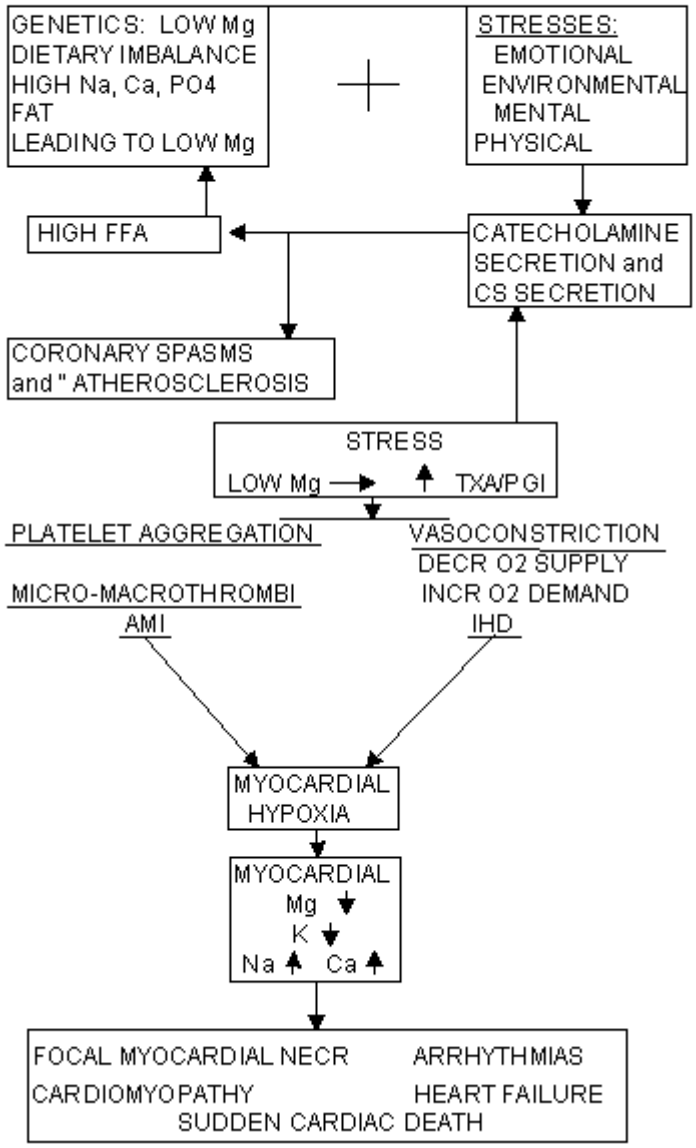
*Platelet Aggregation and the Prostanoids, Affected by Mg and Ca.* Prostacycline (PGI2) and thromboxane (TXA or TXB2) also participate in platelet aggregation, PGI2 inhibiting aggregation; TXA enhancing it. PGI2 is also a potent vasodilator; TXA is a vasoconstrictor. Guenther et al have reported that increased prostanoid synthesis is linked to translocation of Ca into cells (67). Mg deficiency (which causes hypercalcemia in rats) caused increased levels of PGI2 and its metabolite PGF1, but to a far lesser extent than it increased TXA synthesis and release. Studies by Nadler et al (68-70) in normal men have shown that Mg infusion significantly increased excretion of the PGI2 metabolite 6-keto-PGF1a, without altering urinary output of PGI2, and reduced TXB2 synthesis. Franz et al (26) found that physical stress of marathon racing increased TXB2 levels, inversely related to serum Mg levels. Early in the race TXB2 fell slightly, but it rose 9-fold by the end of the race, in association with a fall in serum Mg. PGI2 mediates, at least in part, the hemodynamic (vasodilator) effects of infused Mg (71). Altura et al (72) showed that, in its absence, PGI2-induced relaxation in isolated rat aortic strips is prevented. PGI2 and Mg infusions elicit similar hemodynamic effects (73), and in vitro exposure of (umbilical) vascular endothelial cells to vasodilating concentrations of Mg stimulates release of PGI2 (74,75).

*Catecholamines, Free Radicals, Magnesium and Antioxidants.* Recent work in the laboratories of Weglicki and Bloom (76-83) indicates that oxidative stress, induced by the beta-agonist isoproterenol, causes membrane damage of myocardium, endothelium, and erythrocytes in which release of free radicals participates. Mg deficiency and catecholamines each causes tissue Ca

overload. Both beta-blockers and Ca-channel blockers cause degrees of membrane lipid anti-peroxidative activity (76-81). Furthermore, catecholamine auto-oxidation leads to generation of cytotoxic free radicals (84). That the cardiomyopathy of Mg deficiency, alone, also involves free radicals is indicated by the protective effects of vitamin E and anti-oxidant drugs in Mg-deficient hamsters (80-83). This is pertinent to the observation that high intakes of anti-oxidant nutrients, as well as of Mg, were cardioprotective in a large series of Indian cardiac patients (Singh et al [85,86]) .

**CARDIOVASCULAR REACTIONS TO STRESS INTENSIFIED BY MAGNESIUM DEFICIT**

**Fig. 3. Stress, added to other predisposing factors in magnesium loss leading to: cardiomyopathy-arrhythmias-sudden death.**




**Cardiac Synthesis and Release of Catecholamines; Cardiac Complications with Excess Catecholamines**

Raab first reported very high catecholamine content in the myocardium of a young athlete who died in his sleep after a stressful event (3). The inotropic response to increased demands caused by

stress increases the force of cardiac contraction and oxygen utilization (4). Resulting oxygen debt causes relative myocardial hypoxia that can contribute to a shift of Mg out of cells to the extracellular space and plasma, as occurs with local ischemia of skeletal muscle (87) and in infants with asphyxia (88). The inward shift of Ca, stimulated by catecholamines is important in cardiac contractility, but when excessive, as in patients with ischemic heart disease (IHD) - the chronotropic responses to catecholamines predominate, and there is increased risk of arrhythmia and myocardial damage (89). Even in normal subjects, especially if low in Mg, the chronotropic effect of stress-induced excessive catecholamines may cause arrhythmia and sudden cardiac death (SCD). The increase in FFA caused by stress plays an important role in reducing availability of myocardial Mg. Focal myocarditis and congestive heart failure (CHF), common in patients with pheochromocytoma (90), is the clinical counterpart of the focal myocardial lesions seen in rats given high dosage norepinephrine (91), and in those caused by injection of a beta-adrenergic agonist (isoproterenol), at a dose just sufficient to cause multifocal micronecrosis (40,92). Mg loss is the earliest electrolyte derangement, preceding loss of K and gain of Na and Ca.

Functional stress tests, to which IHD patients are subjected, evoked significantly higher epinephrine and aldosterone levels in cardiac patients than in healthy controls (93). Pre-stress serum Mg levels were 1.6 mEq/L, and fell further at the test end.

**Influence of Personality on Cardiovascular Responses to Stress and Magnesium**

Emotion/anxiety, as well as ischemia, interfere with myocardial oxygen economy (2). Emotion evokes outpouring of catecholamines and CS, which deplete myocardial Mg, as the central cardio-damaging factor of stress, leading to tachycardia, arrhythmia, cardiomyopathy and even SCD. Nervous, emotional individuals who are most prone to cardiovascular disease (Type A) have far less stress tolerance than do Type B persons (94). Exposure to noise and mental stress results in excretion of more catecholamines by Type A than by Type B subjects, who have higher blood Mg (most marked in erythrocytes) than do Type A subjects (56,95). Henrotte et al have found that 70% of variance in rbc Mg levels is familial (56,95,96). Type A students, given a standardized test, had a statistically greater fall in rbc Mg than did Type B students, similarly stressed (56). The self-sustained stress and exaggerated response to external stresses of Type A persons might lead to subnormal Mg status.

Patients with latent tetany of Mg deficiency, who have psychoneurotic complaints (97,98), may also be especially vulnerable to mitral valve prolapse (97,99,100). Children with "nervous" complaints related to psychosocial and school stresses are also prone to hypomagnesemia (101). Durlach (97) suggests that whether Mg deficiency is acute or marginal and chronic (as with long-term suboptimal Mg intake, genetic Mg malabsorption, renal-wasting, or maldistribution), it increases vulnerability to stress, and increases its harmful effects.

**Hypertensive Responses to Emotional Stress; Mg/Ca Effects**

Emotional stress is a factor in hypertension (2,102). Whether clinical hypertension is associated with low or high plasma renin activity (PRA), free i.c. Mg is low (103-107); there is negative correlation of PRA with serum Mg levels (103). In low PRA patients, whose blood pressure was reduced by Ca supplements, and high PRA patients who respond to Mg, free i.c. Mg was inversely correlated with degree of hypertension (104). Low free i.c.Mg was also seen in rats made hypertensive whether by MCS and salt loading, by nephrectomy or renal ischemia (107). Low i.c. free rbc Mg was found in thin and obese hypertensive patients, in hypertensive obese patients with or without diabetes mellitus, and in diabetics (106).

In a study of total rbc Mg levels (a less sensitive parameter than the free rbc Mg) of middle-aged patients with labile hypertension, only those with low total rbc Mg had a blood pressure-lowering response to three months of Mg supplements (108). Workers in a high noise environment, and students preparing for their final examination experienced a rise in blood pressure during the work or study period (109) on diets providing about 5 mg/kg/day, which is above the current official American Recommended Dietary Allowance (RDA) (110). There was no rise in blood pressure in workers, or in students, given Mg supplementation that increased daily Mg intake to 6-7 mg/kg/day (109).

## TOXEMIAS OF PREGNANCY, POSTPARTUM CARDIOMYOPATHY AND SIDS

### Magnesium, Platelet Aggregation and Prostanoids of Toxemias of Pregnancy

Although only indirectly related to acute stress, aside from labor - which can intensify damage associated with toxemias of pregnancy, hypertension, that responds to Mg treatment, is characteristic of pre-eclampsia, as is greater than normal increase of blood pressure following catecholamine injection (111). Increased urinary epinephrine has been found in most patients with convulsive eclampsia (112).

Elucidation of interrelations of Mg on PGI2, TXA and endothelial-derived factors provides insight into the hypertension and hypercoagulability of blood of toxemic pregnancy, and draws parallels to that of cardiovascular disease and diabetes mellitus (113). The reversal of increased blood coagulability of women, with toxemias of pregnancy, by infusions of Mg was demonstrated first by Weaver (114), His pregnant ewes on Mg-low diets had hypertension, renal glomerular endotheliosis, and placental infarcts (115,116).

The increased Mg requirements of pregnancy, and the favorable response of complications of pregnancy to Mg treatment are well known (59). Mg deficiency during complicated pregnancy might be contributory to microvascular thromboses of severe toxemias. Pain from labor, as a stress factor that further increases Mg needs might be a factor in peripartal cardiomyopathy. It is provocative that Woods' rationale for extension of trials of Mg in AMI patients (71) derived partially from its efficacy in eclampsia, and from studies of Mg/PGI2 relations in pregnancy (74,75). Vascular endothelial damage (reflected by elevated fibronectin levels in preeclampsia), is associated with hyper-aggregability of platelets, and linked with prostanoid disturbances and microvascular thrombi in more severe toxemias (75,117). Watson et al (75) found that eclamptic patients have depressed PGI2 levels, whereas serum from MgSO4-treated eclamptic patients increased release of PGI2 by cultured (human umbilical vein) endothelial cells, and that it overcame thrombin induced- platelet adhesion to endothelium. Calvin et al (118) found that plasma fibronectin (released from damaged endothelium) was higher in 18 preeclamptic women than in 19 normal pregnant controls. However, the metabolite of PGI2 (6-keto-PGF1x: which they suggested is possibly a poor index of PGI2 at the microvascular level) was also higher. In vitro studies of the effect of Mg on platelet aggregation in umbilical cord vessel showed that elevated Mg levels increased PGI2 release from the vessels, and increased the anti-aggregating response of platelets to PGI2 (75).

### Relationships of Perinatal Mg Deficiency to Infantile Reactions to Stress

Low birth weight (LBW) infants are more commonly born after complicated pregnancies, that are associated with Mg loss, and to Mg deficient mothers than after Mg-replete pregnancies (59). Not only are such infants more prone to early infantile complications, they are subject to serious reactions to stress. Dietary Mg deficiency of ruminants has caused birth of low birth weight young

(115,116). Rats born to Mg deficient dams are less tolerant of stress, even after infancy (119). Pregnant Mg deficient cows and ewes exhibited 3-fold more clumping of platelets than did controls, and six of 18 lambs, that died in tetany after being fed low Mg diet for 2-12 months, had heart and lung lesions very similar to those produced by collagen-activation of platelets (120). Miller et al (120) suggest that abnormal blood platelet activation may be a significant mortality risk factor in severe gestational and infantile Mg deficit, and draw a parallel between the pathologic findings in Mg deficient lambs with those in the sudden infant death syndrome (SIDS). Caddell proposes that neonatal and postneonatal apnea and respiratory distress syndrome (RDS) are premonitory findings in infants who are later victims of SIDS, and advises Mg treatment in doses low enough not to constitute a risk (0.4 -1 mEq/kg/d for about 2 weeks) in RDS infants with immature kidneys (121-123). Infants with RDS are most commonly born to mothers subject to hypomagnesemia (59,124). SIDS has pathogenic features analogous to sudden cardiac death (SCD) of adults, in which stress and Mg deficiency have been implicated as factors (59,125-127).

**MIGRAINE , COAGULATION AND PROSTANOIDS**

Among the conditioning factors considered in migraine is stress, with its induced intravascular coagulability, associated with hypomagnesemia, platelet hyperaggregability and decreased cerebral blood flow (66,115,128-130). Weaver (115) has correlated the high incidence of migraine in patients with eclampsia (131) with the platelet hyperaggregation seen in both conditions (132). Altura (128) hypothesizes that the Ca-blocking effect of Mg might justify its trial in migraine, to prevent or ameliorate the initiation of migraine attacks.

**NOISE STRESS , HEARING LOSS AND ACCELERATED AGING**

Among the stresses that increase vulnerability to cardiac damage in rats is noise. It increased Mg loss from the heart, and myocardial Ca and collagen deposition (133). Franz found impaired hearing of surviving Mg deficient litter-mates, some of whom had died of sound-induced seizures (134).

Noise stress damaged the inner ear; Mg deficiency intensified ear damage; high Mg intake was protective (133,135-138). Ising and Joachim et al have negatively correlated hearing loss with perilymph and rbc Mg levels (133,138), and suggest that energy requirements of the inner ear are compromised by Mg deficiency-induced increased catecholamine secretion and arteriolar constriction. Mg deficient rats exposed to noise over a long period (i.e. 30 months), age rapidly (133). Cold stressed rats, made Mg deficient enough to cause adverse reactions and death of some in infancy, but to which adaptation took place in survivors, experienced both reduced tolerance of cold stress (as reflected by cardiac damage), and shortened life span (139).

**OBESITY AND STRESS OF STARVATION**

Voluntary starvation to reduce obesity is not the severe stress of famine, or of concentration camps - which has been associated with myofibrillar disruption (140) and SCD (141). During refeeding of surviving victims, strongly negative Mg balances persisted for months on Mg intakes as high as 800 mg/day (142). Even starvation to lose weight has caused arrhythmias and sudden death, possibly associated with lipolysis of ample fat stores, which as with catecholamine released during stress, inactivates available Mg (supra vide). DeLeeuw et al (143), commenting that sudden deaths of total starvation and some low-calorie diets are probably cardiac, showed that total starvation in an obese animal model caused severe myocardial Mg depletion.

Shortand long-term fasting of normal volunteers has caused Mg loss, but not necessarily hypomagnesemia (144,145). Balance studies of grossly obese men who were fasted up to 3 months

showed urinary losses to be responsible for loss of 20% of the body Mg, that resulted in development of carpal spasms requiring MgSO4 infusions for relief (146). Although the Mg status was not explored in the patient whose death after fasting had been preceded by lactic acidosis (147), it is pertinent that high lactate increases urinary Mg loss (148). A healthy young woman developed refractory hypokalemia during starvation for obesity, taking amino acid and protein supplements, despite high dose K treatment (149). Her plasma Mg was normal at the time of death from ventricular fibrillation after elongated QT interval and persistent arrhythmias (seen with hypomagnesemia or hypocalcemia) were treated with Ca infusion. Gross myofibrillar destruction was found, like that described with experimental Mg deficiency (150) and in starvation victims (140). Comparable myocardial lesions were seen in a 38 year-old woman who died in ventricular fibrillation after a liquid protein diet, supplemented with multivitamins, Ca and K (151); i.v. Mg treatment of her refractory ventricular tachycardia had not increased her serum Mg above 1.6 mEq/L. Potentially life-threatening and fatal cardiac arrhythmias have been reported in patients taking liquid protein reducing diets (152-154). Most striking, in a study in which the Mg levels were followed, was persistent magnesuria and normal serum Mg levels despite low intake (153); three of the subjects developed arrhythmia (154).

**GASTROINTESTINAL STRESS REACTIONS; HISTAMINE RELEASE**

Classen's studies of the alarm reaction are pertinent to gastrointestinal (GI) reactions to stress (155). Peptic ulcers in rats are characteristic of the early stress reaction, which sensitizes the mucosa to irritants and other stimuli, especially in Mg deficiency (156-157). Their incidence and extent were decreased by Mg administration. The effect of Mg deficiency on intestinal motility (157) may help to explain the stomach aches and acute abdominal pain in nervous children with marginal Mg deficiency and the beneficial effects of Mg supplementation in these patients (157,158).

How Mg inhibits stress-induction of peptic ulcers is uncertain. Several biochemical changes (decrease of ATP, ADP, and extent of phosphorylation reactions, and increase of cAMP, AMP) preceded the macroscopic appearance of stress ulcers in normal stressed rats (159). The Mg status was not explored, but it is noteworthy that ATP synthesis is Mg-dependent, as is phosphorylation (160), while cAMP levels are low in Mg deficiency (161). In view of the classic use of histamine to test for gastric acid secretion, and the usefulness of histamine receptor-antagonists in management of peptic ulcer disease, it seems plausible that Mg deficiency intensifies stress ulcers through its stimulation of histamine secretion. Mg deficiency in rats increases degranulation of mast cells with histamine release (162-164).

**BRONCHIAL ASTHMA**

Bronchial asthma (165,166), which is very stressful when seriously impeding respiration, has long been associated with hypomagnesemia. Itevokes adrenergic and corticosteroid secretion, which lower tissue Mg levels (supra vide) and it is characterized by histamine release, which has been correlated with hypomagnesemia (supra vide). It is noteworthy that the toxicity of theophylline- a phosphodiesterase inhibitor that lowers myocardial Mg levels, is intensified by beta-adrenergic agonists and CS (167), and resembles the effects of experimental Mg depletion: early tremor, later convulsions, and tachycardia, arrhythmia, myocardial necrosis and death (168-170). The favorable effect of Mg in counteracting toxic theophylline reactions has been attributed to the Ca-blocking effect of Mg (168,169), and to the membrane-stabilizing effect of Mg (169). In addition to counteracting adverse effects of standard drug therapy of asthma, Mg solutions (i.v. or aerosol) have been reported effective, at intervals from 1934 to 1973 (165,171-173), and more frequently in the past ten years (168,169,174-190). Since the early anecdotal reports, there have been case

reports on the ameliorative and even life-saving effect of Mg in intractable asthma (179,182-184,186-188) and favorable double blind studies (174,180). The usefulness of Mg in bronchial asthma, however, has been disputed (191-193). When effective for bronchodilatation and to reduce bronchial reactivity to administered histamine (justifying its adjunctive use with standard drug therapy), doses of Mg sufficient to achieve pharmacologic serum Mg levels were usually used (176-183,185-188,194-196). The need for higher than physiologic levels of Mg to counteract bronchial spasms induced in vitro had been shown by Classen et al (197) for serotonin, acetyl choline or histamine, and by Spivey et al (198) and Lindeman et al (199) for bethanechol, electrical stimulation or histamine. Pertinent to Mg and stress interrelationships in this disease, is the increased stress hormone secretion effected by low Mg/Ca ratios (supra vide). Mathew and Altura (200) have suggested that Mg should have value as an adjunct in treating asthma because it modulates smooth muscle contraction through its Ca-blockage or competition. In their recent review, Landon and Young (201) point out that the increased dietary Ca/Mg ratio of recent years, and loss of Mg caused by diuretics and other drugs, may be a factor in bronchial asthma, in view of Ca-binding to sites that increase acetyl choline release which initiates smooth (bronchial) muscle contraction.

## ATHLETIC STRESS, PERFORMANCE AND MAGNESIUM

### Magnesium Deficiency and Supplementation in Strenuously Exercised Animals

A series of studies by Keen and Lowney et al (202-206) with rats, fed diets providing 50 and 100 ppm and half the adequate intake of 400 ppm for 22 days, determined their endurance when they were run on a treadmill to exhaustion. They showed that decreased exercise capacity can be an early effect of Mg deficiency. Analysis of plasma and muscle levels of Mg of rats that had decreased endurance, sacrificed 2 days or immediately after the stress test, showed slightly lower plasma Mg at both times, and markedly lower muscle Mg immediately after exhaustion (203). Mineral water containing 85 ppm Mg restored endurance (202). Treadmill exercised, Mg deficient rats were shown by Laires et al (207) to have reduced exercise capacity, increased plasma lactate and FFA levels, and decreased plasma and rbc Mg levels. In a test of endurance with the stress of fear (of drowning), Hirneth and Classen (208) showed that Mg intake had to be increased ten-fold (to 4000 ppm) in rats made to swim with a weight attached to their tails, to significantly improve duration of swimming before submersion.

### Human Endurance Exercise, Magnesium, and Metabolism

Refsum et al (210), in 1973, attributed transiently lowered serum Mg and slightly increased rbc Mg, of cross-country skiing racers, to shift of Mg from e.c. to i.c. fluid. Well trained athletes were found by Casoni et al (210) to have significantly increased mean rbc Mg and slightly decreased serum Mg after a 25-km marathon, compared to pre-race levels. Serum Mg decreased and rbc Mg increased in 40 trained long-distance runners, 19 to 71 years of age, studied by Hoffmann and Boehmer (211) after each of two 25 km marathon races. Lijnen et al (212) found lower rbc and plasma Mg in teen-aged marathon runners just after the race than before, that returned to pre-race values 12 hours later. Urinary Mg decreased immediately after the race, and increased 12 hours later. Joborn et al found that long-term steady state exercise had little effect on Mg levels, but there was a decrease during the first hour of recovery (20).

High altitude intensive training resulted in negative Mg balance, in Mader et al's study (213). Hypomagnesemia sufficient to cause convulsions was seen in a man subjected to 4 hours intense exertion under conditions of heat (Jooste et al [214]). Non-supplemented marathoners lost muscle protein (Bertschat et al [215]).

Important studies of long-term Mg loss by trained adults undergoing sustained heavy exercise have been reported by Stendig-Lindberg et al (216-219). Apparently healthy young subjects who underwent a 7-month graded physical training program before undertaking a 120-km march (of 22 hours duration) had Mg intakes of 340 mg in food and 364 mg Mg in water (216). Despite the higher than usual intake, the mean serum Mg pre-march (1.66 mEq/L) fell significantly 1 hour after the march ended to (1.4mEq/L). After rising to almost pre-march levels at 24 hours, it fell again, at 72 hours to 1.3 mEq/L; 89% had hypomagnesemia. They exhibited elevated serum creatine kinase (CK) activity, suggesting that the serum Mg rise at 24 hrs resulted from exertional rhabdomoyolysis or loss of membrane integrity. Significantly lowered serum Mg (1.51 mEq/L) persisted for 3 months (217). A study after a 70 km march extended the findings to aspartate amino transferase (S-AST), alanine amino transferase (S-ALT), creatine kinase activity (S-CK), and VO2 ml/min-1. kg-1 (VO2 max) (218). Maximal aerobic power, hemoglobin, hematocrit, total protein and albumin were unchanged throughout. Immediately after the march, serum Mg did not change, but S-AST, S-ALT, hours after the march, serum Mg fell significantly; it remained low after 18 days, with no intervening marches or dietary changes. The means of S-ALT and S-CK rose & S-CK rose slightly. At 72significantly. For the first time there was a significant rise of blood sugar, and of serum triglycerides, and a second rise of total cholesterol. In a long-term follow-up of two additional groups of young men subjected to the same training and 120 km march as reported in 1985 (260), significant depression of serum Mg persisted as long as 10-11 months after the march in the two test groups; serum triglycerides showed a delayed rise (219).

**Short-term Intensive Exercise and Magnesium**

Plasma Mg was unchanged, but rbc Mg decreased significantly during exercise on an ergometer in a study by Golf et al (10). Boehmer (220), however found that an hour's strenuous exercise sharply lowered serum Mg in a large group of well-trained athletes, whose resting serum Mg values were normal. On the other hand, increased plasma Mg, attributed to reduction of plasma volume and influx of Mg to the vascular pool during short-term intense exercise on a bicycle ergometer, was reported by Joborn et al (19) and Ansquer (221), who commented that when there was a fall in serum Mg, it was accompanied by increased rbc Mg, the total blood Mg remaining constant, indicating an intercompartmental shift. Treadmill running until exhaustion was shown by Deuster et al (222) to transiently decrease plasma Mg significantly, with over 85% of the loss caused by shift to rbc. There was significantly increased urinary Mg on exercise, and of post-exercise blood lactate and oxygen consumption during recovery versus a control period. Exercise-induced inter-compartmental Mg shifts in blood Mg returned to pre-exercise values in 2 hours; urinary Mg loss on the exercise day returned to baseline the next day. They suggested that the exercise-induced loss of urinary Mg might depend on exercise intensity and relative contribution of anaerobic metabolism to total energy expended during exertion. Lukaski et al (223), who treadmill-tested 44 healthy male university athletes and 20 untrained men, observed that athletes' average maximum oxygen consumption was greater than in non-athletes, and was significantly correlated with plasma Mg, suggesting that Mg may enhance O2 delivery to working muscles in trained subjects. Conn et al (224) found that VO2max was significantly higher in pre-adolescent swimmers than in controls for both sexes, despite comparable plasma, rbc and whole blood Mg. Laires et al (225) assessed the effect of swimming for 30 minutes on plasma Mg and lipids in 6 well-trained swimmers before, just after, 30 minutes after, and 24 hours after exercise. Serum Mg decreased significantly shortly after exercise, returning to base line the next day; rbc Mg did not change. Plasma total cholesterol decreased significantly 30 minutes after exercise, with a significant positive correlation between plasma Mg and plasma HDL-cholesterol that disappeared after exercise but reappeared 24 hours later.

**Magnesium Supplementation and Dietary Magnesium Intakes of Athletes**

*Mg Supplements and Athletic Performance.* The effect of K + Mg aspartate supplements on the capacity for intense exercise (90 minutes) was reported in 1968 by Ahlborg et al (226) in six young men, the day before, and daily on four days of testing on a bicycle ergometer until complete exhaustion and/or muscle pain required stopping. Those supplemented on day 3 exhibited 50% increased work capacity before muscle pain developed, versus those given placebo. It was postulated that Mg accelerated glycogen synthesis or spared glycogen in muscle, thereby sparing energy. That premise was justified by the demonstrated fall in muscle glycogen and increase in lactate after 20 minutes of heavy exercise on a bicycle ergometer, without supplementation, shown by Bergstrom et al in 1971 (227).

Endurance (power) athletes under constant strain, given Mg supplements by Boehmer in 1979 (228) did not exhibit the declining Mg levels seen in the power athletes not so supplemented, and had better performance and endurance. In the study of de Haan et al (229), K and Mg aspartate supplementation did not improve muscle performance during short intensive exercise, as Vieth et al (230) had shown it to do in endurance athletes. In a double-blind study of marathon racers, whom Terblanche et al (231) described as Mg-replete, 365 mg/d of Mg had no effect on performance or recovery. In another double-blind study, of Mg supplemented athletes, Wodick and Grunert-Fuchs (232) used a running board and bicycle ergometry and found that 480 mg of Mg as the aspartate-HCl salt/day for 4 weeks significantly improved physical capacity. Four weeks of supplementation with Mg aspartate-HCl was found to increase rbc Mg and decrease maximal ventilation by 11% as compared to a pre-Mg test in 14 male rowers in maximal-tests on a rowing ergometer (Golf et al [233]). Plasma and urinary lactate increased from 1 mM/l to 15 mM/l, and blood oxygen content decreased.

The effect on blood coagulation and fibrinolytic factors of supplementing male swimmers for a month with 480 mg of Mg/day or with a placebo before a 1500 meter race was studied by Pohlmann et al (234). Those receiving Mg had both increased serum and rbc Mg levels, and showed anti-coagulating effects.

**Dietary Mg Intake of Athletes**

Surveys of dietary intakes of athletes have shown that as many as half consumed diets delivering less than the 1980 RDA (235) estimated for sedentary adults, which was lowered in the current edition(110). Those undergoing active anabolism and/or subjected to stress have substantially higher Mg needs. Studies of diets of athletes have shown that such needs are commonly not met by their diets (222,236-242). Supplementation of athletes to keep their Mg status optimal, to ensure their best performance without interfering muscle cramps and premature fatigue, and to prevent muscle damage, has been recommended or implied by those reporting improvement in the Mg-treated group in placebo-controlled studies (supra vide). Since athletes undergo severe physical stress, as well as the psychological drive to win, and most ingest sub-optimal amounts of Mg, they are vulnerable to Mg deficiency. Diets very rich in Mg, or prophylactic use of Mg supplements should be advised to be sure of Mg intake of not less than 6 to 10 mg/kg/day, and possibly more (97,221,236-238,243-246).

**TRANSPORTATION /SUDDEN DEATH**

Sudden death of pigs and cattle being transported under the stressful conditions of crowding has been reduced in incidence by Mg supplementation with Mg aspartate HCl or $MgCl_2$ in sufficient dosage to raise serum Mg (247). Calmer behavior of Mg treated animals was observed in 49.5% of groups during fattening and in 37.6% during transport and in the slaughterhouse. (248,249). Up to half of losses of young chickens (broilers) have been attributed to a sudden death syndrome,

possibly from stress. A study of 5180 broilers whose high protein and carbohydrate diets were supplemented with Mg aspartate HCl, for 40 days during their fattening period, disclosed significant reduction of losses from sudden death (250).

**CONCLUDING COMMENTS**

Stress-intensification of Mg inadequacy may well be to blame for the SCD associated with stress, even in young healthy athletes. This report has evaluated data implicating high Ca/Mg ratios in increased adverse responses to stress. Stress hormone intensification of Mg loss, and stimulation of their secretion by high Ca/Mg constitute a self-reinforcing loop. High Ca/Mg ratios lead to increased blood coagulation and vasotonus, which are also influenced by prostaglandins and fibronectin, that are affected by low Mg levels. It is provocative that pharmacologic doses of Mg, are therapeutic in conditions that are intensified by loss of Mg, and in which Mg inadequacy might well be a contributory factor. High serum Mg levels, such as are required to control eclampsia, have been shown to be effective adjunctive therapy in bronchial asthma unresponsive to standard therapy; prompt high dosage i.v.Mg has prevented or reduced the incidence of post-AMI complications and significantly improved survival in double-blind trials (251-256).

Worth consideration and trial is the possibility that higher than usual Mg intake - whether from a Mg-rich diet or from supplementing the usual diet with Mg salts, possibly with anti-oxidant nutrients, might be protective against damage caused by the usual vicissitudes of life and unusual stresses. Might higher Mg (and vitamin E) intakes decrease the risk of arrhythmias and SCD in humans? Large intervention studies, like those undertaken to elucidate the effects of lowering saturated fat intake on cardiovascular disease and cancer, are needed to determine the extent to which adding Mg (and anti-oxidant nutrients) to already recommended dietary changes, might further improve health and increase stress-tolerance. Such studies are particularly urgent in view of the recent National Institute of Health recommendation (257) that the optimum Ca intake be further increased to 1500 mg/day (to prevent osteoporosis), which disregards the low American Mg intake, less than 300 mg/day. This would bring the Ca/Mg ratio to 5/1 - which is above the 4/1 ratio of Finland, the land with the highest ischemic heart disease death rate for young to middle-aged men (258,259).

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# Effects of Magnesium Supplementation in Hypertensive Patients

**Assessment by Office, Home, and Ambulatory Blood Pressures**

Yuhei Kawano[1], Hiroaki Matsuoka[1], Shuichi Takishita[1], Teruo Omae[1]

[1]*From the Division of Hypertension and Nephrology, National Cardiovascular Center, Suita, Osaka, Japan*

**Abstract**

*Abstract*—An increase in magnesium intake has been suggested to lower blood pressure (BP). However, the results of clinical studies are inconsistent. We studied the effects of magnesium supplementation on office, home, and ambulatory BPs in patients with essential hypertension. Sixty untreated or treated patients (34 men and 26 women, aged 33 to 74 years) with office BP >140/90 mm Hg were assigned to an 8-week magnesium supplementation period or an 8-week control period in a randomized crossover design. The subjects were given 20 mmol/d magnesium in the form of magnesium oxide during the intervention period. In the control period, office, home, and average 24-hour BPs (mean±SE) were 148.6±1.6/90.0±0.9, 136.4±1.3/86.8±0.9, and 133.7±1.3/81.0±0.8 mm Hg, respectively. All of these BPs were significantly lower in the magnesium supplementation period than in the control period, although the differences were small (office, 3.7±1.3/1.7±0.7 mm Hg; home, 2.0±0.8/1.4±0.6 mm Hg; 24-hour, 2.5±1.0/1.4±0.6 mm Hg). Serum concentration and urinary excretion of magnesium increased significantly with magnesium supplementation. Changes in 24-hour systolic and diastolic BPs were correlated negatively with baseline BP or changes in serum magnesium concentration. These results indicate that magnesium supplementation lowers BP in hypertensive subjects and this effect is greater in subjects with higher BP. Our study supports the usefulness of increasing magnesium intake as a lifestyle modification in the management of hypertension, although its antihypertensive effect may be small.

Magnesium is related to various physiological functions, including cardiovascular regulation. It may play an important role in control of neuronal activity, cardiac excitability, neuromuscular transmission, muscular contraction, vascular tone, BP, and peripheral blood flow.[1] Mg ions compete with Ca ions for membrane-binding sites, lower levels of intracellular $Ca^{2+}$, and cause vasodilation. It has been suggested that deficiency in Mg and abnormalities in Mg metabolism play pathophysiological roles in ischemic heart disease, congestive heart failure, sudden cardiac death, arrhythmias, preeclampsia and eclampsia, insulin resistance and diabetes, and hypertension.[1]

An inverse relationship between dietary Mg intake and the level of BP or the prevalence of hypertension has been observed in epidemiological studies.[2 3 4] It has also been shown that hypertensive patients often have reduced serum and intracellular levels of $Mg^{2+}$ compared with normotensive subjects.[5 6] Measurements of serum ionized Mg and intracellular free $Mg^{2+}$ may provide better estimation for the Mg deficiency than conventional measurement of serum Mg.[6 7] In experimental studies, dietary Mg deficiency raises BP of normotensive animals, whereas Mg supplementation lowers BP in hypertensive rats.[8 9] However, the results of clinical studies on the effects of Mg supplementation in hypertensive patients and subjects with high normal BP have

been inconsistent. Significant reductions in BP have been shown in several studies,[10 11 12] but not in others.[13 14] Although adequate dietary intake of Mg was recommended in the report of the Joint National Committee,[15] increasing Mg intake is not accepted as a general application in the treatment of hypertension.[16]

Earlier clinical studies concerning Mg supplementation relied on casual BP measurements. Monitoring of 24-hour ambulatory BP and self-measurement of BP at home have advantages compared with casual BP measurement because they provide multiple BP records, have good reproducibility, and eliminate observer bias and the placebo effect.[17] These methods appear to be particularly useful in the evaluation of nonpharmacological interventions, as we have shown.[18 19] To our knowledge, only 1 study used ambulatory BP monitoring to assess the effects of Mg supplementation,[20] and the effects of Mg on home BP have not been reported. In the present study, we investigated the effects of Mg supplementation on 24-hour ambulatory BP and home BP, as well as casual office BP, in hypertensive patients who were untreated or insufficiently treated in a randomized crossover design.

## Methods

### Subjects

Sixty-two Japanese men and women with mild to moderate essential hypertension participated in this study. They were 35 to 74 years old, either treated or untreated, and had office SBP >140 mm Hg and/or DBP >90 mm Hg on at least 2 occasions before entering the study protocol. Two patients withdrew from the study because of gastrointestinal symptoms (diarrhea) during Mg supplementation. The remaining 60 subjects completed the study protocol.

The clinical characteristics of the 60 patients are shown in Table 1⇓. Twenty subjects were untreated, while 40 subjects were treated with antihypertensive drugs. Among the treated subjects, 18 were receiving monotherapy and 22 were receiving combination therapy. Ca antagonists were the most frequently prescribed drugs (n=30), followed by β-blockers (n=14), angiotensin-converting enzyme inhibitors (n=9), diuretics (n=6; 5 thiazide, 1 spironolactone), and α-blockers (n=5). Antihypertensive therapy was continued without any alterations throughout the study protocol.

Table 1 - Clinical Characteristics of Study Subjects

| **Parameter** | |
|---|---|
| Age, y | 35–74 (58.1±1.1) |
| Gender, M/F | 34/26 |
| Body weight, kg | 63.8±1.3 |
| Body mass index, kg/m$^2$ | 24.7±0.4 |
| Antihypertensive drugs | Yes 40, No 20 |
| Drinking habit[1] | Yes 25, No 35 |

[1] Yes indicates regular drinkers (≥1 drink/d); no, abstainers or occasional drinkers.

### Protocol

The study protocol was approved by the Clinical Research Committee of our institute. Informed consent was given by each subject before participation in this study. An 8-week Mg supplementation period and an 8-week control period were assigned in a randomized crossover manner. Thirty subjects entered the control period first, and the other 30 subjects entered the Mg

period first. During the Mg supplementation period, 20 mmol/d (480 mg) Mg was given in the form of MgO (400 mg BID) to each subject. Placebo was not given during the control period because the placebo effect is usually negligible in the monitoring of ambulatory or home BP,[17 21] and the majority of subjects were already taking antihypertensive drugs.

Casual office BP and 24-hour ambulatory BP were measured at the end of the control and Mg supplementation periods. Home BP was measured throughout the study protocol. Blood samples and 24-hour urine samples were collected at the end of each period.

**Measurements**

Office BP was measured twice with the subject in the sitting position by a physician with a mercury sphygmomanometer. Home BP was measured by the patients in the sitting position 3 times in the early morning and also in the late evening with semiautomatic devices using the oscillometric method. Ambulatory BP was measured every 30 minutes for 25 to 26 hours by the oscillometric method using the TM-2421 (A&D Co Ltd). Accuracy and performance of this device have been demonstrated previously.[22] The accuracy of each recorder was also checked by simultaneous measurement with a mercury sphygmomanometer, and all recorders showed a difference of <10 mm Hg. The same recorder was used in each subject to avoid errors due to differences in equipment. Serum and urinary electrolyte levels were determined with a TBA-80 M autoanalyzer (Toshiba).

**Data Analysis**

Averages of 2 measurements were used for analysis of office BP. For home BP, averages of the records for the last 7 days in each period were used. The first 1-hour record of ambulatory BP was discarded for the analysis of 24-hour BP because it may be substantially higher than the usual BP. The daytime BP was defined as that from 6:30 am to 10 pm, and the nighttime BP was defined as that from 10:30 pm to 6 am in this study.

Data are expressed as mean±SEM. Student's paired or unpaired *t* test was used for comparison of 2 groups of data. Linear regression analysis was used to assess correlations between 2 parameters. Multiple regression analysis was used to identify independent determinants for the change in BP with Mg supplementation. A value of $P<0.05$ was considered statistically significant. Analyses were performed using StatView software (Abacus Concepts Inc).

**Results**

Serum and urinary electrolyte levels in the control and Mg supplementation periods are shown in Table 2⇓. Serum concentration and urinary excretion of Mg increased significantly after Mg supplementation. The average change in serum Mg was 6%, and that in urinary Mg was 60%. Serum and urinary levels of Na, K, and Ca, as well as urinary creatinine excretion, were similar between the 2 periods.

Table 2 - Serum and Urinary Electrolyte Levels in Control and Mg Supplementation Periods

| Parameter | Control Period | Mg Period |
|---|---|---|
| Serum | | |
| Na, mmol/L | 142.1±0.2 | 142.1±0.2 |
| K, mmol/L | 4.25±0.05 | 4.36±0.05 |
| Ca, mmol/L | 2.37±0.02 | 2.36±0.02 |

| | | |
|---|---|---|
| Mg, mmol/L | 0.84±0.01 | 0.89±0.01[1] |
| Urine | | |
| Na, mmol/d | 182.5±9.5 | 188.7±9.9 |
| K, mmol/d | 54.2±2.0 | 54.7±2.1 |
| Ca, mmol/d | 4.95±0.32 | 5.10±0.35 |
| Mg, mmol/d | 2.92±0.13 | 4.67±0.21[1] |
| Cr, g/d | 1.20±0.05 | 1.21±0.05 |

- Cr indicates creatinine.
- 1 $P<0.001$ between the 2 periods.

Table 3⇓ shows office, home, and ambulatory BPs in the control and Mg supplementation periods. These levels correlated significantly with each other, although the correlation coefficient was from 0.31 (office SBP versus 24-hour SBP) to 0.45 (office DBP versus home DBP). Office, home, average 24-hour, and daytime SBP as well as DBP were significantly lower in the Mg period than the control period. Average differences in SBP assessed by the 3 methods were 2 to 4 mm Hg, and those in DBP were 1 to 2 mm Hg. Changes in nighttime SBP and DBP were comparable to those in daytime BP, although the changes in nighttime BP were not statistically significant.

Table 3 - Office, Home, and 24-Hour Ambulatory BP in Control and Mg Supplementation Periods

| Parameter, mm Hg | Control Period | Mg Period | Difference |
|---|---|---|---|
| Office | | | |
| SBP | 148.6±1.6 | 144.9±1.7 | 3.7±1.3[2] |
| DBP | 90.0±0.9 | 88.3±0.9 | 1.7±0.7[1] |
| Home | | | |
| SBP | 136.4±1.3 | 134.4±1.4 | 2.0±0.8[1] |
| DBP | 86.8±0.9 | 85.4±0.8 | 1.4±0.6[1] |
| 24-h | | | |
| SBP | 133.7±1.3 | 131.2±1.1 | 2.5±0.8[2] |
| DBP | 81.0±0.8 | 79.6±0.8 | 1.4±0.6[1] |
| Day | | | |
| SBP | 137.7±1.2 | 135.2±1.3 | 2.5±1.1[1] |
| DBP | 84.0±0.8 | 82.5±0.9 | 1.5±0.7[1] |
| Night | | | |
| SBP | 125.9±1.9 | 123.4±1.5 | 2.5±1.3 |
| DBP | 74.8±1.1 | 73.6±0.9 | 1.2±0.7 |

- Day indicates 6:30 am to 10 pm; Night, 10:30 pm to 6 am.
- 1 $P<0.05$,
- 2 $P<0.01$.

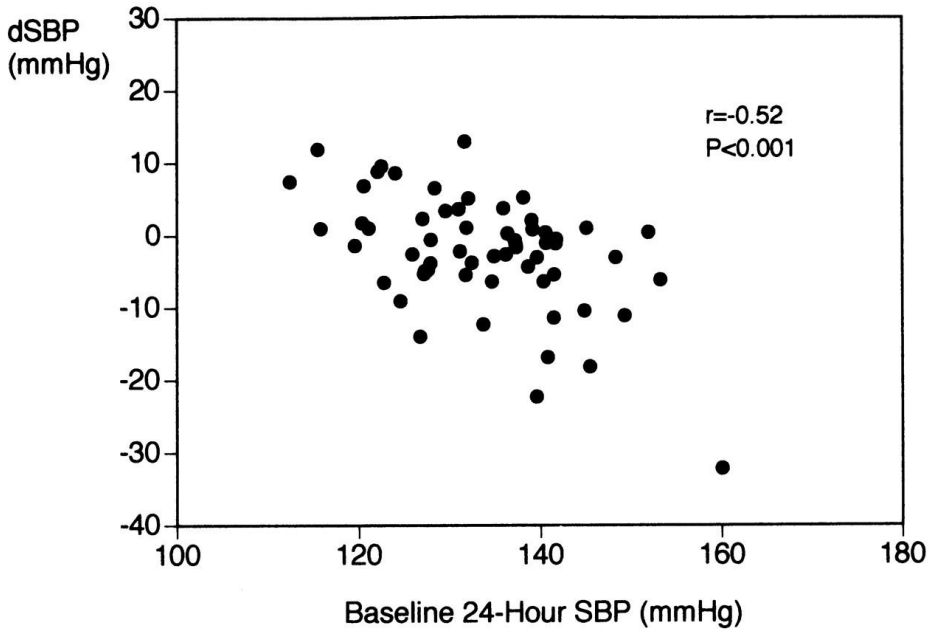
Levels of electrolyte and BP in men and women are shown in Table 4⇓. Baseline serum Mg was lower and urinary Mg was higher in men than in women, although these differences were not significant. Office, home, and 24-hour BPs decreased significantly with Mg supplementation in men, but these changes were not significant in women.

Table 4 - Body Weight, Electrolyte Level, and BP in Control and Mg Supplementation Periods in Men and Women

| | Men | | Women | |
|---|---|---|---|---|
| **Parameter** | **Control** | **Mg Supplement** | **Control** | **Mg Supplement** |
| Body weight, kg | 68.8±1.6 | 68.7±1.7 | 57.4±1.4[5] | 57.4±1.5[5] |
| Serum | | | | |
| Na, mmol/L | 141.6±0.3 | 141.7±0.2 | 142.7±0.3 | 142.5±0.4 |
| K, mmol/L | 4.27±0.06 | 4.30±0.06 | 4.24±0.10 | 4.44±0.07 |
| Ca, mmol/L | 2.36±0.02 | 2.33±0.02 | 2.37±0.02 | 2.38±0.02 |
| Mg, mmol/L | 0.83±0.01 | 0.90±0.01[3] | 0.85±0.01 | 0.88±0.01[2] |
| Urine | | | | |
| Na, mmol/d | 199.5±14.2 | 204.6±15.5 | 162.6±11.0 | 167.8±9.7 |
| K, mmol/d | 56.2±2.8 | 56.8±2.9 | 52.0±3.1 | 52.4±3.0 |
| Ca, mmol/d | 5.08±0.52 | 5.20±0.47 | 4.80±0.41 | 4.98±0.53 |
| Mg, mmol/d | 3.17±0.21 | 4.60±0.29[3] | 2.70±0.14 | 4.70±0.22[3] |
| Cr, g/d | 1.43±0.06 | 1.42±0.07 | 0.92±0.04[5] | 0.94±0.04[5] |
| Office BP, mm Hg | | | | |
| SBP | 145.0±1.8 | 140.9±2.0[1] | 153.5±2.6[4] | 150.1±2.7[4] |
| DBP | 89.4±0.9 | 86.4±1.1[1] | 90.5±1.7 | 90.0±1.5 |
| Home BP, mm Hg | | | | |
| SBP | 136.3±1.9 | 134.0±1.9[1] | 136.5±2.1 | 134.9±2.0 |
| DBP | 87.2±1.3 | 85.7±1.0[1] | 86.4±1.5 | 85.2±1.4 |
| 24-h BP, mm Hg | | | | |
| SBP | 135.4±1.8 | 131.6±1.6[1] | 131.6±1.7 | 130.8±1.6 |
| DBP | 82.6±1.0 | 80.5±1.0[1] | 78.8±1.3 | 78.4±1.3 |

- 1 $P<0.05$,
- 2 $P<0.01$,
- 3 $P<0.001$ between the control and Mg periods;
- 4 $P<0.05$,
- 5 $P<0.001$ between men and women.

The Figure⇓ shows the relationship between changes in 24-hour BP with Mg supplementation and levels of 24-hour BP in the control period. The changes in both SBP and DBP correlated negatively with their baseline levels. The changes in 24-hour BP also correlated negatively with changes in serum Mg level (Table 5⇓). Correlations between changes in 24-hour BP and age, control levels of serum Mg, control levels or changes in urinary Mg, or control levels of urinary Na were not significant.



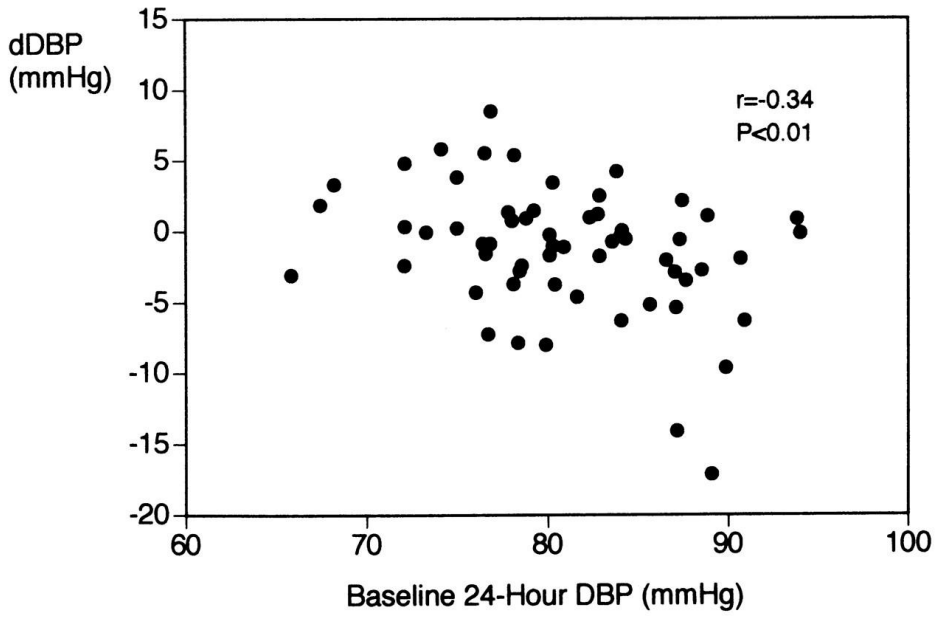




Figure 1 - Relationship between 24-hour BP in the control period and changes in 24-hour BP with Mg supplementation. dSBP indicates change in 24-hour SBP; dDBP, change in 24-hour DBP.

Table 5 - Correlations Between Changes in 24-Hour BP With Mg Supplementation and Age, Baseline BP, Serum and Urinary Mg, and Urinary Na Excretion

| | **Change in 24-h SBP** | | **Change in 24-h DBP** | |
|---|---|---|---|---|
| **Parameter** | ***r*** | ***P*** | ***r*** | ***P*** |
| Age | −0.187 | 0.153 | −0.196 | 0.133 |
| Control 24-h SBP | −0.523 | <0.001 | −0.343 | 0.007 |
| Control SMg | 0.198 | 0.129 | 0.221 | 0.090 |
| Change in SMg | −0.324 | 0.011 | −0.337 | 0.008 |
| Control UMg | 0.093 | 0.483 | 0.003 | 0.982 |

| | | | | |
|---|---|---|---|---|
| Change in UMg | −0.091 | 0.491 | −0.089 | 0.502 |
| Control UNa | −0.137 | 0.298 | −0.190 | 0.147 |

- SMg indicates serum Mg; UMg, urinary magnesium; and UNa, urinary sodium.

Table 6⇓ shows results of subgroup analysis regarding the changes in 24-hour BP with Mg supplementation. Age, gender, antihypertensive medication, drinking habit, and the order of the control and Mg periods did not significantly influence the changes in 24-hour BP, although the Mg-induced BP reduction tended to be greater in older subjects, men, and subjects taking antihypertensive medication. Subjects with high (above average) 24-hour SBP in the control period showed significantly greater reduction in 24-hour SBP (−5.3±1.5 mm Hg) than those with low 24-hour SBP. Similarly, subjects with high 24-hour DBP showed greater reduction of 24-hour DBP (−2.7±0.9 mm Hg) with Mg supplementation than those with low 24-hour DBP.

Table 6 - Changes in 24-Hour BP With Mg Supplementation: Subgroup Analysis

| **Parameter** | **24-h SBP, mm Hg** | **24-h DBP, mm Hg** |
|---|---|---|
| Age, y | | |
| ≥60 | −4.1±2.0 | −2.5±1.0 |
| <60 | −1.1±0.9 | −0.4±0.6 |
| Gender | | |
| Men | −3.7±1.6 | −2.1±0.9 |
| Women | −0.8±1.1 | −0.4±0.6 |
| Medication | | |
| Yes | −3.6±1.3 | −1.9±0.7 |
| No | −0.4±1.7 | −0.4±1.0 |
| Drinking habit | | |
| Yes | −2.5±1.6 | −1.5±1.0 |
| No | −2.5±1.4 | −1.2±0.7 |
| Order | | |
| Control-Mg | −2.9±1.5 | −1.9±0.8 |
| Mg-Control | −2.0±1.4 | −0.8±0.8 |
| Control 24-h BP | | |
| High | −5.3±1.5[2] | −2.7±0.9[1] |
| Low | 0.4±1.3 | −0.2±0.7 |

- Medication indicates antihypertensive medication; High, SBP ≥134 mm Hg (n=30) and DBP ≥81 mm Hg (n=27); Low, SBP <134 mm Hg (n=30) and DBP <81 mm Hg (n=33).
- 1 $P<0.05$,
- 2 $P<0.01$ between subgroups.

In multiple regression analysis, the baseline level of 24-hour SBP was an independent determinant for the change in 24-hour SBP with Mg supplementation. The baseline 24-hour DBP was a significant determinant for the change in 24-hour DBP. Other variables were not significant determinants for the change in 24-hour SBP or DBP.

**Discussion**

In the present study, supplementation with Mg for 8 weeks significantly lowered BP, with increases in serum Mg concentration and urinary Mg excretion in hypertensive patients. The reduction in BP was detected by 3 different methods, ie, measurement of casual office BP, self-measurement of home BP, and 24-hour ambulatory BP monitoring. Our results provide additional support for the antihypertensive effect of high dietary Mg intake, although the reduction in BP may be small.

Dietary Mg intake appears to be declining in developed countries.[4] In the United States, it was estimated to be 475 to 500 mg/d at the turn of the century,[23] but it was 283 mg/d for men and 215 mg/d for women in 1989 to 1990.[24] In Japan, estimated Mg intake in 1980 was 240 mg/d.[25] The recent recommended daily allowances for Mg for adults are 280 mg for women and 350 mg for men in the United States[26] and 4 mg/kg in Japan.[27] In earlier intervention studies, amounts of supplemental Mg were from 15 mmol (360 mg) to 40 mmol (960 mg) daily. A dose of 15 or 20 mmol was often used because higher doses may cause adverse effects such as diarrhea. In the present study, 20 mmol/d (480 mg) Mg was given to the study subjects, most of whom completed the protocol without adverse symptoms. This dose is considered to be within the upper range of physiological intake, although it may increase average Mg intake by about 200%.

Urinary Mg excretion in the control period was approximately 3 mmol/d in the present study. This level is similar to that observed in US studies[28 29] but was lower than that seen in European studies[10 30] or observational studies in China and Cameroon.[31 32] These differences may be attributed to different lifestyles among populations. Individual level of serum Mg in the control period was within the normal range, except in a few patients whose level was slightly low. Therefore, the study subjects did not seem to have severe Mg deficiency. It has been shown that measurements of serum ionized Mg taken using ion-selective electrodes and erythrocyte-free $Mg^{2+}$ by $^{31}P$ NMR provide more precise estimation of body Mg status than conventional measurement of serum Mg.[7] Unfortunately, we did not determine serum ionized Mg or intracellular free $Mg^{2+}$ in the present study.

Results of Mg supplementation studies based on casual BP measurement have been inconsistent.[33] Significant reduction in BP was reported in several studies[10 11 12 28] but not in others.[13 14 29 30] Lind et al[34] observed that Mg supplementation had no general effects on BP, but it lowered BP in subgroups with low urinary Mg excretion. Average changes in BP produced by Mg supplementation were −12 to −3 mm Hg for SBP and −8 to −3 mm Hg for DBP in positive studies. They were −7 to +3 mm Hg and −7 to +1 mm Hg, respectively, in negative studies. Doses of supplemental Mg were 20 to 40 mmol/d in the positive studies, except 1 study (15 mmol) in patients receiving diuretic treatment,[10] while they were 15 to 20 mmol in the negative studies. The study subjects had mild to moderate hypertension in most trials, but some negative studies included subjects with high normal BP.[12 30] In our study, casual office BP decreased by 3.7/1.7 mm Hg on average after Mg supplementation at a dose of 20 mmol/d for 8 weeks in hypertensive patients. These findings taken together, Mg supplementation appears to lower BP at least in some hypertensive subjects, although its antihypertensive effect may be small. Subjects with Mg-depleted status caused by low dietary intake or diuretic use may respond to oral Mg intake with greater BP reductions.

In our study, small but significant reductions in BP were also revealed by repeated home BP measurement and 24-hour ambulatory BP monitoring. These methods are considered to be more reliable for the assessment of pharmacological and nonpharmacological treatments of hypertension compared with casual BP measurement, which may overestimate or underestimate the effects of treatment because of several factors such as poor reproducibility, observer bias, white-coat phenomenon, and placebo effects.[17 21] In the present study, the average reduction in 24-hour BP was 2.5/1.4 mm Hg, and changes in daytime and nighttime BPs were comparable. Our results are consistent with a report by Haga,[20] who examined effects of Mg supplementation (25

mmol/d for 2 weeks) on 24-hour BP in a small number of hypertensive patients. In the present study, we examined the effects of Mg supplementation on home BP and showed small but significant reductions (2.0/1.4 mm Hg on average). Our results also support the usefulness of home and ambulatory BP monitoring, since these methods detected changes in BP of <2 mm Hg in a moderate number of study subjects.

Several mechanisms may be involved in the antihypertensive effect of Mg. Mg ions lower resting levels of intracellular $Ca^{2+}$ by competing with $Ca^{2+}$ for membrane-binding sites and modulating Ca binding and release from the sarcoplasmic reticulum.[1] Thus, it can induce vasodilation as an intracellular Ca blocker. At the cell membrane, $Mg^{2+}$ regulates ion flux through voltage-gated, acetylcholine-activated, $Ca^{2+}$-activated, and ATP-activated $K^{+}$ channels. These actions may also be involved in the cardiovascular effects of $Mg^{2+}$. Cardiac and vascular smooth muscle cells are vulnerable to deficits in extracellular $Mg^{2+}$, and the deficits in $Mg^{2+}$ result in elevation of intracellular $Ca^{2+}$ in these cells.[1]

It has been shown that hypertensive patients have reduced serum and intracellular levels of Mg compared with normotensive subjects.[5 6] In addition to the low Mg intake, various factors such as high salt intake and use of alcohol and thiazide diuretics may also cause the Mg-deficient status by promoting renal Mg excretion.[1] The BP-lowering effect of Mg supplementation was apparent in subjects with low urinary Mg excretion[34] and in subjects receiving long-term diuretic treatment.[10] In the present study, relationships between control levels of serum or urinary Mg and changes in 24-hour BP were not significant, but changes in serum Mg were correlated inversely with the changes in 24-hour BP. Our findings suggest that the actual increase in body Mg is more strongly related to the antihypertensive effect of Mg supplementation than the baseline level of serum or urinary Mg. The changes in 24-hour BP with Mg supplementation tended to be greater in treated than in untreated patients. However, this tendency did not seem to be due to diuretic use because thiazide diuretics were prescribed in only 5 of 40 treated subjects and the changes in BP in these 5 subjects were not marked. Sodium and alcohol intakes did not significantly affect the Mg-induced BP reduction in our study. The absence of severe Mg deficiency in the study subjects may account for the only slight reductions in BP with Mg supplementation and lack of clear association between baseline Mg status and the changes in BP.

The reductions in 24-hour BP with Mg supplementation were correlated with baseline levels of BP in the present study. The ambulatory BP decreased by 5.3/2.7 mm Hg in subjects with higher than average baseline BP, whereas it did not change in those with low baseline BP. Our results were consistent with an earlier study in which Mg supplementation lowered BP in hypertensive patients but not in normotensive subjects.[20] Although the precise mechanisms responsible for the different BP responses to Mg supplementation were not clarified, antihypertensive drugs including Ca antagonists are known to be more effective in patients with higher BP and have little effect on normotensive subjects. Our study suggests that the BP-lowering effect of high Mg intake is enhanced with elevation of baseline BP.

The antihypertensive effect of Mg supplementation was evident in men but not in women in our study. It also tended to be greater in older subjects than in younger subjects. Gender and age are possible determinants of BP response to mineral intake, as shown in the case of dietary Na.[35] However, the influence of gender and age were not significant in multiple regression analysis.

In summary, oral Mg supplementation significantly decreased office, home, and 24-hour BPs in hypertensive patients, and this effect was greater in subjects with higher baseline BP. Our study supports the usefulness of increasing dietary Mg intake as a part of lifestyle modifications in the

management of hypertension. However, the therapeutic value of high Mg intake may be limited because its antihypertensive effect appears to be small.

**Selected Abbreviations and Acronyms**

BP = blood pressure
DBP = diastolic blood pressure
NMR = nuclear magnetic resonance
SBP = systolic blood pressure

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# Magnesium – its role in CKD

Angel L. M.De Francisco[1], Mariano Rodriguez[2]

[1]*Servicio de Nefrología. Hospital U Valdecilla . Santander (España)*
[2]*Servicio de Nefrología. Hospital U Reina Sofía. Córdoba (España)*

**Abstract**

Magnesium containing compounds present promising oral phosphate binders for the treatment of hyperphosphataemia in patients with chronic kidney disease (CKD). However, the impact of magnesium in CKD patients still remains unclear in clinical routine practice. Therefore, this publication provides a practicable overview of knowledge about the physiological role of magnesium in general and in particular in CKD patients. Prevalence of hypomagnesaemia is high in the general population and especially in intensive care unit patients, but often not being detected. Magnesium deficiency increases the risk for several diseases, like diabetes mellitus type 2, hypertension and atherosclerosis. Moderate hypermagnesaemia, however, seems to have beneficial effects on vascular calcification and mortality rates in CKD patients. On the other hand, higher serum magnesium levels are reported to be linked to lower PTH levels and results on the effects on bone are controversial. In addition, low magnesium levels are associated with low bone mass, osteoporosis and vascular calcification. In dialysis patients serum magnesium levels are dependent mainly on the dialysate magnesium concentration. To confirm the potential delay of arterial calcification and improved survival outcomes by long-term intervention with magnesium powered randomized studies are required in dialysis patients. Since a recent trial revealed that a phosphate binder containing a combination of magnesium carbonate and calcium acetate was as effective as the polymer-based agent sevelamer hydrochloride and had an equally good tolerability profile, it is time for a re-examination of the role of magnesium in CKD patients.

**Key Words**: Magnesium, CKD, CKD-MBD, Phosphate, Phosphate binders, Hyperparathyroidism, Vascular calcification.

## INTRODUCTION

Magnesium, the fourth most abundant cation in the body, plays an important role in numerous enzymatic reactions, transport processes and synthesis of proteins, DNA and RNA. In contrast to its physiological role, the clinical importance of magnesium is often underestimated. Disorders of magnesium are hardly mentioned in most educational books of medicine. Furthermore serum magnesium concentrations are not measured routinely in hospitalized patients and thus most magnesium abnormalities are remaining undetected (Whang et al. 1990). During the last 20 years substantial knowledge has been accrued about magnesium and new avenues have been opened for patients. The present publication gives an overview about magnesium metabolism and disorders in magnesium balance, especially in chronic kidney disease (CKD) patients. The role of magnesium-containing phosphate binders in CKD patients is re-examined, since their use offers a chance to circumvent problems associated with other agents of this class. This review represents a brief compendium of the more detailed magnesium supplement written by experienced scientists and clinicians (Luft 2012).

## MAGNESIUM METABOLISM AND PHYSIOLOGY

Magnesium fulfils various intracellular functions. It stabilizes enzymes in many ATP generating reactions, antagonizes calcium in muscle contraction, modulates insulin signal transduction and cell proliferation, and is important for cell adhesion and membrane transport (Jahnen-Dechent et al. 2012). About 99% of total magnesium is located in bone, muscles and non-muscular soft tissue. Extracellular magnesium accounts for about 1% and is primarily found in serum and red blood cells (Elin 1988).

Humans have to consume magnesium regularly to prevent a deficiency. The Institute of Medicine recommends 310–360 mg and 400–420 mg for adult women and men, respectively. Intestine, bone and kidneys maintain magnesium homeostasis (Figure 1): Magnesium is absorbed in the gut, stored in bone and muscles and excreted by the kidneys. Intestinal absorption is dependent on magnesium status: The lower the magnesium level, the more is absorbed. However, regulation of intestinal magnesium uptake is highly complex and still not fully understood. The majority of magnesium is absorbed by a passive paracellular mechanism, which is driven by the electrochemical gradient (Jahnen-Dechent et al. 2012). A minor but important fraction of magnesium is actively transported via transcellular channels (de Baaij et al. 2012). Besides intestinal uptake renal excretion is crucial in maintaining magnesium balance. In kidneys a mechanism comparable to the intestinal uptake regulates magnesium reabsorption. Up to 90% of the filtered magnesium is reabsorbed in a passive paracellular fashion. Fine-tuning takes place in a dedicated part of the nephron, the distal convoluted tubule. Here, magnesium-specific transporters carefully keep magnesium reabsorption within a sizeable range: renal excretion of the filtered load may vary from 0.5%-70%. In moderate CKD (stage 1-3) loss of renal function is compensated by an increased fractional excretion of magnesium, while this mechanism fails in advanced CKD resulting in hypermagnesaemia. In dialysis patients, serum magnesium levels mainly depend on dialysate magnesium and dietary intake.

The most common test for the assessment of the magnesium status is the measurement of serum magnesium levels – a practicable and inexpensive test. However, it should always be kept in mind that serum magnesium levels do not reflect the total body magnesium status.

Reference ranges for serum magnesium levels are 0.65–1.05 mmol/L for total magnesium and 0.55–0.75 mmol/L for ionized magnesium in adult blood serum (Tietz 1990; Mai-Zurawska 1994). The prevalence of hypomagnesaemia in hospitalized patients is high (9-65%; Hashizume et al. 1990) especially in patients on postoperative intensive care units (Chernow et al. 1989). In the general population, hypomagnesaemia frequently occurs in patients with diabetes, chronic gastrointestinal diseases, alcoholism and after the use of certain types of drugs. A small but interesting group of patients have a hypomagnesaemia that is the result of genetic mutations. Specifically, mutations in genes that encode ion transporters in the distal convoluted tubule can explain many familial forms of hypomagnesaemia (de Baaij et al. 2012). In particular mutations in the TRPM6 magnesium channel have been shown to account for the largest part of the genetic forms of hypomagnesaemia. Hypermagnesaemia is often associated with undetected renal impairment and excessive oral administration of magnesium-containing drugs (e.g. laxatives or antacids) or with advanced CKD (Jahnen-Dechent et al. 2012; Xing et al. 2001). Clinical signs of both hypo- and hypermagnesaemia are non-specific, can be similar or even absent. Signs include loss of appetite, fatigue and weakness, later on, as magnesium deficiency worsens, numbness, cramps, seizures, personality changes, arrhythmias and coronary spasms. Severe hypermagnesaemia might lead to depression or loss of deep tendon reflexes, hypotension, gut paralysis and ECG-changes. At very high and very low serum magnesium levels severe neuromuscular dysfunction, hypotonia and even pseudoparalysis, respiratory depression, areflexia and coma may develop.

**SERUM MAGNESIUM AND DISEASE RISK**

Various epidemiological studies demonstrated associations between low serum magnesium levels and an increased risk for metabolic syndrome, type 2 diabetes mellitus (T2DM), hypertension and atherosclerosis (Geiger et al. 2012). However, the use of magnesium as a therapeutic agent is only indicated for pre-eclampsia and specific forms of arrhythmias (figure 2). Cardiovascular disease and T2DM are common comorbidities of CKD and therefore the role of magnesium in these diseases is discussed in more detail.

Hypomagnesaemia was found in 14-48% of T2DM patients versus 3-15% of the healthy control group and has been implicated in adversely affecting several diabetic complications (Pham et al. 2007). A retrospective study with 550 T2DM patients without known kidney disease revealed, that lower magnesium levels were associated with further deterioration of renal function. Patients with higher serum magnesium levels (between 0.82–1.03 mmol/L) had the slowest progression and the best glycaemic control. Therefore these serum levels were suggested as target levels for diabetic patients (Pham et al. 2005). A meta-analysis of seven studies (n=286,668) found, that an increased magnesium intake of 100 mg/day is inversely associated with the incidence of T2DM (Larsson et al. 2007). The authors suggested that magnesium-rich food might reduce the risk for T2DM. Whether patients with established T2DM benefit from additional magnesium supplementation was evaluated in a meta-analysis of nine randomized trials enrolling 370 T2DM patients (Song et al. 2006). Although dosage and inclusion criteria varied and the numbers of patients in single studies were relatively small, magnesium supplementation (median dose of 15 mmol/day) used as an adjunct therapy for 4–16 weeks was found to be significant regarding lowering fasting glucose levels (Song et al. 2006). Thus, daily magnesium supplementation seems to be beneficial in pre-diabetic and T2DM patients.

Also, many cardiovascular disorders are associated with low magnesium levels. The authors of a recent epidemiological study (n=212,157) found that low magnesium levels predicted cardiovascular and all-cause mortality (Reffelmann et al. 2011). They also showed that low serum magnesium concentrations − regardless of other risk factors − were associated with long-term gain of left ventricular mass, a significant predictor for adverse cardiovascular events.

Hypomagnesaemia has also been linked to high blood pressure. In the ARIC study (n=15,248) serum magnesium levels were inversely related to systolic blood pressure (Ma et al. 1995). Further, a meta-analysis of 20 randomized studies (n=1,200) revealed that magnesium supplementation is associated with significant dose-dependent blood pressure reductions (Jee et al. 2002). However, other more recent trials failed to demonstrate blood-pressure-lowering effects of magnesium supplementation alone but suggest that they enhance the effect of antihypertensive medications (Dickinson et al. 2006; Rosanoff 2010).

Atherosclerosis is a well-known risk factor for cardiovascular disease, potentially triggering myocardial infarction and stroke. The pathogenesis of atherosclerosis is complex and like endothelial dysfunction and hyperlipidaemia, hypomagnesaemia has been identified as a major risk factor. According to the follow-up of the ARIC study (n=13,922), patients with the lowest serum magnesium level had the highest risk for coronary artery disease (Liao et al. 1998). Furthermore, Ascherio et al. (1998) found a negative association between dietary magnesium intake and risk of stroke in a prospective study (n=43,738). Inverse associations were all stronger in hypertensive than normotensive men and were not materially altered by adjustment for blood pressure.

Hypomagnesaemia also seems to be involved in the pathogenesis of ischaemic heart disease by altering lipoprotein composition and modifying post myocardial arrhythmia. For this reason, magnesium therapy has been extensively studied in the context of acute myocardial infarction in various clinical trials (Woods et al. 1994, Geiger et al. 2012). However, the conclusion after the last

clinical trials (ISIS 4 Collaborative Group 1995) was that magnesium sulphate cannot be generally recommended for the routine administration after acute myocardial infarction.

**MAGNESIUM IN CHRONIC KIDNEY DISEASE (CKD)**

Although excretion of magnesium via the kidney is highly adaptable, this ability deteriorates when renal function declines significantly (Cunningham et al. 2012). In moderate CKD (stage 1-3), an increase in fractional excretion compensates for the loss of renal function such that magnesium levels are maintained within the normal range (Coburn et al. 1969). Interestingly, there are differences in diabetics and non-diabetics. A significant correlation between a low creatinine clearance and a high serum magnesium was found in non-diabetics but not in diabetics. In these, serum magnesium levels were significantly lower despite reduced creatinine clearances ($p<0.001$; Dewitte et al. 2004).

In more advanced CKD (stage 4-5) renal compensatory mechanisms become inadequate. Hypermagnesaemia develops frequently in patients with creatinine clearances less than 10 mL/min (figure 3). In dialysis patients, magnesium concentrations are dependent mainly on dialysate magnesium. Magnesium crosses the dialysis and peritoneal membrane readily. Various magnesium dialysate concentrations have been investigated in patients undergoing haemodialysis (HD) or peritoneal dialysis (PD) (Table 1). Magnesium dialysate concentrations of 0.75 mmol/L may cause mild hypermagnesaemia, whereas a concentration of 0.25 mmol/L mostly causes hypomagnesaemia. Results for the magnesium dialysate concentration of 0.5 mmol/L are less consistent but magnesium levels are mostly within the normal range. The inconsistent results of several studies suggest that other factors, such as nutrition and magnesium supplements (e.g. antacids), may also play an important role in determining serum magnesium levels in dialysis patients.

One important subject in CKD patients is the relationship of magnesium with parathyroid activity. Multiple abnormalities contribute to the development of secondary hyperparathyroidism (sHPT), a common complication of CKD. Serum calcium, calcitriol, fibroblast growth factor 23 (FGF23) and serum phosphate have key roles in regulating parathormone (PTH) synthesis and secretion (Cunningham et al. 2012). Calcium is the dominant activator of the calcium sensing receptor (CaSR), but magnesium also activates CaSR, though with a potency 2–3fold less than calcium. Even though, serum magnesium levels may have a regulatory role in PTH secretion.

The relationship between serum magnesium and PTH levels in patients undergoing HD or PD were investigated in several studies (Cunningham et al. 2012). Navarro et al. (1999a+b) found a significant inverse relationship between serum magnesium and PTH levels ($p<0.001$) in 110 HD as well as 51 PD patients using 0.5 mmol/L and 0.75 mmol/L magnesium dialysate, respectively. However, most other studies used concomitant changes of magnesium and calcium dialysate concentrations (Wei et al. 2006). Therefore result interpretations are difficult and further research is necessary to elucidate the influence of magnesium on PTH levels independent of calcium changes.

55% of the body's magnesium content is found in bone, the largest magnesium store in the human body. But magnesium only represents a tiny proportion (below 1%) of the total bone mineral that mainly consists of calcium and phosphate in form of hydroxyapatite. Studies about magnesium concentrations in the bone of CKD patients revealed highly variable results indicating that several factors influence the uraemic bone metabolism. The role of magnesium in the pathogenesis of renal bone disease has been studied in recent years. Magnesium seems to be crucial for the regulation of osteoblast and osteoclast activity and bone remodelling. Magnesium deficiency in rat

caused impaired bone growth, osteopenia and skeletal fragility (Rude et al. 1999). Epidemiological studies have linked insufficient magnesium in the diet to low bone mass and osteoporosis (Rude et al. 2009). However, many other factors, like vitamin D status, PTH level, calcium and phosphate concentrations and metabolic acidosis, contribute also to bone metabolism and the exact role of magnesium needs to be investigated in more depth.

**MAGNESIUM AND VASCULAR CALCIFICATION IN CKD**

Cardiovascular disease is the leading cause of death in both CKD and PD/HD patients (Foley et al. 1998). Patients with CKD undergoing dialysis have 2- to 5-fold more coronary artery calcification than age-matched individuals. Calcifications in the intimal and medial vessel layer are major direct or indirect contributors to cardiovascular disease and mortality in CKD patients.

The progressive loss of kidney function is accompanied by elevated FGF-23 levels, a decrease in phosphate excretion and a dysregulation of bone metabolism. Disturbances in mineral and bone metabolism clearly promote vascular calcification. The pathogenesis of vascular calcification is a cell-mediated and actively regulated process that closely resembles the formation of normal bone tissue (Massy et al. 2012). A central role play vascular smooth muscle cells (VSMCs) that compose the medial layer of the vessel wall and converse into osteoblast-like cells – a phenotype that is commonly found in calcified vessels. Abnormalities in mineral metabolism, particularly hyperphosphataemia and the loss of mineralization inhibitors, initially lead to the formation of calcium phosphate nanocrystals that can transform to more organized and stable apatite crystals. Nanocrystals are taken up by VSMCs via endocytosis and initiate the transdifferentiation into osteoblast-like cells, ultimately resulting in vessel calcification.

Several in vitro and animal studies suggest a protective role of magnesium on vascular calcification by multiple molecular mechanisms (figure 4): Firstly, magnesium inhibits the formation of apatite crystals and forms smaller, more soluble deposits (Peters et al. 2001). Secondly, magnesium functions as a calcium antagonist thus inhibiting the entry of calcium into the cells (Altura et al. 1987). Thirdly magnesium restores the balance between the expression of calcification promoters and inhibitors (Kircelli et al. 2011). In addition, magnesium acts on the CaSR and activation of CaSR by calcimimetics has been shown to inhibit VSMC calcification (Ivanovski et al. 2005).

Clinical studies provided evidence for the protective effect of magnesium on vascular calcification. Mitral annular calcification and increase of carotid intima–media thickness were shown to be strongly associated with low magnesium levels in HD patients (Tzanakis et al. 2004; Tzanakis et al. 1997). Long-term magnesium supplementation reduced carotid intima–media thickness and thus may retard arterial calcification in CKD patients (Turgut et al. 2008). Epidemiological studies in HD patients revealed that elevated magnesium levels are associated with survival advantages, whereas low magnesium levels are independent predictors of death (Ishimura et al. 2007). Overall, elevated serum magnesium levels and/or magnesium supplementation may delay arterial calcification and lead to survival advantages in dialysis patients. However, adequately powered randomized studies are required to confirm these results.

**MAGNESIUM AS A PHOSPHATE BINDER**

Declining renal function is associated with increasing phosphate levels and most patients will have hyperphosphataemia from CKD stages 4 onwards. Phosphate binders are needed in CKD patients to prevent hyperphosphataemia leading to disturbed bone and mineral metabolism, cardiovascular disease and sHPT. Phosphate binders have been used for many years, but the ideal binder in terms of efficacy, patient adherence, safety and costs has not been found (Hutchison et al. 2012).

phosphate binders containing aluminium or calcium have well-known drawbacks, like Aluminium-toxicity or increased calcium load causing hypercalcaemia and associations with vascular calcification. Newer agents avoid calcium burden but are very expensive. Magnesium-containing phosphate binders offer a chance to circumvent some of the problems associated with other agents.

Early studies in the 1980s substituted magnesium hydroxide for aluminium, because of its reported toxicity. Magnesium hydroxide was effective in lowering phosphate and PTH levels, but was associated with poor gastrointestinal tolerability. Later studies used magnesium carbonate combined with calcium carbonate or calcium acetate to improve the tolerability. The results of some of the most important recent studies are described below.

**Efficacy of magnesium carbonate in combination with calcium carbonate or calcium acetate**

A randomized parallel-group study compared the efficacy of magnesium carbonate/calcium acetate compared to calcium carbonate monotherapy in 50 HD patients (Deuber et al. 2004). Treatment with magnesium carbonate/calcium acetate resulted in significantly lower serum phosphate, calcium and iPTH levels compared to monotherapy ($p<0.05$ each). Serum magnesium concentrations in the combination group were significantly increased, but remained within the normal range (Deuber et al. 2004).

The CALMAG study, a large randomized multicentre study, compared the efficacy of calcium acetate/magnesium carbonate to sevelamer hydrochloride in 255 HD patients (de Francisco et al. 2010). The primary efficacy endpoint was to show non-inferiority of calcium acetate/magnesium carbonate compared to sevelamer hydrochloride in lowering phosphate levels to below K/DOQI targets after 24 weeks of treatment. This was fulfilled, and there was no significant difference between groups regarding the lowering of serum phosphate levels at the end of the study ($p=0.069$) (figure 5). The area under the curve (AUC) for serum phosphate ($p=0.0042$) and the number of visits above K/DOQI (≤1.78 mmol/L, $p=0.0198$) and KDIGO targets (≤1.45 mmol/L, $p=0.0067$) were significantly lower with calcium acetate/magnesium carbonate compared to sevelamer hydrochloride.

**Safety of magnesium carbonate in combination with calcium carbonate or calcium acetate**

Tolerability, hypermagnesaemia and calcium load are relevant safety parameters of magnesium-containing phosphate binders. The tolerability was shown by different studies. Combined magnesium/calcium carbonate was compared to calcium carbonate monotherapy in a 2-year, randomized, crossover trial in 29 HD patients (Delmez et al. 1996). Serum levels of calcium, phosphate and magnesium were similar in both phases, but the ingestion of calcium was significantly less in the combined phases ($p<0.0001$). In spite of the relative high magnesium intake of 465±52 mg/day (mean±SD), the combined treatment was generally well tolerated: No patients reported any gastrointestinal symptoms including loose stools, diarrhoea or bloating.

A pilot study investigated the calcium load of magnesium/calcium carbonate compared to calcium acetate monotherapy (Spiegel et al. 2007). 30 HD patients were randomized to receive either combination (n=20) or monotherapy (n=10) for 12 weeks. Phosphate control of the combination therapy was at least as good as that of calcium acetate alone, but calcium consumption was significantly lower in the magnesium/calcium carbonate group (908±24 versus 1743±37 mg/day, mean±SD; $p<0.0001$) resulting in significantly lower calcium levels ($p<0.003$). Both regimens were generally well tolerated with a similar incidence of gastrointestinal symptoms.

The CALMAG study compared safety parameters of calcium acetate/magnesium carbonate to sevelamer hydrochloride (de Francisco et al. 2010). Both regimens were equally well tolerated with a similar number of adverse events. No symptomatic hypermagnesaemia was observed. Although a small increase in serum magnesium occurred in the calcium acetate/magnesium carbonate group, the mean concentration was well below possibly symptomatic levels (1.29 ± 0.25 versus >1.5-2.0 mmol/L).

Spiegel et al. (2009) studied the effects on coronary artery calcification (CAC) and vertebral bone mineral density (V-BMD) of magnesium plus calcium carbonate in a small, 18-month, open-label pilot study with 7 HD patients. They found no significant progression of the CAC score and no significant change in V-BMD, suggesting that magnesium may have a favourable effect on these parameters, though the size of the study precludes any firm conclusions and larger studies are needed to confirm these findings.

**Cost-effectiveness**

Healthcare costs become an ever more important factor. Magnesium containing phosphate binders are relatively inexpensive compared with sevelamer- or lanthanum-based agents. Calculated costs for the treatment with magnesium carbonate/calcium acetate were about 80% lower compared to sevelamer hydrochloride (de Francisco et al. 2010). Though magnesium-calcium-based salts have proven efficacy and safety, there are still some concerns about hypermagnesaemia and serum magnesium monitoring. Serum magnesium levels are not routinely measured in many dialysis clinics. Some may argue that routine monitoring is not necessary since the safety margin appears significant, but this is unlikely to satisfy the concerns of nephrologists. The cost of introducing magnesium monitoring would, however, be offset by the lower cost of magnesium-containing phosphate binders over sevelamer or lanthanum, and most modern multi-channel biochemistry analyzers can report magnesium if required without major cost implications(as an example: magnesium in serum (photometric assessment/AAS)—Germany (Synlab, Augsburg): GOÄ 3621 1.00 (GOÄ = Gebührenordnung für Ärzte, private; scale of charges for physicians)= 2.33 €; France (Biomnis, Ivry-sur Seine)= 1.89 €).

**CONCLUSION**

Despite its physiological importance, the clinical role of magnesium in terms of benefits and harms of higher or lower magnesium levels particularly in CKD patients has been underestimated for many years. Disorders of magnesium balance, especially hypomagnesaemia, are common, but hardly identified in routine clinical practice. Furthermore magnesium deficiency was shown to be a risk factor for several common metabolic diseases, whereas magnesium supplementation and mild hypermagnesaemia might have beneficial effects in CKD patients with regard to calcification and mortality. On the other hand, the growing understanding of the effects of hyperphosphataemia has raised the need for efficient and well-tolerated phosphate binders. The combination of calcium acetate and magnesium carbonate presents a phosphate binder, which is at least as effective as sevelamer hydrochloride and equally well tolerated, but saves about 80% of treatment costs. Magnesium levels were only slightly elevated and there was no difference in gastrointestinal tolerability compared to sevelamer hydrochloride. If necessary, routine magnesium monitoring is feasible without major cost implications. The possible beneficial effects of magnesium regarding calcification, PTH-levels and survival in CKD patients on the one hand and the promising results about efficacy, tolerability and cost-effectiveness on the other are potential advantages which make magnesium-containing phosphate binders an attractive treatment option in routine clinical practice. However, more clinical research is needed to confirm and understand the clinical effects of magnesium administration.

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**Key concepts**

- Hypomagnesaemia prevalence is high and associated with several diseases, however it is often undetected.
- Moderate hypermagnesaemia seems to have positive effects on vascular calcification and mortality rates.
- In dialysis patients serum magnesium is highly influenced by magnesium dialysate concentration.
- Mg-carbonate/Ca-acetate is an efficient, safe and cost-effective phosphate binder.

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# Calcium intake in health maintenance – a systematic review

Kirsti Uusi-Rasi,[1,*] Merja U. M. Kärkkäinen,[2] and Christel J. E. Lamberg-Allardt[3,*]

[1]*UKK Institute for Health Promotion Research, Tampere, Finland*
[2]*Academy of Finland, Helsinki, Finland*
[3]*Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland*
[*]***Christel J. E. Lamberg-Allardt,*** *Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 66 (Agnes Sjöbergsgatan 2), FI-00014 University of Helsinki, Finland*
[*]***Kirsti Uusi-Rasi,*** *UKK Institute for Health Promotion Research, P.O. Box 30, FI-33501 Tampere, Finland*

**Abstract**

**Background**

Calcium (Ca) is an essential nutrient for the human body. Despite lively research, there is uncertainty about Ca requirements in terms of desirable health outcomes including an upper intake level above which the potential for harm increases.

**Objectives**

The aim was to conduct a review to update requirements and desirable or harmful health effects of Ca on the current scientific evidence.

**Methods**

We searched Medline and Swemed from January 2000 to December 2011 and included all systematic reviews that reported Ca supplementation or usual Ca intake on health outcomes. Meta-analyses, randomized clinical trials and cohort studies were included in the second search between May 2009 and March 2011 and an additional search covering studies till the end of 2011. This review concentrated on studies reporting independent effects of Ca, although a few recent trials report sole effects of Ca on health outcomes, most trials use Ca in combination with vitamin D vs. placebo.

**Results**

In total, we reviewed 38 studies addressing the effects of Ca on bone, pregnancy-related outcomes, cancers, cardiovascular outcomes, obesity, and mortality. There was a lot of heterogeneity in the study protocols, which made it difficult to draw any strong conclusions. According to the literature, high Ca intake seems to have a small positive effect on bone mineral content (BMC) or bone mineral density (BMD) in children and postmenopausal women. We did not find any consistent evidence on the effects of Ca on bone health in premenopausal women or men. Also, the evidence that Ca supplementation reduces fracture incidence is scarce and inconsistent. Maternal diet may influence the peak bone mass of offspring but more studies are required. There was no overall effect of Ca intake on cancers. Ca was associated with a decreased risk of breast cancer and a slightly increased risk of prostate cancer in two of the three studies. No associations were found with other cancers. We found no consistent association between cardiovascular outcomes and Ca

intake except for blood pressure. A small decrease of 2–4 mmHg in systolic blood pressure was found in pregnant and in hypertensive subjects with Ca supplementation. Reviewed studies did not show consistent evidence relating Ca intake to either mortality or obesity.

**Conclusion**

Based on this evidence, there is no need to change the Nordic recommendations for Ca intake. However, due to heterogeneity in the studies it is difficult to interpret the results and provide single summary statement.

**Keywords:** Calcium intake, calcium requirements, disease prevention, osteoporosis, bone mineral density, health maintenance, health outcomes

**Aims**

The overall aim was to review the recent scientific data on requirements and health effects of calcium (Ca) to update the current dietary reference values valid in Nordic countries.

The specific objectives of the review on health effects on Ca in human nutrition are as follows:

1. Review the scientific evidence to determine, based on a set of agreed criteria, dietary reference values for Ca for different life stages (infants, children, adolescents, adults, elderly, and during pregnancy and lactation);
2. Assess the requirement of Ca for adequate growth, development, and maintenance of health; and
3. Assess the health effects of different intakes/exposures of Ca.

**Scientific background**

At full-term birth, the human infant accrues about 26–30 g of Ca, most of which is present in the skeleton as calcium hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$), which provides the rigidity necessary for the skeleton to function mechanically. When the Ca transfer from the placenta ceases at birth, the newborn infant is dependent on dietary Ca. Ca is an important regulator of several body functions, such as muscle contraction, function of the nervous system, and blood clotting. Due to its vital importance, Ca concentration in intracellular and extracellular fluid is tightly regulated. Bone tissue serves as a reservoir and as a source of Ca for these critical metabolic needs through the process of bone remodelling. The most important regulators of Ca metabolism (Fig. 1) in humans are parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D).

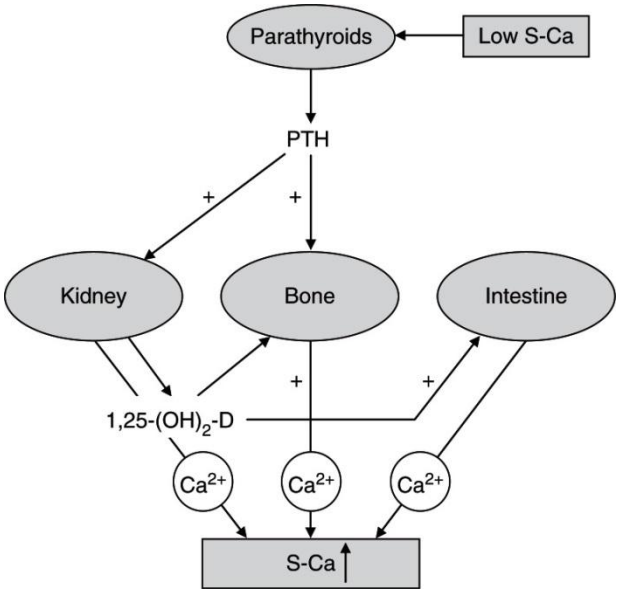


Fig. 1 - Endocrine feedback system that maintains serum calcium concentrations: involvement of $1,25(OH)_2D$ and parathyroid hormone (PTH).

Bone is a dynamic and metabolically active tissue; it is constantly renewed at an average rate of 8–10% per year, and the body's need for Ca relative to skeletal growth and remodeling varies by life stage. The major physiological activities include bone accretion during skeletal growth and maintenance of bone mass after growth is completed. Ca balance studies have shown that Ca retention is significantly higher in adolescents than in adults with the same Ca intake. Later in adult life, net Ca is lost from the body when bone formation no longer balances with bone resorption. There are two different types of bone tissue, which are formed with the same cells and matrix elements, but they differ in their structure and function. The peripheral skeleton constitutes 80% of the skeletal mass, and is composed primarily of cortical bone. About 70% of the central skeleton is, in turn, trabecular bone. Trabecular bone, with its greater surface area, is metabolically more active and is thus more responsive to changes in mineral homeostasis, whereas the cortical bone fulfills mainly the mechanical and protective functions. In adults, bone modeling occurs less frequently than bone remodeling, particularly in trabecular bone. While the turnover rate of 2–3% per year in cortical bone is consistent with maintenance of mechanical properties, the turnover rate in trabecular bone is much higher.

Dietary calcium is classically associated with dairy products, and food supplies such as milk, yoghurt, and cheese are rich sources of Ca, providing the majority of Ca in the general diet in the Scandinavian countries. In Finland (2) and Sweden (3), milk products provide more than 60% of the dietary calcium intake and in Norway (4) approximately 70%. The mean intakes in adult women in these countries vary from 800 to 1,000 mg/day and in men from 1,000 to 1,200 mg/day.

**Research/key questions for calcium**

1. What is the relationship between Ca intake and different outcomes in different population and age groups?
2. What is the relationship between Ca intake and well-established markers of different functional or clinical outcomes in different population and age groups?
3. What is the effect of supplemental Ca on different outcomes in different population and age groups?

4. What is the effect of Ca intake from different sources on Ca metabolism in different population and age groups?
5. Which is the interaction of Ca intake from different sources with iron intake or iron status on health outcomes in different population and age groups?
6. Which is the UL (tolerable upper intake level) for Ca intake for different health outcomes in different population and age groups?

**Methods**

**Definitions**

This review focuses on publications reporting independent effects of Ca, although a few recent trials report sole Ca effects on health outcomes, most trials use Ca in combination with vitamin D vs. placebo.

The *indicators of exposure* were dietary calcium, fortified foods, and supplementation exposure.

Publication types: Questions 1–4: SRs, Cochrane database systematic reviews, meta-analyses ; Question 5: cohort studies (longitudinal or prospective studies), intervention studies; Question 6: for adverse effects: randomized controlled trial s(RCTs). Time frames for search : January 2000–April 2009; May 2009–June 2010; and July 2010–December 2011.

The following outcome measures were included:

- Pregnancy outcomes and growth
- Bone health
    - All fractures
        - Hip fractures
        - Vertebral fractures
    - BMD/osteoporosis
    - Bone mass
    - Bone quality
- Muscle strength
- All cancers
- Breast cancer
- Colorectal cancer
- Prostate cancer
- Autoimmune diseases
- Diabetes type II
- Obesity/weight control
- Total mortality
- Cardiovascular disease clinical outcomes

The search terms are defined in Appendix A.

The following *life stages* were included: infants, children, adolescents, adults, elderly, and pregnancy and lactation.

The population groups in the search were primarily Caucasian.

**Search methods and terms**

Two expert reference librarians designed and conducted the electronic search strategy based on the research questions provided by the three investigators. The following electronic databases were searched: Medline and Swemed. The search was conducted using medical subject heading terms (MESH; see Appendix A). The search was done in two batches, the first covering January 2000–April 2009 and the second May 2009–June 2010 and July 2010–February 2011. A complementary search was done at the end of January 2012 covering the period between the first searches until the end of December 2011. The search is documented in Appendix A. In the first search, the investigators focused only SRs and in the second SRs and randomized control trials, published after May 2009, except the study question 5 where all types of studies were included due to the less number of hits.

Furthermore, we used snowballing for SRs and RCTs, which was not in the original search.

**Selection of articles/studies, data collection, and analyses**

The investigators screened all abstracts individually from the searches, and later all the three investigators made a common decision on the full-text articles that had been required from the librarian. From the batches of full-text articles, we included those that met the criteria for SRs. In addition regarding study question 5, RCTs, intervention studies, and cohort studies were included. The full-text articles were examined individually and the three investigators made a common decision on which articles should be included and which to be excluded. Eligible criteria for full-text articles were SR, matching the research questions and healthy population (not patients or medication). In the case of clinical studies and cohort studies, only studies from Europe, Australia, and North America were included. We recorded the reason for rejection of all full-text articles (Fig. 2).

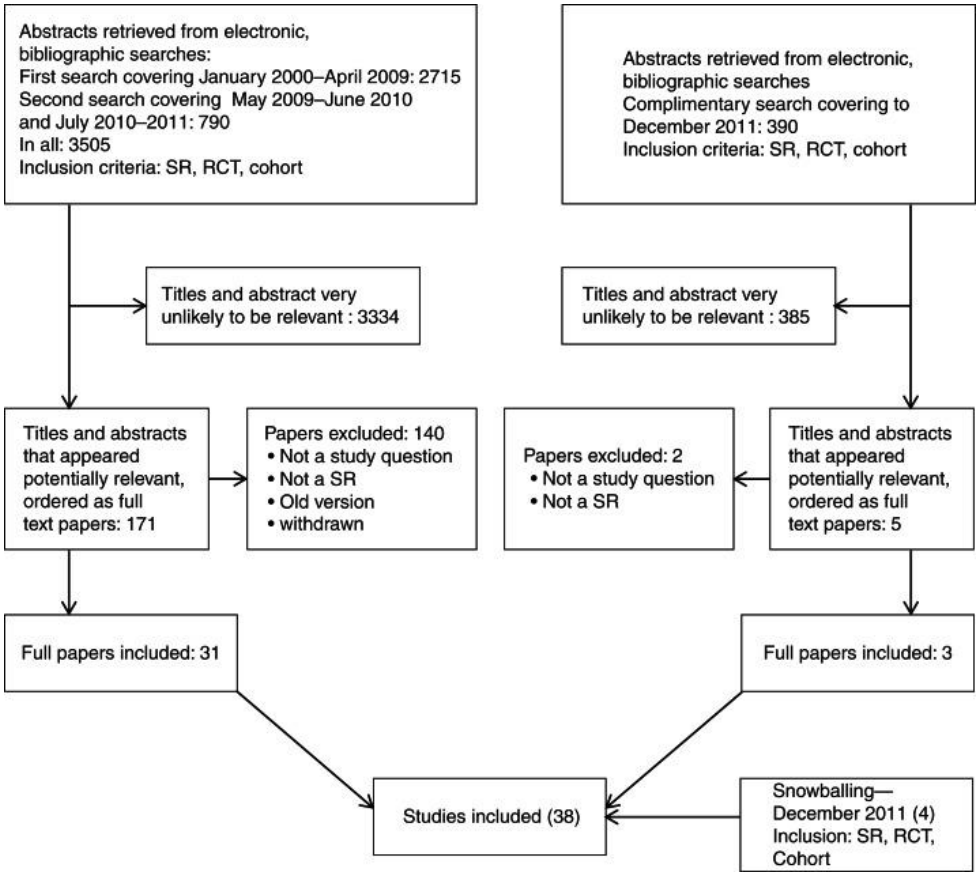


Fig. 2 - Flow chart of the study selection.

Three authors first independently assessed trial quality and extracted data including adverse events.

**Quality assessment of studies**

The results of systematic *reviews and meta-analyses* were quality assessed and evaluated using the NNR5-modified AMSTAR quality assessment tolls and incorporated in the evidence tables. Quality assessment of the RCTs and cohort studies was done according to the NNR guidelines.

**Participants**

We focused on populations in Europe and North America. However, if other populations were included in the SRs, we were usually not able to separate them.

**Exposure**

Dietary calcium or calcium supplements such as calcium carbonate, calcium citrate, calcium chloride, or calcium phosphates.

**Study quality**

There was a lot of heterogeneity in study subjects, methods, and protocols, making it difficult to interpret the results and provide single summary statement. The sources and dose of Ca varied widely among the studies, as well as the methods used to assess the amount consumed. For many risk factors, the quality and strength or direction of association varies among studies. Study cohorts were mainly Caucasian participants.

**Publication bias**

Publication bias cannot be ruled out, since relevant studies were searched by the electronic databases, such as Medline and Swemed, unpublished or ongoing studies were not identified. The search was to the end of December 2011.

**Reporting and summarizing the evidence**

The evidence is summarized in the evidence tables, Appendix C, tables 1–6. The included and excluded studies are listed in Appendix B.

**Effects of Ca exposure on outcome measures**

**Health outcomes**

Characteristics of the included studies for each health outcome are shown in Evidence Tables 1–6.

**Bone health**

The results of bone health are shown in Evidence Table 1.

**Calcium accrual and bone health in childhood**

**Calcium accrual**

Since more than 99% of body Ca is present in the skeleton, an adequate Ca intake during the growth period may be critical in maximizing BMC. In their longitudinal study, Vatanparast et al. (5) reported the average accumulation of Ca (standard deviation, SD) in adolescent Caucasian boys and girls (9–18 years). Boys accrued 198.8 (74.5) g bone mass (BMC) per year, equivalent to 175.4 (65.7) mg calcium per day with the maximum accrual of 335.9 g from age 13 to 14. Girls accrued 138.2 (64.2) g BMC per year, equalling 121.8 (56.6) mg calcium per day with the maximum annual BMC accrual of 226.0 g from age 12 to 13.

**Bone health**

We found only one SR (graded A) and one meta-analysis (graded C) addressing bone health in children. We did not find any data reporting effects of Ca supplementation on bone fractures as an outcome in children.

A systematic review of Winzenberg et al. (6) included RCTs of Ca supplementation either with Ca supplements or dietary Ca compared with a placebo, with a treatment period of at least 3 months. The participants were healthy children aged 3–18 years, and bone outcomes were measured after at least 6 months of follow-up. Nineteen RCTs (*N*=2,859 participants, out of them 1,367 were randomized to supplements, 1,426 to placebo) met inclusion criteria. Ca supplementation doses ranged from 300 to 1,200 mg/day using calcium citrate, calcium carbonate, calcium phosphate, calcium lactate gluconate, calcium phosphate milk extract, or milk minerals as a source of Ca. None of these studies used dairy foods as a supplement.

There was no statistically significant effect of Ca supplementation on BMD] ($mg/cm^2$) at the femoral neck (10 studies) or lumbar spine (11 studies) standardized mean differences (SMD; 95% CI) being 0.07 (from -0.05 to 0.19) and 0.08 (from -0.04 to 0.20), respectively. However, there was a small effect on total body BMC (g) (9 studies) SMD being 0.14 (0.01–0.27), as well as on upper limb density (12 studies) (0.14; 0.04–0.24). This effect is comparable to about 1.7% greater increase in supplemented groups. Only the effect in the upper limb persisted after supplementation ceased (0.14; 0.01–0.28). In the subgroup analyses, treatment effects during supplementation were greater at all sites in females than males, though not statistically significantly. Baseline Ca intake, physical activity, pubertal stage, type of supplementation (milk extract or other), duration of supplementation, and exceeding the Ca threshold did not significantly modify the effect. They concluded that although there was a small effect of Ca supplementation in the upper limb, the increase in BMD is unlikely to result in a clinically significant decrease in fracture risk.

Huncharek et al. (7) did a meta-analysis evaluating the effects of calcium/dairy supplementation on bone health using BMC (g) as the primary outcome. Initial pooling of 12 RCTs (*N*=2,460) yielded a summary mean difference (95% CI) in BMC between the Ca supplemented and placebo arms of 2.05 g (from -3.26 to 7.36 g). Owing to the heterogeneity of the pooled data, the calculated summary mean difference in BMC is of dubious validity. When the papers with low baseline Ca intake were pooled (three studies), a summary mean difference was 49.9 g (24.0–76.6 g) in total body BMC. Four RCTs contained data on lumbar spine BMC, but due to substantial differences across these reports, the authors could not further characterize the effect of Ca supplementation at the lumbar spine. They concluded that increased calcium/dairy intake significantly increases total body and lumbar spine BMC in children with low baseline intakes. However, it is improbable to demonstrate significant differences between groups when both groups are receiving physiologically adequate amounts of Ca.

In conclusion, there was a small effect of Ca supplementation in the upper limb BMD and total body BMC. From a physiological point of view Ca is essential for the skeleton. Other factors, such as

vitamin D, may be crucial in the assessment of the role of Ca intake. Adequate vitamin D intake may be a crucial factor when assessing the role of Ca intake.

**Bone health in pregnancy and fetal growth**

We did not find any SR regarding Ca intake in pregnancy, but one RCT (graded C) and one longitudinal follow-up study (graded C).

In addition to genetic and lifestyle factors, adequate maternal nutrition during pregnancy may be an important contributor to fetal growth and bone health. In an RCT, healthy primiparous women (<20-week gestation) with Ca intake below 600 mg/day received either a Ca supplement of 1,500 mg/day (*N*=231) or a placebo (*N*=230) (8). There were no bone mass or density measurements of either the mother or her offspring. However, in addition to other biometric measurements, femoral and humeral diaphysis length of the fetus was measured. No differences were found in fetal biometric measurements between the supplemented or placebo groups. Similarly, neonatal characteristics and anthropometric measurements at delivery were comparable in both groups. They concluded that Ca supplementation of 1,500 mg/day in pregnant women with low Ca intake did not appear to impact on fetal somatic or skeletal growth.

A longitudinal follow-up of a Tasmanian birth cohort, mainly of Caucasian origin, was used to describe the association between maternal dietary intake in the third trimester of pregnancy and bone mass in their offspring at the age of 16. Maternal Ca intake was positively associated with lumbar spine BMD, but no association was found between femoral neck and total body bone mass. After including all significant nutrients into the same multivariate regression model, Ca intake was no more significant (9).

**Bone health in adulthood**

Optimally, after puberty and throughout most of adulthood, bone formation and resorption are balanced. During this period, bone mass is consolidated, and Ca requirements are relatively stable. Peak bone mass, the maximum amount of bone that can be accumulated, is reached in early adulthood (10, 11). The ability to attain peak bone mass is affected by genetic background and by lifestyle factors, such as physical activity and total Ca intake. Bone is a dynamic tissue, and a number of clinical studies suggest that increasing bone mass early in life has a transient effect, but does not confer protection against later bone loss and osteoporosis (12). The Ca content of bone at maturity is approximately 1,200 g in women and 1,400 g in men (13, 14). In men, this level remains relatively constant until the onset of age-related bone loss later in life, and in women until the onset of menopause. Caucasian women appear to lose as much as 3–10% of their trabecular bone per year during the first few years after menopause and about 1% of their cortical bone per year during the first decade after menopause. After this accelerated bone loss period, the loss again levels off during the postmenopausal years. Lifetime losses may reach 30–40% of peak bone mass among women and 20–30% among men (15).

**Bone health in premenopausal women**

Data are scarce with respect to bone health (mass and fractures) in premenopausal women. We did not find any SR data from this millennium reporting effects of Ca supplementation on premenopausal women's bone health.

**Bone health in postmenopausal women**

Postmenopausal women are the most common target populations for research regarding bone health. We found one SR (graded C) and two meta-analyses (graded A and C).

The SR by Waugh et al. (16) evaluated risk factors for low BMD among 40- to 60-year-old women. They found an inconsistent evidence of an association between the current Ca intake and BMD (four studies) and insufficient evidence of an association between the past Ca intake and BMD (one study). None of these studies were intervention studies, and Ca intake was self-reported. They concluded that evidence of an association between the current Ca intake and BMD was inconsistent and insufficient for past Ca intake and BMD.

The meta-analysis of Shea et al. (17) included 15 RCTs with Ca supplementations (*N*= 1,806, out of them 953 were Ca supplemented). The pooled mean (95% CI) difference in bone loss between the supplemented and placebo groups in percentage change from baseline was 1.66% (0.92–2.39%) for the lumbar spine (nine studies), 1.64% (0.70–2.57%) for the hip (eight studies), and 1.91% (0.33–3.50%) for the distal radius (six studies) favoring the Ca supplementation. They concluded that Ca supplementation alone had a small positive effect on bone density.

Chung et al. (18) included in their large review the Ottawa EPC report (19) and did an update search of RCTs published after that. The review did not separate Ca trials from Ca in combination with vitamin D trials. The Ottawa EPC report concluded that overall, there is good evidence that combined vitamin $D_3$ and Ca supplementation resulted in small increases in BMD of the spine, total body, femoral neck, and total hip. The update search of Chung et al. (18) did not find evidence to change this conclusion, although quantitative synthesis was limited due to variable treatment durations and BMD sites.

**Bone health in men**

The data evaluating the effect of Ca intake on bone health in men are scarce. We found one SR (graded C) of Papaioannou et al. (20). They included 14 studies in their assessment of BMD and Ca intake in men (5 longitudinal and 9 cross-sectional studies). Cross-sectional studies showed inconsistent evidence. Five studies showed a positive association between Ca intake and BMD of the lumbar spine and hip, whereas four studies did not find an independent association between Ca intake and BMD at these bone sites. Also, the five longitudinal studies showed inconsistent evidence. In two studies, Ca intake was associated with bone loss. In particular, men in the lower quartiles of Ca intake (<1,100 mg/day) had approximately double the rate of bone loss. On the other hand, three studies did not predict bone loss.

Men with low Ca intake were underrepresented in these studies. When the participants were classified into quartiles by dietary Ca intake, the limit of the lowest quartile was <1,100 mg/day, which is mainly seen to be a sufficient Ca intake. The authors concluded that the existing data were inconsistent and did not show that Ca intake (dietary or supplements) could predict bone loss.

**Fractures**

We found one SR (graded A), three meta-analyses (one graded A, two graded C), one cohort study (graded C), and one cross-sectional study (graded C) relating Ca intake and fractures.

Shea et al. (17) found only five RCTs that measured fracture rate in a meta-analysis from 2002. The study found no significant reduction in either vertebral fracture risk ratio (RR, 95% CI) being 0.77 (0.54–1.09) or in non-vertebral fractures, RR 0.86 (0.43–1.72). They also concluded that the magnitude of reduction in fracture risk with Ca supplementation alone remains an open question.

Tang et al. (21) reported in their meta-analysis whether Ca or Ca in combination with vitamin D prevents fractures and bone loss. Twenty-nine studies were included in the analyses, out of them 17 reported all types of fractures as an outcome and 24 reported BMD. In total, 64,897 individuals 50 years or older, of them 92% women, were included. In 16 trials, participants received Ca-only supplements. Effect of Ca only on fracture risk reduction (RR; 95% CI) was 0.90 (0.80–1.00). The difference in RR between Ca-only supplementation and Ca with vitamin D combination was very small (0.90 vs. 0.87) and was not significant. The treatment effect was greater in participants whose daily intake was low (less than 700 mg/day). They concluded that the fracture risk reduction was greater in the trial in which the compliance rate was high. In addition, the treatment effect was better with Ca doses of 1,200 mg/day or more.

Instead of all types of fractures, Bischoff-Ferrari et al. (22) assessed in their meta-analysis the relationship of Ca intake to the risk of non-vertebral and hip fractures. In the included five RCTs, Ca supplementation varied between 800 and 1,600 mg/day and was compared with the placebo. Among 6,740 participants (5,666 primarily postmenopausal women and 1,074 men), the pooled risk ratio (RR; 95% CI) for non-vertebral fractures was non-significant, 0.92 (0.81–1.05). The pooled risk of four RCTs with separate results for hip fracture (*n*=6,504, 139 hip fractures) was 1.64 (1.02–2.64). Pooled results from seven prospective cohort studies (*N*=170,991 women, 2,954 hip fractures) found no association between Ca intake and hip fracture risk (RR per 300 mg total Ca per day=1.01; 0.97–1.05). They concluded that Ca intake was not significantly associated with hip fracture risk in women or men based on pooled results from prospective cohort studies. The pooled results from RCTs showed no reduction in hip fracture risk with Ca supplementation, and an increased risk was possible.

The Ottawa EPC report (19) was included in the SR of Chung et al. (18). The authors concluded that Ca supplementation in combination with vitamin D (most studies used $D_3$) is effective in reducing fractures and falls in institutionalized populations, but there is inconsistent evidence in reducing falls in postmenopausal women and older men. The update search of Chung et al. (18) identified one new RCT of female Navy recruit, aged 17–35, which showed that vitamin D (800 IU/day) in combination with Ca (2,000 mg/day) supplementation can reduce the risk of stress fractures from military training compared to placebo.

In a Swedish cohort study, Warensjö et al. (23) evaluated the association between Ca intake and risk of fractures (*n*=61,433 women born between 1914 and 1948) and osteoporosis (subcohort of 5,022 women). The participants were divided into quintiles by Ca intake, and the third quintile was used as a reference group (range of mean Ca intake: 882–992 mg/day). The HR for the first event of any fracture was 1.18 (1.12–1.25) and for hip fracture 1.29 (1.17–1.43) in the lowest quintile (Ca intake<751 mg/day). The risk for osteoporosis was also higher the OR being 1.47 (1.09–2.00). The highest quintile of Ca intake did not further reduce the risk of fractures of any type, or of osteoporosis, but was associated with a higher rate of first-event hip fracture (HR 1.19; 1.06–1.32).

In their cross-sectional study, Zhong et al. (24) evaluated the association of Ca intake and dietary protein intake with risk of fractures in postmenopausal women aged 50 years or older. They did not find a relationship between total Ca intake and risk of fractures. However, the cross-sectional nature does not permit assessment of causality, and establishing the effect of Ca supplementation on fractures would require large, relatively long trials measuring fracture incidence.

**Pregnancy-related outcomes**

We found two high-quality and one good quality systematic reviews on the effects of Ca during pregnancy (Evidence Table 2). The other included studies, one RCT and one cohort study, had

serious flaws in their study design. Only the results of at least good quality studies are reviewed here.

**Offspring**

Buppasiri et al. (25) reviewed 21 RCTs involving 16,602 pregnant women. They found a significant increase of approximately 65 g (95% CI 16–114 g) in birth weight in children whose mothers had used Ca supplements during pregnancy compared to babies of non-users (19 trials, 8,287 women; Evidence Table 2). No significant differences were, however, found in infant with low birth weight between the two groups.

In the systematic review by Bergel et al. (26) of two RCTs and three observational studies, no consistent effect was found on the blood pressure of infants. However, in children aged 1–9 years, higher maternal Ca intake (dietary or from supplements) was associated with lower systolic blood pressure (mean: 1.92 mmHg, 95% CI 0.71–3.14 mmHg). No association with diastolic blood pressure was found. This finding was supported by another systematic review of Hofmeyr et al. (27), which found that the risk of childhood systolic blood pressure greater than 95th percentile was reduced (514 children: RR 0.59, 95% CI 0.39–0.91).

**Mother**

Hofmeyr et al. (27) reviewed 13 randomized trials comparing at least 1 g daily of Ca during pregnancy with a placebo. They found that the risk of high blood pressure was reduced by 35% (12 trials with 15,730 women, RR=0.65, 95% CI 0.53–0.81), the effect being greatest in women with low baseline Ca intake. In four small trials among women at high risk of developing pre-eclampsia (568 women: RR 0.45, 95% CI 0.24–0.83), the risk of pre-eclampsia was reduced by 55%.

The results on preterm birth were inconsistent. Hofmeyr et al. (27) reported that the average risk of preterm birth was reduced in the Ca group overall (11 trials, 15,275 women: RR 0.76; 95% CI 0.60–0.97). In a systematic review by Buppasiri et al. (25), there were no statistically significant differences between women who received Ca supplementation and those who did not in terms of reducing preterm births (less than 37-week gestation) (RR 0.90; 95% CI 0.73–1.11; 12 studies, 15,615 women; random-effects model) and also in less than 34-week gestation (RR 1.11; 95% CI 0.84–1.46; three trials, 5,145 women).

**Lactation**

We did not find any SR regarding relationships between Ca intake and lactation.

**Cancer**

Nine studies (five SR, one meta-analysis, three cohort studies) with cancer as an outcome were included (Evidence Table 3). Of these 9 studies, 2 SRs were ranked as high quality and three cohorts as good quality studies. Ca intake did not have a consistent association with different types of cancers; some of the studies showed protective effect and in some studies Ca was associated with an increased risk of some type of cancers.

In the three studies of which one was meta-analysis (28), one systematic review (18) and one cohort study (29) having *breast cancer* as an outcome, Ca intakes were associated with a lower risk of breast cancer (28) in premenopausal women only (18) and tended to have an association in the third study (29).

Ca intake was associated with lower risk of *recurrence of colorectal adenomas* in one high-quality SR (30) and in another SR (31) but not with the risk of *colorectal cancer* in SR (30) in the general population.

High Ca intake was associated with slightly increased risk of *prostate cancer* in two of the three studies (18, 32, 33). Only one of these studies was ranked high quality.

No significant associations were found with *endometrial cancer* (34), *lung cancer* (35), or *total cancer* (18).

**Cardiovascular outcomes**

Thirteen studies (seven SRs, three RCTs, three cohort studies) addressed different types or cardiovascular outcomes (Evidence Table 4). Three of the SRs were ranked giving high-quality evidence; one SR, one RCT, and one cohort were ranked as good quality studies. No consistent evidence of Ca intake with other cardiovascular outcomes other than systolic blood pressure was found in these studies.

Ca intake or Ca supplementation was not significantly associated with increased or reduced risk of *aortic valve calcification*, *coronary artery calcification* (CAC) (36), increased *serum lipids* (36, 37), *stroke or death* (36, 38) or *cardiovascular disease mortality* (39), *atherosclerosis vascular disease* (40), *cardiovascular events* (41) or *abdominal aortic calcification or coronary aortic calcification* (42). In one study (43), Ca supplementation was associated with *increased risk of myocardial infarction* but not in the others (40, 41). In the only included study that had diabetes as an outcome (44), the higher Ca intake was associated with reduced risk of type 2 diabetes.

The cardiovascular outcomes with Ca intake during pregnancy are reported in pregnancy-related outcomes.

Ca supplementation lowered *systolic blood pressure* with 2–4 mmHg in hypertensive and in pregnant women (18, 27, 45) in three high-quality SRs. One RCT found a small effect on blood pressure in those with below median Ca intakes (37).

**Obesity and control of body weight**

Three systematic reviews, of these two high quality (18, 46, 47) and two randomized clinical trials (37, 48) with body weight and/or obesity as an outcome, were included in this review (Evidence Table 5). No consistent effect was found. In only one SR (seven RCTs, 794 subjects) (47), a mean difference of −0.74 and −0.93 kg body fat favoring higher Ca intake was found. The studies reviewed did not show convincing evidence or a favorable effect of Ca intake on body weight.

**Total mortality**

Mortality as an outcome was assessed in five studies (two SRs and three cohort studies), of which one SR was ranked high quality (Evidence Table 6). No consistent effect was found of Ca intake on death of various causes. The results varied from increased risk of *death of cardiovascular causes* (43), to no effect (18, 43), to about 10–25% decreased risk *all-cause death* (38, 39, 49) with higher Ca intake.

**Upper intake level**

We did not find any dose–response data to evaluate the safety limits of upper intake level. We included only clinical outcomes, such as all-cause mortality, cancer, and cardiovascular events. The results of these outcomes have been described in previous sections. Briefly, we did not find that calcium/dairy intake is associated with an increased risk of mortality. On the contrary, all-cause mortality was lowest in those with the highest use of Ca (38, 39, 49). The results with relationship to cancer showed either no relationship or high Ca intake having a protective association. Prostate cancer was an exception with inconsistent results. However, observational studies suggested an increased risk of prostate cancer among men with high daily Ca intake of more than 2,000 mg (32, 33).

**Discussion**

The aim of this review is to find a scientific base for a Nordic recommendation for dietary intake of Ca. We analyzed the literature on the relationships between Ca intake and different health outcomes. We focused on published systematic reviews but included a few RCTs and cohort studies. Some of the SRs included observational studies as well as RCTs. The quality of the SRs varied from A to C, while none of the other type of studies reached the score of A.

**Main findings in relationship to the research questions**

**Dose–response assessment including exposure range**

The difference in Ca intake between the studies, some estimating total Ca intake, some concentrating on supplemented Ca, and some neglecting the supplemental use assessing only dietary intake, complicates the interpretation of Ca effects. Also, the methodology of assessing dietary Ca intake varies widely from dietary records of several days and validated food frequency questionnaires (FFQs) to enquire the use of milk in general. Amount of milk and milk products, the richest and most important Ca source in Nordic countries, is fairly stable simplifying the estimation of Ca intake, which is still challenging. Although the participants are willing to record their diet to the best of their ability, there may be a discrepancy between actual and reported Ca intake. The most common method is an FFQ with varying number of items on the list. If the FFQ is validated for the study at issue, it mainly works well, but often the FFQ is done for different purposes, for different age groups or even for different ethnic groups. Single 24-h recalls have a problem, because individual estimates may not represent usual intake.

The use of terminology is not always clear. Some studies use the term Ca supplementation but also include the use of vitamin D in combination with Ca supplements. Thus, it is not possible to evaluate the sole Ca effect. Owing to increasing interest in vitamin D and health, recent studies focus on the use of Ca in combination with vitamin D, or the comparison is done between Ca and CaD without pure placebo groups. Self-reported uses of Ca supplements may have been asked with one question without any further enquiry about name or quantity of the ingredient.

**Health outcomes**

**Bone health**

Methodological inadequacies are the biggest limitation in evaluating factors affecting bone health. Dual energy X-ray absorptiometry (DXA) provides a reasonable overall description of bone density, which is a measure of mineral content of the bone, but overlooks geometric alterations that can influence bone strength. However, it is ultimately the whole bone structure, that is, its shape and size, mean cortical thickness, cortical, and trabecular density not its mass, mineral content, or areal

density (BMC or BMD) that determines bone mechanical competence. Although BMD correlates directly with the change in bone mass, it does not take into account possible dimensional, material, or structural properties of bone, and is subject to inherent uncertainty (50, 51). A consideration of geometric properties emphasizes that both the amount and location of bone mineral are important. Age-related loss of bone mass is not necessarily accompanied by a proportionate age-related loss of bone strength because geometric adaptation may compensate for the loss. Age-related cortical thinning of long bone shafts, for example, is associated with increased bone girth, a compensatory change that tends to increase structural rigidity (52).

Although BMD measured with planar DXA is the most prevalent outcome in bone research, it does not represent well all determinants of bone geometric properties. However, thus far there is limited research evaluating effects of dietary or supplemental Ca on bone strength or structure.

In their reviews among children, Winzenberg et al. (6) included studies with Ca supplements, while Huncharek et al. (7) reviewed works with dietary Ca and dairy products. Ca intake affected bone mass positively, and although the effect was small, Ca intake was crucial in childhood and youth. Furthermore, the benefit was especially seen in children with low baseline Ca intake. It is likely that Ca intake is a necessary but insufficient condition for the development of a strong skeleton. However, the results do not support the general use of Ca supplementation in healthy children. Adequate vitamin D intake may have an essential factor when assessing the role of Ca intake. There are also some findings that low Ca intake is more harmful when associated with low vitamin D status (53).

Most studies on the effects of Ca intake on adult bone mass concerned postmenopausal women. We were not able to find any recent SRs about Ca intake and bone health in premenopausal women after the review of Welten et al. from the year 1995, where they analyzed the relationship between Ca intake and bone mass among young adults between 18 and 50 years of age (54). They included 33 studies and only 4 of them were intervention studies. The cross-sectional studies in women suggested a positive association between Ca intake and bone mass. Also, the few intervention studies found a consistent positive effect of a Ca supplement of about 1,000 mg/day on bone mass. These studies suggest that Ca intake should be at least 800 mg/day to optimize bone mass.

Due to high heterogeneity, inconsistent results, lack of RCTs, and limited number of other kind of studies (one SR included five longitudinal and nine cross-sectional studies), it is not possible to make any conclusions in the case of men.

In postmenopausal women, a positive treatment effect on BMD was evident in most studies. Ca supplementation had a relatively small, but possibly meaningful, effect in reducing bone loss (17). Ca in combination with vitamin $D_3$ supplementation resulted in small increases in BMD of the spine, femoral neck, and total hip. Based on included trials, it was less certain whether vitamin D3 supplementation alone has a significant effect on BMD (18, 21).

**Fractures**

The evidence that Ca supplementation reduces fracture incidence is less and inconsistent. Pooled results from cohort studies did not find significant associations between the total Ca intake and hip fracture risk in women or men (22). Also, a longitudinal follow-up of a Swedish cohort showed increased risk of fragility fractures and osteoporosis in women with Ca intake below 751 mg/day. In that cohort, women with the highest Ca intake (>1,137 mg/day) had increased risk of first-event hip fracture (23). On the other hand, in the meta-analysis of Tang et al. (21), the treatment effect was

better with doses at or above 1,200 mg/day than with lower doses, and in individuals who were elderly, had low dietary Ca intake (<700 mg/day), or were compliant with Ca supplementation (>80%) (21). However, participants were mainly women, and the data are limited for men. Furthermore, Ca supplementation with vitamin D may be effective in reducing fractures in institutionalized populations although the influence remains controversial in general population (18).

As a general conclusion, Ca is essential for bone health. Although Ca is only one factor contributing to bone mass and strength, it is essential for correct bone development, and especially in individuals with a low intake, sufficient Ca intake will reduce, but not prevent bone loss. Increased Ca intake suppresses the number of bone-remodeling sites and augments premature mineralization of immature bone, leading to an apparent increase in bone density. Net bone accumulation will be greater as Ca intake increases to the point of genetic program governing growth. Further increases in Ca intake will produce no further bone accumulation. This may be different for different stages of growth (55). It has also been found in postmenopausal women that the rate of bone loss is significantly lower in the first year of Ca supplementation than in the second year, especially at regions where more than half of the bone content is of the trabecular type. Furthermore, the long-term (3–4 years) preservation of bone by Ca supplements has been found mainly at sites with a predominance of cortical bone, such as the radius and total body, with an annual turnover rate of only 2–3%. This slow rate of cortical bone turnover implies that a steady state is reached many years later than in trabecular bone. The long-term effect of additional Ca on bone density and fracture prevention is therefore not readily revealed by or extrapolated from randomized bone density studies (56).

Ca supplementation may transiently increase BMD by reducing the rate of bone remodeling. Increases in bone mass appear to occur primarily in cortical bone sites, are most apparent among populations with low Ca intake, and do not seem to persist beyond the Ca supplementation period.

**Pregnancy-related outcomes**

**Offspring**

The evidence regarding maternal Ca intake and offspring's health is scarce and contradictory. In their RCT, Abalos et al. (8) did not find any difference in fetal growth between fetuses of women with or without Ca supplements. However, a small increase of 65 g in birth weight was found in SR of Buppasiri et al. (25). This increase is most likely not clinically important, and due to high heterogeneity this result must be interpreted with caution. There was no difference in other pregnancy and infant outcomes (25).

Some results support an association between maternal Ca intake during pregnancy and offspring systolic blood pressure among children (26, 27). The evidence is more consistent among children older than 1 year (26).

**Mother**

Ca supplementation is associated with a significant protective benefit in the prevention of pre-eclampsia especially in women with low baseline Ca intake (18, 27). The average risk of high blood pressure was also reduced with Ca supplementation compared with a placebo (18, 27). Since pre-eclampsia is a dangerous life-threatening disorder, in which hypertension arises, Ca supplementation may be one factor in reducing the risk of this serious medical condition. Since pre-eclampsia is a dangerous life-threatening disorder, in which hypertension arises, in women with

low Ca intake Ca supplementation may be one factor in reducing the risk of this serious medical condition.

However, there was no evidence that Ca supplementation had any effect on maternal weight gain during pregnancy (25).

**Lactation**

Profound changes in Ca metabolism and bone mineral status accompany pregnancy both during gestation and after delivery and lactation. Accumulating data suggest that the losses are independent of maternal Ca intake, and largely reversible in subsequent lactation and after the end of breast-feeding, possibly in connection with the return of menses (57, 58). This does not imply that good nutrition, including the maintenance of adequate Ca intake, is important during lactation. However, the accumulating data suggest that breast-feeding women need not consume excess Ca (59).

**Cancer**

In general, no association has been found between increasing Ca intakes and cancer incidence or mortality (Chung et al. 2009). However, there are inconsistent findings considering different cancer types. In women, there was a trend toward lower breast cancer risk for those with high Ca intake (18, 29, 60).

The results concerning prostate cancer are inconsistent showing slightly increased association, no association or inverse association between Ca intake and risk of prostate cancer (18, 32, 33). The results of Kristal et al. do not simplify the interpretation. They report that dietary Ca intake was positively associated with low-grade cancer but inversely associated with high-grade cancer.

The results are not unambiguous in aspects of colorectal cancer, either. Although there are some findings that high Ca intake may contribute, to a moderate degree, to the prevention of adenoma polyps recurrence especially in patients with a history of adenomas, no apparent effect was seen in advanced adenomas or in populations at lower risk (18, 30, 31). More evidence is needed to recommend the general use of Ca supplements to prevent colorectal cancer.

The current evidence for a role of Ca in endometrial carcinomas is too limited to draw any conclusions (34). Similarly, more evidence is needed about the relationship between Ca intake and lung cancer, although increased total Ca intake reduced lung cancer risk in a subgroup of current smokers and a beneficial trend was seen in women (35).

**Cardiovascular outcomes**

**Hypertension and blood pressure**

Fairly consistent evidence suggests that Ca supplementation lowered systolic blood pressure among hypertensive adults, but no significant effect has been found on diastolic blood pressure or in normotensive individuals (18, 37, 45).

More data are needed about influences of age, sex, Ca dose, background dietary Ca, and supplement versus dietary source on blood pressure.

No consistent evidence of the relationship between Ca intake and other cardiovascular outcomes was found (36, 37, 39–41, 43). Even a possible increased risk concerning cardiovascular events is inconclusive.

**Cardiovascular events**

Recently, concern has arisen of an increased risk of cardiovascular events associated with Ca supplementation. Bolland et al. (43) suggested an upward trend in cardiovascular events in older people taking Ca. However, there are several limitations. Cardiovascular events were not a primary outcome, the events may not have been well adjudicated, the studies included are small and the event frequency is low. Moreover, the total Ca intake was not reported, and the supplementation was generally 1,000–1,200 mg/day. The events may therefore be associated with intakes higher than the supplemented dose, perhaps more than 2,000 mg of calcium per day. So, it is difficult to conclude that Ca intakes per se in the range of 1,000–1,200 mg/day can be associated with cardiovascular events (60). There are also opposing findings with no increase in cardiovascular events (40, 41), and supplements may even improve some vascular risk factors, e.g., blood pressure (37, 45).

**Diabetes**

Although Pittas et al. (44) found an inverse association between Ca or dairy intake and type 2 diabetes or metabolic syndrome, they concluded that the evidence is limited because most observational studies are of low quality, whereas duration of intervention studies was short, included few participants, or did post-hoc analyses.

**Body weight/obesity**

No convincing findings regarding benefits of Ca supplementation on body weight loss or weight maintenance were found. Ca supplementation did not alter weight or fat gain (18, 37, 46, 47).

**Total mortality**

The evidence relating total mortality is inconsistent. There is some proof about increased death of cardiovascular events (43), also a protective effect of Ca intake, especially on all-cause mortality (39, 49, 61), or no effect (18, 43). There are considerable differences between the works showing a protective influence, which complicates the interpretation. Mursu et al. (49) reported the use of Ca supplements in relationship to total mortality in older women, whereas Kaluza et al. (39) assessed dietary Ca intake in middle-aged or older men. The follow-up time of 65 years of van der Pols et al. (38) is considerable. Although the results suggest a beneficial effect of high dairy consumption in childhood on total mortality in adulthood, environmental and socio-economic circumstances have changed during the follow-up period, but these changes are impossible to be considered in the analysis. A much smaller challenge is the difficulty in separating the Ca effect from the total diet. One possible interpretation is that high dairy consumption is one factor in a healthy balanced diet. Also, it is difficult to compare the results of Bolland et al. (43) (supplement users, mainly women) and Chung et al. (18), whose result is based on total Ca intake in one cohort of adult men and their spouses.

**Adverse effects**

Ca is mainly thought of to be a safe nutrient to be used as a supplement, although recently there has been much of debate in the literature about the possible adverse effects of high Ca

supplementation. Discordant data provided limited proof of the unfavorable effects of high calcium/dairy intake. So far, only a few studies on the whole have reported adverse intestinal discomfort, such as constipation and gas, being apparently related to the Ca supplements (18).

Kidney stone formation is an adverse outcome, most notably among postmenopausal women. However, the levels of Ca intake that may cause kidney stones within a normal population cannot be specified with certainty and are known to be vary depending on a number of factors, including baseline renal function, pre-existing disease conditions, and interactions with drugs (61).

In the past, milk-alkali syndrome was often a side effect of treating peptic ulcer disease with antacids containing Ca. It is rarely seen today, because newer, better medications that do not contain Ca are available for treating ulcers. A more common scenario today is when someone takes excess calcium carbonate in an attempt to prevent osteoporosis. Calcium deposits in the kidneys and in other tissues can occur in milk-alkali syndrome. High levels of vitamin D can worsen this condition. This has been reported in persons who take 2 g or more of calcium per day. Though the modern management of peptic ulcer disease has radically changed over the past decades, milk-alkali syndrome may still occur, especially in patients who self-medicate for symptoms of dyspepsia (62).

**Doses**

The total Ca intake has not been assessed in most of the studies. There is a huge variation in daily Ca intake. Few studies have assessed total Ca intake including dietary and supplemented Ca. Many studies using supplements have not asked about dietary Ca intake, but on the other hand some studies assessing dietary Ca intake have neglected the possible use of supplements. Dose–response studies have not been performed.

**Study duration**

Study duration varied from a short few weeks' interventions to follow-ups of several years. Short interventions may be enough to show changes in blood pressure or serum lipids, whereas evaluation of Ca intake on bone density takes at least a few months and cardiovascular events, cancer or fragility fractures need an exposure of several years.

**Heterogeneity**

Owing to heterogeneity in the studies, it is difficult to interpret the results and provide single summary statement. The sources and dose of Ca vary widely among the studies, as well as the methods used to assess the amount consumed. For many risk factors, the quality and strength or direction of association varies among studies. Study cohorts were mainly Caucasian participants.

Substantial differences exist across the studies in many important factors. Many of the studies enrolled participants with sufficient or near-sufficient Ca intake, whereas in others baseline Ca intake was well below the recommended level, especially in reports from Asian populations which were included in the SRs. Also, the relatively large range in supplemental doses may complicate the comparison. Compliance has mostly not been reported or taken into account.

**Limitations**

Most studies tested Ca supplementation, not total Ca intake, i.e., dietary Ca and supplementation. However, the effect of Ca intake may differ according to baseline Ca intake. Evaluation of Ca intake

in groups, where mean Ca intake is above the recommended daily intake may mask individuals with low habitual intake or Ca deficiency. In addition, several studies examined Ca with vitamin D supplementation, or the participants are allowed to use multivitamins and even other Ca or vitamin D supplements were used simultaneously, which makes it difficult to interpret the results.

**Conclusions**

We did not find solid evidence for changing the Nordic recommendations from 2005. Although calcium/dairy supplementation had a marginal impact on bone health in the studies, milk and dairy products has to be considered to be an optimal source of Ca in the Nordic setting with important effects on bone health. Regarding children, the effect of Ca supplementation on the skeleton remained small, but most studies included children with adequate Ca intake. It is likely that Ca supplementation has a much greater impact on bone mass accretion in children with low baseline Ca intake. Similarly in adults, the treatment effect seems to be greater in participants with low daily Ca intake.

It is also difficult to separate the effect of Ca from the combination of Ca and vitamin D.

In addition to skeletal health, there is evidence that high Ca intake has a protective influence in reducing blood pressure and pre-eclampsia. The results suggesting that high supplementary Ca intake has a harmful effect on health outcomes, especially on cardiovascular health or prostate cancer are not solid. However, Ca supplementation does not seem to affect weight loss or weight maintenance, and regarding relationships between Ca intake and other cancers, the evidence is inconsistent or contradictory.

However, if individual dietary Ca intake exceeds the recommendation (depending on the age group), there is no need to recommend Ca supplements for safety reasons. We recognize that these conclusions, which are based on the current evidence, might change as additional data about long-term effects of Ca supplementation become available.

**Implications for research**

1. The adverse effects of increased Ca intake on health outcomes.
2. Studies including both dietary and supplemental Ca.

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**Notes**

To access the appendices to this article please see Supplementary files under Article Tools online

**Conflict of interest and funding**

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**Contributions of authors**

All authors contributed to methodological appraisal and data extraction.

All authors decided independently and then by consensus which studies met inclusion criteria. All authors assessed quality and extracted data from included studies. K. U.-R. prepared the draft of bone health outcomes, and M. K. and C. L-A. prepared the draft of other health outcomes. All authors drafted the discussion, commented on the draft review, and suggested changes.

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# Deep ocean mineral water accelerates recovery from physical fatigue

Chien-Wen Hou,[1] Yung-Shen Tsai,[1] Wei-Horng Jean,[2] Chung-Yu Chen,[1] John L Ivy,[3] Chih-Yang Huang,[#4,5,6] and Chia-Hua Kuo[#1,4]

[1]*Laboratory of Exercise Biochemistry, Taipei Physical Education College, Taipei, Taiwan*
[2] *Department of Anesthesiology, Far Eastern Memorial Hospital, New Taipei, Taiwan*
[3] *Department of Kinesiology and Health Education, University of Texas at Austin, Austin, Texas, USA*
[4] *Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan*
[5] *School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan*
[6] *Department of Biotechnology, Asia University, Taichung, Taiwan*

**Abstract**

**Background**

Deep oceans have been suggested as a possible site where the origin of life occurred. Along with this theoretical lineage, experiments using components from deep ocean water to recreate life is underway. Here, we propose that if terrestrial organisms indeed evolved from deep oceans, supply of deep ocean mineral water (DOM) to humans, as a land creature, may replenish loss of molecular complexity associated with evolutionary sea-to-land migration.

**Methods**

We conducted a randomized, double-blind, placebo-controlled crossover human study to evaluate the effect of DOM, taken from a depth of 662 meters off the coast of Hualien, Taiwan, on time of recovery from a fatiguing exercise conducted at 30°C.

**Results**

The fatiguing exercise protocol caused a protracted reduction in aerobic power (reduced $VO_{2max}$) for 48 h. However, DOM supplementation resulted in complete recovery of aerobic power within 4 h ($P < 0.05$). Muscle power was also elevated above placebo levels within 24 h of recovery ($P < 0.05$). Increased circulating creatine kinase (CK) and myoglobin, indicatives of exercise-induced muscle damage, were completely eliminated by DOM ($P < 0.05$) in parallel with attenuated oxidative damage ($P < 0.05$).

**Conclusion**

Our results provide compelling evidence that DOM contains soluble elements, which can increase human recovery following an exhaustive physical challenge.

**Keywords:** Deep seawater, Origin of life, Trace elements, Hydrothermal vent hypothesis

**Introduction**

A living organism can be regarded as a gathering of diverse molecules originating from the earth that works cooperatively to decrease entropy against the catabolic stresses from an ever-changing environment. Deep ocean mineral water (DOM) has been suggested to contain the primordial source of chemical components contributing to the creation of life [1,2]. Besides the major minerals, more than 70 trace elements existing in the ocean water have been documented [3]. The question regarding how many chemical components are necessary or required to support the best complexity of human life is not completely defined.

Presently, there is no information as to the effect of DOM on the physiological function of animals or humans following extreme environmental or physiological challenges. The most consistent observations reside around the anti-atherogenic effects of DOM against dietary challenges [4-7]. Compared to desalinated surface ocean water with a similar profile of major minerals (magnesium, potassium, calcium, sodium, chloride, and sulfate ions), desalinated DOM has been found to have a much superior effect on preventing the development of atherosclerosis in rabbits challenged with a high cholesterol diet [4]. This result suggests that the highly diverse trace elements found in DOM are responsible for its anti-atherogenic capabilities and have significant physiological effects on terrestrial animals. It is possible the surface waters of the oceans where sunlight is permeable are devoid of these important trace elements as a result of the photosynthetic activity of many marine organisms [8].

Due to environmental limitations marine and terrestrial organisms rely on different nutritive sources to maintain life [9]. Paleobiological evidence, however, strongly suggests terrestrial life evolved from marine ancestor [10]. Although sharing common cellular constituents with marine organisms, terrestrial survivors had to acquire alternative nutritive sources from the land to compensate for the loss associated with ancient sea-to-land migration. We proposed that if deep oceans contain the evolutionary preferred constituents for terrestrial descendents, DOM supplementation can be complementary to achieve the best biological complexity for land animals. To test this hypothesis, we conducted a human study in which we determined the time required for physical performance to recover after a dehydrating exercise when desalinated DOM or placebo drink was supplied for rehydration.

## Methods

### Subjects

Subjects taking alcohol, medication, or nutritional supplements were excluded from the study. Twelve healthy male volunteers (age 24 ± 0.8 y; height 171.8 ±1.5 cm; weight 68.2 ±2.3 kg; $VO_{2max}$ 49.7 ± 2.2 ml · $kg^{-1}$ · $min^{-1}$) were enrolled as participants in the study. Baseline $VO_{2max}$ were measured 72 h before the beginning of the study. Written informed consent was obtained after explanation of the purpose and experimental procedures of the study. This study was approved by the appropriate university Institutional Review Boards and performed in accordance with principles of the Declaration of Helsinki.

### Drink

The desalinated DOM, taken from the West Pacific Ocean (662 meters in depth), was kindly provided by Taiwan Yes Deep Ocean Water Co., Ltd. (Hualien, Taiwan). DOM was filtered by a micro-filter (removal of microorganism) and an ultra-filter (removal of macromolecule and virus) before use. Molecules sized above 1.5 KD were removed after the two filtration procedures. To mask the taste difference between DOM and placebo, the same amount of sucrose, artificial flavors, citrate, citrus juice, calcium lactate, potassium chloride, vitamin C, and mixed amino acids was

added to each. Tap water purified by reverse osmosis process was used for making the placebo drink.

**Experimental design**

An exercise-challenge protocol used by Nose et al. was modified for this study [11]. Subjects were required to run on a motorized treadmill at 40% $VO_{2max}$ at a room temperature of 30°C until a body mass decline of 3% (maximal running time: 240 min). During recovery, subjects consumed pure water or DOM containing the ingredients listed above at an amount equivalent to 1.5 fold of their body mass loss [12]. Water supplements were evenly divided into 4 sub-supplements and ingested at 30-minute intervals. Measures of physical performance (aerobic power and lower-body muscle power), physiological stress, and muscle damage were determined 4, 24, and 48 h during the recovery period. To control for possible confounding effects of individual variation, a randomized double-blind crossover design was employed with trials spaced 7 d apart.

**Physical performance**

Aerobic power (maximal oxygen consumption, $VO_{2max}$) and peak lower-body muscle power were the physical performance measures selected for determining the degree of physical fatigue recovery. $VO_{2max}$ was evaluated by the Bruce graded treadmill running protocol. This protocol consists of a 5-min warm up and incremental increases in speed and grade every 3 min until exhaustion. Verification that $VO_{2max}$ was achieved was a Respiratory Exchange Ratio (RER) greater than 1.1 and a plateau in $VO_2$ with increasing workload. Samples of expired gases were analyzed using a MetaMax3B (Cortex Biophysik, Nonnenstrasse, Leipzing, Germany). Peak lower-body muscle power was assessed using a Bertec force plate (4060-NC2000, Bertec Corporation, Columbus, Ohio, USA) with a sampling rate of 1,000 Hz. Each subject performed 3 repetitions of maximal squat jumps from a 90° knee flexion angle to full extension. Subjects were signaled when to jump by a light placed 2 meters in front of them at eye level. There was a one-minute rest between jumps. Velocity and power of each jump was calculated from vertical ground reaction forces ($V_{GRF}$) according to the impulse-momentum theorem ($V_{GRF}$ × time = body mass times ΔV, ΔV is the change in vertical velocity) (Innovative Sports Training, Inc, Chicago, IL, USA). Instantaneous velocity was determined by adding ΔV to the previous time interval, starting at zero at the beginning of the jump. Instantaneous power was derived from the product of $V_{GRF}$ measured by the force plate and the calculated instantaneous velocity [13]. The peak value of instantaneous power during the entire period of each jump was selected as peak power. The peak power values of the 3 jumps were averaged for statistical calculation.

**Biochemical analysis**

Venous blood samples were assayed for plasma myoglobin (Immunology Consultants Laboratory, Inc. OR, USA), thiobarbituric acid reactive substances (TBARS) (Cayman Chemical Company, Ann Arbor, MI, USA), cortisol (IBL-America, Inc. MN, USA), erythropoietin (eBioscience, Vienna, Austria), IL-6 (eBioscience, Vienna, Austria), and testosterone (Nova Tec Immundiagnostica GmbH, Dietzenbach, Germany) with enzyme-linked immunosorbent (ELISA) readers (Tecan Genios, Salzburg, Austria). Plama CK was analyzed enzymatically using a bench top DT-60II analyzer (Johnson and Johnson, NY, USA).

**Statistical analyses**

All values are expressed as percent of baseline (mean ± standard error). A two-way analysis of variance with repeated measures was used for comparisons between DOM and pure water at

specified time points during recovery. A paired t test with Bonferroni's correction was used to compare treatment differences at each time point. Probability of a type I error less than 5% was considered statistically significant.

**Results**

The geographic location of DOM is illustrated in Figure1.1. Concentrations of the minerals and trace elements of DOM are shown in Table 1.1. Our physical challenge protocol successfully induced a prolonged physical fatigue in aerobic power of our control trial (RO purified water) for 48 h of recovery (Figure2A, $P < 0.05$). DOM supplementation completely restored the loss of aerobic power to baseline within 4 h. Lower-body muscle power was not affected by our physical challenge protocol, yet DOM supplementation increased the power performance by ~10% above baseline (Figure2B) at 4 h and 24 h during the recovery ($P < 0.05$).

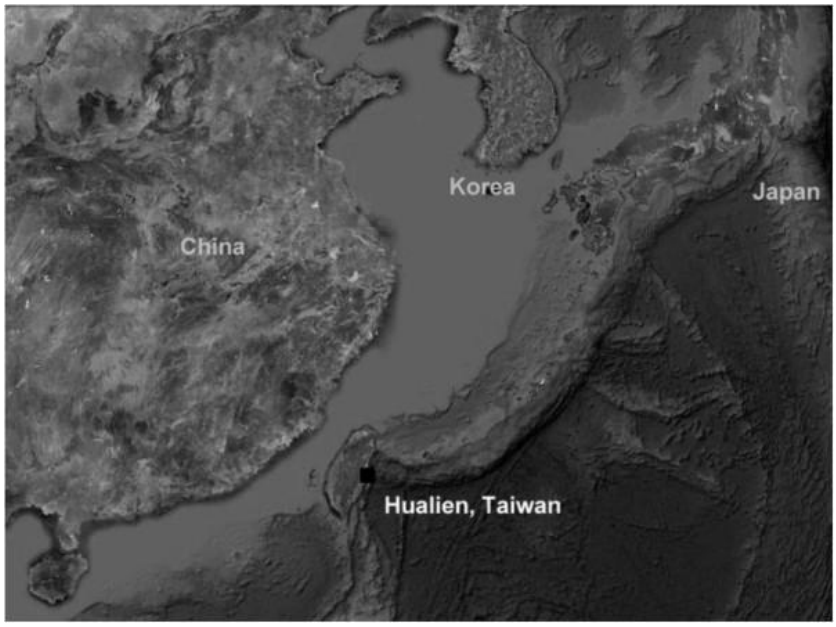


**Figure 1** - **Geographic location of DOM collection.** The black square designates the site of seawater collection, providing the shortest piping distance from land down to the deep site of the ocean (a depth of 662 meters off the coast of Hualien, Taiwan) along the circum-Pacific belt (known as Pacific Ring of Fire) in East Asia.

| Mineral | Placebo (mg/L) | DOM (mg/L) |
|---|---|---|
| Na | 38.3 | 119 |
| K | 75.6 | 115.6 |
| Ca | 53.1 | 54.6 |
| Mg | 3.24 | 140 |
| Trace element | Placebo (μg/L) | DOM (μg/L) |
| Li | N. D. | 17 |
| Rb | N. D. | 16 |
| B | N. D. | 1590 |
| Osmolarity | 226 (mOsm/L) | 249 (mOsm/L) |

**Table 1** - Minerals and trace elements in deep ocean mineral water (DOM) drink

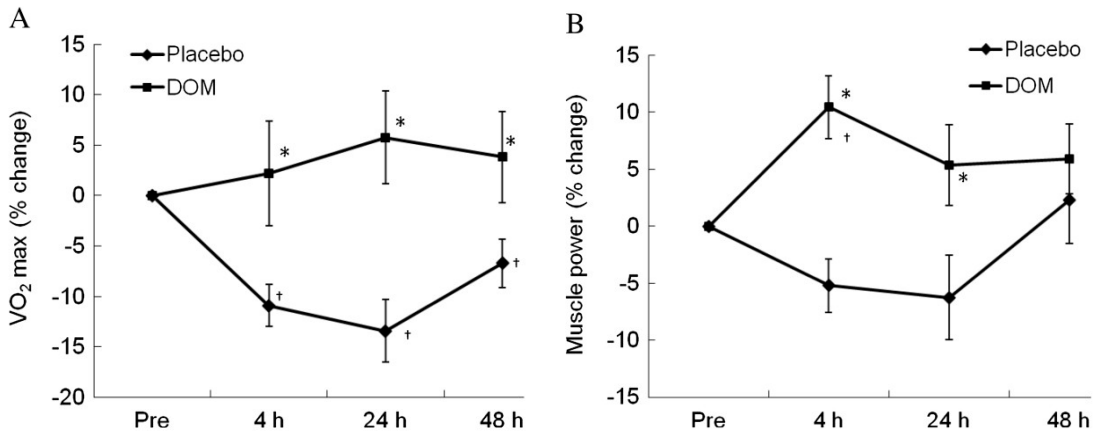


**Figure 2** - Human physical performance. DOM accelerated the recovery of aerobic capacity after a fatiguing exercise (**A**), and increased lower-body muscle power performance (**B**) during recovery. *significance against Placebo, $P < 0.05$; †significance against Pre, $P < 0.05$. N. D.: non-detectable.

Stress hormone responses are shown in Figure3 and confirms the same physiological stress produced during each trial. For both control and DOM trials, the exercise challenge temporally elevated plasma IL-6 levels (14%, $P < 0.05$) at 4 h of recovery to a comparable extent (Figure3B). This increase subsided to baseline within 24 h. Similarly, we observed a rise in erythropoietin (EPO) of 14% ($P < 0.05$) at 4 h of recovery for both treatments. By 24 h of recovery, however, EPO had fallen below baseline and was still below baseline at 48 h of recovery ($P < 0.05$). Both cortisol and testosterone dropped at 4 h during recovery (by 46% and 52%, $P < 0.05$), and had returned close to baseline by 24 h and 48 h following exercise. Again, there was no treatment differences associated with these hormones.

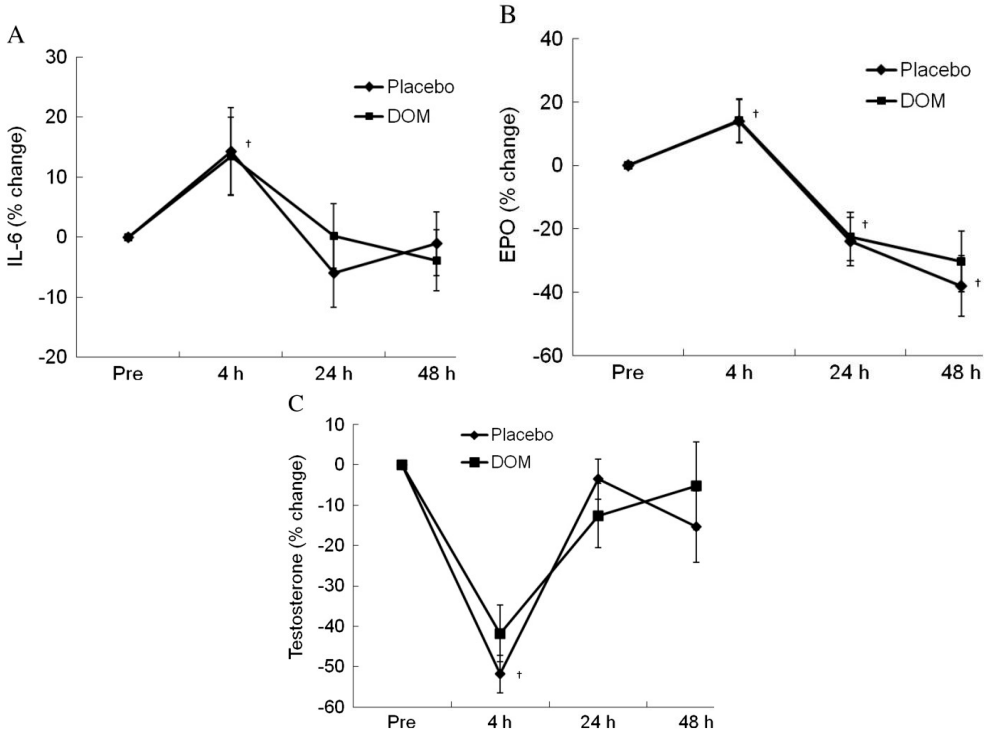


**Figure 3** - Stress hormones. Exercise challenge elevated plasma IL-6 (**A**) and EPO levels (**B**, $P < 0.05$) for both trials to a similar extent. Testosterone dropped on both trials during recovery (**C**, $P < 0.05$), and returned to baseline by 24 h during recovery. No group differences in stress hormone responses were found after the physical challenge. †significance against Pre, $P < 0.05$.

Plasma CK and myoglobin, known as exercise-induced muscle damage markers are shown in Figure3.3. A gradual rise in CK was observed 48 h following exercise in the control trial (Figure4A), while DOM eliminated this increase ($P < 0.05$). A marginal increase in myoglobin was observed at 4 h and 24 h following exercise in the control trial, while following the DOM treatment myoglobin was significantly below the control level at 4 and 24 h of recovery (Figure4B). Results for the oxidative marker thiobarbituric acid reactive substances (TBARS) are shown in Figure4C. TBARS increased significantly during the control trial at 4 h and 24 h of recovery ($P < 0.05$), while increasing only at 4 h of recovery during the DOM trial.

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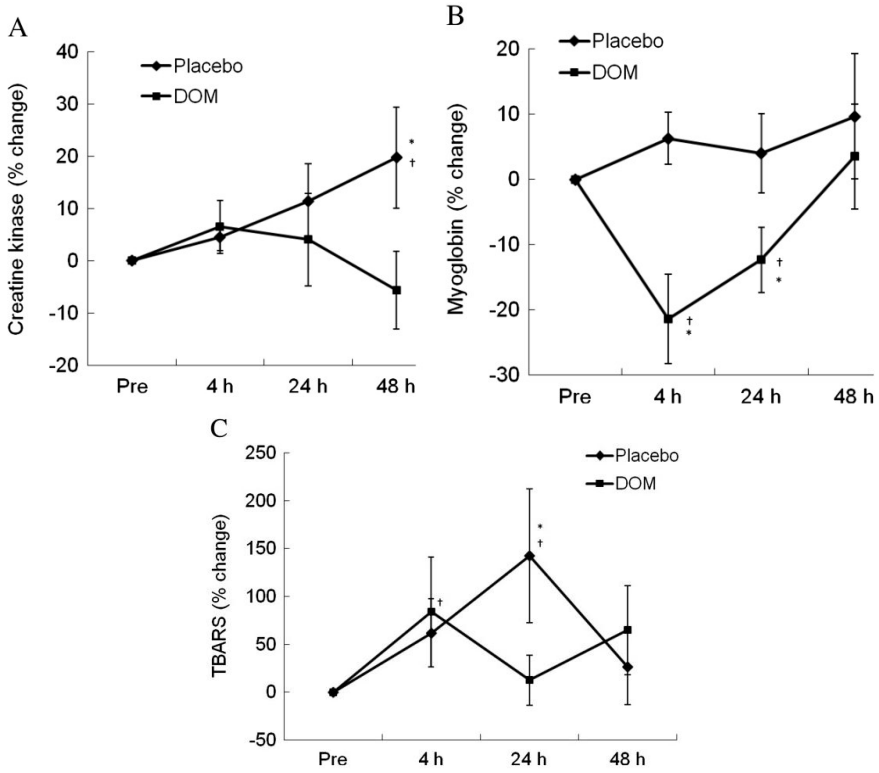


**Figure 4** - **Muscle damage markers.** Exercise-induced muscle damage was suppressed by DOM, as indicated by attenuated CK (**A**) and myoglobin (**B**) responses during recovery. DOM also attenuated oxidative damage (TBARS) increased by exercise (**C**). *significance against Placebo, $P < 0.05$; †significance against Pre, $P < 0.05$.

**Discussion**

In this study, we propose that if terrestrial organisms evolved from deep ocean [10], supply of deep ocean mineral water (DOM) to humans may replenish loss of molecular complexity associated with evolutionary sea-to-land migration, and optimizes the biological fitness. Here, we provide evidence that desalinated DOM, taken from 662 meters below sea-level, can substantially accelerate recovery from physical fatigue in aerobic power and enhance lower-body muscle power after a prolonged bout of dehydrating exercise. This improvement appears to be associated with a complete elimination of exercise-induced muscle damage, suggesting that DOM contains components, which can complement and enhance the molecular and cellular complexity of humans to minimize entropic stress produced during prolonged physical activity in the heat.

The key components of DOM contributing to the observed ergogenic benefits are not exactly known. In the study, the DOM taken from the west rim of the Pacific Ocean is characterized by enriched contents of boron, magnesium, lithium, and rubidium. In DOM the content of boron (1.59 mg/L), which is now considered an essential nutrient for humans, is 5–10 fold that found in human serum (~0.2-0.3 mg/L) [15]. Boron is known to attenuate exercise-induced rise in plasma lactate in animals [16] and to prevent magnesium loss in humans [17]. Serum magnesium concentration and dietary magnesium intake are known correlates of muscle strength [18,19]. Therefore, the minerals and trace elements in DOM may work cooperatively to sustain normal human performance.

The observed effect of DOM on accelerating fatigue recovery is closely associated with the eradication of exercise-induced muscle damage [20,21]. Elevation of these muscle damage markers

normally occurs in parallel with increased oxidative damage [22]. Our results on thiobarbituric acid reactive substances (TBARS) fits well with those on markers of muscle damage ($P < 0.05$). Higher content of magnesium, lithium, and rubidium in DOM may be associated with strengthened antioxidant capability against oxidative stress during post-exercise recovery [23-25]. In animals, lack of magnesium in their diet leads to increased free radical production [26], while magnesium supplementation eliminates free radical production induced by ischemia reperfusion [23] and alcohol drinking []. Lithium can increase the free radical scavenging capability in animals [25] and thus help to increase the resilience of a cell against destructive free radical attack [28].

One significant feature of DOM is the enriched rubidium content compared to fresh water. Rubidium concentration increases considerably in seawater as the depth of the ocean approaches 450 meters. The concentration of this trace element in human plasma ranges from 40–310 μg/L [29], about 2.5-20 fold higher than that found in DOM. However, rubidium has a high retention rate in the human body, taking 39-134 days for 50% of infused rubidium to be excreted into urine and feces [30]. Compared to rats fed rubidium, rats fed a rubidium-free diet exhibit higher urea nitrogen in plasma [31], suggesting that rubidium is essential to preserve biological integrity against daily entropic stress. The rubidium concentration in the human brain decreases with age [32], and supplementation of rubidium chloride has been found to increase spontaneous physical activity in animals [33]. Additions of lithium and rubidium into seawater have been shown to increase frequency of movement in jellyfish [34]. The recommended dietary allowance for rubidium has not yet been defined for humans. Rubidium demonstrates interchangeability with potassium in a variety of biological systems meaning that rubidium deficiency can be compensated by supplementation of potassium in many species [35]. Compared to potassium, rubidium may be an evolutionary preferred nutritive source for animals.

The oceans are the largest water reservoirs on earth, which consists of a great diversity of water-soluble chemical components, feeding a vast quantity of marine organisms [8,36]. However, nutrients in the clear ocean surface water have most likely been exhausted by a high rate of photosynthesis [8, 37]. Compared to the surface layer of the oceans, DOM may exert greater metabolic benefit, evidenced by its superior action on eliminating oxidative stress and preventing vascular damage in terrestrial animals challenged with a high cholesterol diet [4]. This observation implies that the water-soluble components unique to (or enriched in) DOM may play an important role in supporting metabolic functions of terrestrial animals when they are faced with a various physiological and metabolic challenges.

The limitation of the study is the loci-specific distribution of minerals and trace elements in the ocean, thus preventing us from being able to generalize that DOM from all sites of the world can confer the same ergogenic benefits as presented. Geographic specificity is suggested by a report documenting relatively lower silver, cobalt and nickel concentrations in the North Atlantic Ocean than the other major oceans [38]. Furthermore, the profile of minerals and trace elements is also varied with the depth of the ocean [37,39], and hydrothermal activity and diffusion from bottom sediments can also influence the composition of minerals and trace elements in the ocean waters [40]. Experiments using Antarctic Ocean waters have also suggested that not all deep ocean water will provide comparable biogenic benefits [41].

On the application side, we confirm the benefit of acute DOM supplementation on decreasing physical fatigue with elimination of post-exercise oxidative damage. However, it has been reported a diminished training effect when antioxidant was supplemented to trained men [42], suggesting that free radicals may play a role for training adaptation. Thus, whether or not decreasing oxidative stress by DOM supplementation may confer negative effects on exercise training adaptation demands more investigation.

**Conclusion**

Our findings demonstrate that desalinated DOM can increase human robustness against an entropic physical challenge, and this positive outcome appears to be associated with its protection against exercise-induced muscle damage. DOM consists of many minerals and trace elements that could not be *de novo* synthesized by the human body. Thus the momentary imbalance between loss and gain of essential minerals and trace elements after prolonged exercise may underlie the delayed recovery from physical fatigue in humans. In line with the "deep ocean life of origin hypothesis", the results of this study imply that DOM can provide required nutrients for humans that will speed recovery from entropic physical stress.

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