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ABSTRACT

Methods of assessment of magnesium status in humans

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Currently, dietary magnesium in the Western countries does not meet the recommended intake. Numerous data point out the relationship between low magnesium intake and metabolic syndrome, obesity and type 2 diabetes supporting a risk of latent magnesium deficiency with Western diet behavior. Thus, there is an urgent need to progress in development and validation of relevant magnesium status biomarkers. In regard to body magnesium distribution (mainly in bone and soft tissues) the laboratory assessment of magnesium status is problematic. The conciliation between an easy obtained sample, rapid and robust laboratory test, and the parameter representative for intracellular magnesium is extremely difficult to reach. Recently a systematic review assessed the usefulness of magnesium status biomarkers in healthy subjects. Although limited data were available, plasma and erythrocyte magnesium, and urinary magnesium excretion which responded to dietary manipulation appear to be useful biomarkers in the general population. It was not possible to draw any conclusions about usefulness of other biomarkers. This in-depth review highlighted that well-designed RCTs of sufficient size with varying doses and duration of supplementation and with evaluation of the magnesium intake using a whole-diet profile are still required. The development of biomarkers, permitting to obtain cell magnesium levels as well as body magnesium pool evaluation, relevant to study large populations, remains a major challenge for the assessment of magnesium status. Contrary to many other minerals, no indirect biomarkers of magnesium status are identified. Extensive progress of genetics and genomics opens interesting perspectives for the search of these biomarkers. With emerging knowledge of genetic factors determining magnesium status, it will be important in future studies to take into account the genetic background of studied subjects. The data from human genetics will initiate a new era in understanding the relationship between genetics, nutrition and diseases in determining magnesium status.

Reevaluation of the concept of chronic latent magnesium deficiency

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I initially proposed the concept of chronic latent magnesium deficiency (CLMD) in 1988 as individuals who have a small, chronic, negative magnesium (Mg) balance due to a significant decrease in the ingested Mg over the past century, but a serum Mg concentration within the reference interval (0.75-0.95 mmol/L) indicating normal Mg status. However, the Mg deficiency (latent) was documented by an abnormal Mg load test. During the past 24 years, a number of research studies have helped to define and to indicate the rationale for this concept. I will review the research that supports the concept of CLMD and the possible impact on the health of the individual. Further, I have revised the criteria for CLMD to be a serum Mg concentration <0.85 but >0.75 mmol/L (the lower half of the current reference interval for the serum Mg concentration) since the Mg load test is essentially not used clinically. To alert physicians to CLMD, I believe we need an evidence-based reference interval for the serum Mg concentration that is likely to be between 0.85-0.95 mmol/L for long term health.

Regulation of Mg²⁺ homeostasis in a bacterial pathogen

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Salmonella enterica is the etiologic agent of gastroenteritis and typhoid fever in humans. There is a connection between Salmonella pathogenicity and Mg²⁺ homeostasis because: (i) the major regulator of virulence is activated in response to low extracytoplasmic Mg²⁺; (ii) this regulator
controls transcription of two of three Mg$^{2+}$ transporters and the activity of the third one; and (iii) two of the three Mg$^{2+}$ transporters are necessary to cause disease.

Expression of the Mg$^{2+}$ transporter genes is ultimately governed by cytosolic signals detected by the leader regions of the corresponding messenger RNAs (mRNAs). These mRNAs have the ability to adopt alternative stem-loop structures that dictate whether the associated coding regions are transcribed. Which structure forms is determined by specific ions and/or metabolites in the cytosol. The mRNA leader corresponding to the Mg$^{2+}$ transporter MgtA responds to both Mg$^{2+}$ and the levels of proline-charged tRNA$^{Pro}$. The mRNA leader corresponding to the Mg$^{2+}$ transporter MgtB responds to the same two signals as well as to cytosolic ATP. The ability to sense ATP is necessary for Salmonella virulence as the mgtB gene is expressed inside macrophages whereas mgtA is not. These findings suggest that Salmonella requires three different Mg$^{2+}$ transporters because they operate under distinct conditions and have special properties, which enable Salmonella to explore a variety of niches.

SLC41A1, a key to the future of Na$^+$/Mg$^{2+}$ exchanger research
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Na$^+$/Mg$^{2+}$ exchanger (NME) was known to be existent for a long time. Its presence in various cell types was demonstrated at the physiological level, however, a genetic entity encoding for NME remained unknown. Presently we demonstrated that NME is encoded by magnesium sensitive gene SLC41A1. This gene was shown to be a part of the novel Parkinson's disease susceptibility locus PARK16 and also shown to have regulated transcription by androgens. Because of the latter its direct or indirect relationship to the etiology of prostate cancer might be speculated. In the lecture following will be summarized: (1) present knowledge on the molecular biology of SLC41A1, (2) its physiological relevance for the cell, respective tissues and organs. An attention will be given to presentation of the research perspectives addressing connections between SLC41A1 and the relevant diseases.

TRPM7, the most vital ion channel
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TRPM7 is a member of the melastatin-like transient receptor potential (TRPM) subfamily. The protein is a ubiquitously expressed divergent cation channel that constitutes the major Mg$^{2+}$ uptake mechanism in mammalian cells. It has both an ion channel and a functional a-kinase domain. The protein is critical for Mg$^{2+}$ regulation of mammalian organisms, with the channel domain facilitating cellular Mg$^{2+}$ influx and the kinase domain regulating Mg$^{2+}$ absorption. TRPM7 activity is directly linked to the energy status of a cell, since the channel is strongly suppressed in the presence of physiological amounts of Mg·ATP. TRPM7 has been implicated as a regulator of cell proliferation. This is based on the channel’s function in Mg$^{2+}$ transport, since cell growth can be restored by Mg$^{2+}$ supplementation. Based on the combined work from many laboratories, TRPM7 arises as a novel and promising therapeutic target where pharmacological modulators of TRPM7 could have significant potential in treating cancer, cardiac fibrillations and ischemic conditions. Given the lack of selective TRPM7 inhibitors, we developed and optimized a fluorescent dye-based assay using TRPM7-overexpressing cells and screened a chemical library of 1,122 marine organism-derived extracts and fractions. The confirmed hit rate was 0.8%, including an organic extract of Sarcothelia edmondsoni, a soft coral endemic to Hawaii. Bioactivity profiling led to the identification of the diterpene waixenicin A as the major active component. In patch-clamp experiments, we established that waixenicin A demonstrates (1) Mg$^{2+}$-dependent inhibition of TRPM7 currents in both native and over-expressing cells, (2) nanomolar potency at physiological intracellular Mg$^{2+}$ concentrations, and (3) selectivity toward TRPM7 versus other related channels. Consistent with TRPM7 inhibition, the compound blocked cell proliferation in cancer cell lines. Based on the compound’s ability to inhibit cell proliferation through Mg$^{2+}$-dependent block of TRPM7, waixenicin A or structural analogs may have cancer-specific therapeutic potential.
**Magnesium and aging**

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Magnesium (Mg) deficiency has a negative impact on the energy production pathways required by the mitochondria to generate ATP and reduces the threshold antioxidant capacity of the aging organism and its resistance to free-radical damage. Mg itself acts as an antioxidant against free radical damage of the mitochondria. Chronic inflammation and oxidative stress have both been identified as pathogenic factors in aging and in several age-related diseases. Chronic Mg deficiency results in excessive production of oxygen-derived free radicals and low-grade inflammation. Aging is very often associated with Mg inadequacy and with increased incidence of a number of chronic diseases, as well as with muscle mass and performance loss (sarcopenia), altered immune responses, and vascular and metabolic conditions, such as atherosclerosis, type 2 diabetes and the cardiometabolic syndrome. The most common mechanism leading to Mg depletion in the elderly population is dietary Mg deficiency, although secondary Mg deficit in aging may result from other causes. We discuss here the possible mechanisms and consequences of the modifications of Mg metabolism with age, the difficulties in the measurement of Mg status, and the current evidence suggesting that age-related chronic Mg deficits may be proposed as one of the physiopathological links that may help explain the interactions between inflammation and oxidative stress with the aging process, and with the genesis of age-related diseases.

**Successful treatment of hypertension with magnesium - a targeted approach**

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Objective/Methods: Three published meta-analyses of Mg for hypertension (HT) have all reported small but significant reductions in blood pressure with Mg supplementation. The high levels of heterogeneity in these 3 meta-analyses show clearly that simply combining studies without first qualitatively sorting for different categories cannot be fully informative. In an earlier study we sorted Mg for hypertension (HT) studies by hypertensive status, Mg dose and anti-hypertensive medication status and found sub-categories of studies where Mg supplementation lowered high blood pressure significantly, others where it did not. While proceeding to meta-analysis of these categories, we have found a sub-set of High Responder studies and performed a meta-analysis. Results: Seven studies with n=135 hypertensive (HT) subjects taking anti-hypertensive medications (Ace Inhibitors, beta-blockers, Ca channel blockers and diuretics) with mean starting systolic blood pressure (SBP) >155 mmHg showed a mean change of -19.24 mmHg [95% CI = -15.49 to -22.99] p<0.0001 and an Effect size test Cohen’s d = 1.23, i.e. a large and highly significant effect. Meta-analysis of diastolic blood pressure (DBP) for these same 7 studies showed mean change in DBP of -8.27 mmHg [95% CI = -6.08 to -10.46], p<0.0001, with an Effect size test Cohen’s d = 0.90. Other studies, approaching but not meeting these starting values, show very different results. An analysis of how these high-responder studies might differ from the lower-responding studies of Mg on HT subjects is presented. Conclusion: The published meta-analyses seem to have missed this sub-set of high-responder studies and two of the meta-analyses have included studies on normotensive subjects. Including normotensive subjects and/or excluding high-responder studies in the three published meta-analyses of Mg for hypertension studies quite possibly has diluted significant effects of Mg for hypertension in their resulting conclusions.

**Magnesium status and their implications in clinical diseases**

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Objective: Magnesium (Mg) is involved in the regulation of numerous biochemical and physiological functions. It plays a significant role in the genesis of various clinical diseases. In order to maintain various functions of Mg, both extracellular and intracellular Mg concentrations are regulated by complex control mechanisms. To
date, all clinical studies have been hampered by findings that (1) serum Mg are not sensitive enough to define Mg deficiency, (2) Mg status are ill-defined, and (3) not all “Mg-deficiency” patients respond to Mg supplementation. Material and Methods: The expression levels of Mg transporter genes in mice and human lymphocytes were determined. Total RNA was extracted from lymphocytes. Then, RNA was reverse-transcribed to cDNA. Expression levels of nine Mg\(^{2+}\) transporter genes (SLC41A1, SLC41A2, SLC41A3, CNNM2, MAGT1, TRPM6, TRPM7, NIPA1, and N33) were determined by quantitative real-time PCR (Q-PCR). The statistical significance of differences between groups is determined by one-way analysis of variance (ANOVA) followed by Dunnett’s test. A p value less than 0.05 is considered significant. Results: SLC41A2, CNNM2, MAGT1, TRPM6, TRPM7, and N33 are up-regulated in the low magnesium diet fed mice. In addition, CNNM2, MAGT1, TRPM6, TRPM7, and N33 can be reversed after 4 weeks supplement of Mg. SLC41A1, SLC41A2, SLC41A3, CNNM2, TRPM6, NIPA1, and N33 are up-regulated (4-14 folds) in the first trimester of pregnant women. However, MAGT1, TRPM6 are slightly down-regulated in pregnant women. SLC41A1, SLC41A2, CNNM2, TRPM6, NIPA1, MAGT1, and TRPM6 are up-regulated in patients with osteoporosis. Conclusion: The knowledge of Mg status should improve our understanding of mechanisms and the treatment of some clinical diseases. As Mg is a safe supplement, a targeted increase in Mg supplementation should have a significant effect on “Mg-deficiency” patients. In addition, interactions of Mg status and dynamic Mg transporter gene expression during Mg supplementation may be explored.

**Magnesium and cancer**

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Magnesium (Mg) and calcium (Ca) belong to the same family in the periodic table and share the same homeostatic regulating system. Moreover, Mg and Ca antagonize each other in (re)absorption, inflammation and many other physiologic activities. We hypothesized that the competition between Mg and Ca for absorption in the gut, measured by dietary ratio of Ca to Mg, modifies the effects of Ca and Mg intakes on colorectal cancer carcinogenesis. In the US population, we found that Ca and Mg intakes may only be related to a reduced risk of colorectal adenoma when the dietary ratio of Ca to Mg intake was below 2.78; and Ca treatment in a randomized clinical trial was found to reduce colorectal adenoma recurrence risk only when baseline dietary Ca/Mg ratio was under 2.63. We also found that high serum Ca/Mg ratio was associated with an increased risk of high-grade prostate cancer after controlling for both serum Ca and Mg. Furthermore, we found in two population-based cohort studies conducted in a Chinese population with a low Ca/Mg intake ratio (median ratio = 1.7), Ca/Mg intake ratio modified the associations of intakes of Ca and Mg with risk of total mortality, including mortality due to cancer (unpublished results). Also, we recently observed evidence that Mg interacted with vitamin D in relation to risk of chronic diseases. In addition to nutrient-nutrient interactions, we found Ca/Mg ratio significantly interacted with a functional polymorphism in the transient receptor potential melastatin 7 (TRPM7) gene in relation to risk of colorectal adenoma and hyperplastic polyp. Currently, we are further evaluating this gene-nutrition interaction among colorectal adenoma patients in an ongoing US NIH R01 supported personalized intervention trial. In another ongoing US NIH R01 project, we have successfully identified several nutrition (Ca, Mg or Ca/Mg ratio)-gene interactions in association with risk of colorectal adenoma in a two-stage design. Our findings indicate that Mg interact with both Ca and vitamin D in relation to risk of several cancers and other chronic diseases. The results will be discussed in detail.

**Fetal neuroprotection with magnesium sulphate - what is the evidence?**

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Very preterm infants have rates of neurologic impairments and disabilities that are too high relative to infants born at term. As more very preterm infants now survive they are contributing disproportionately to the burden of illness in childhood, and in later life.
Neuroprotective strategies for the very preterm infant are urgently required. Basic science research suggests that magnesium sulphate before birth may be neuroprotective for the preterm fetus. Magnesium ions are essential for many key cellular processes, and overall are associated with more than 300 enzymatic systems. Magnesium may influence mechanisms implicated in cell death or dysfunction, and magnesium also has some beneficial haemodynamic effects. Some, but not all observational studies in humans suggest a protective effect of antenatal magnesium sulphate on cerebral palsy. However, magnesium sulphate also has deleterious effects. There are five randomised trials (RCTs) of antenatal magnesium sulphate where long-term neurological effects in surviving infants have been reported. Of the five RCTs (6145 fetuses), in four studies (4446 fetuses) the primary intent of the study was neuroprotection of the fetus. Antenatal magnesium sulphate therapy given to women at risk of preterm birth substantially reduces the risk of cerebral palsy in the child (relative risk [RR] 0.68; 95% confidence interval [CI] 0.54 to 0.87; \( P = 0.002 \); five trials; 6145 infants). Moreover there is a significant reduction in the rate of substantial gross motor dysfunction (RR 0.61; 95% CI 0.44 to 0.85; \( P = 0.003 \); four trials; 5980 infants). No statistically significant effect of antenatal magnesium sulphate therapy is evident on pediatric mortality, or on other neurological impairments or disabilities in the first few years of life. There are no significant effects of antenatal magnesium sulphate on combined rates of mortality with neurologic outcomes, except in the studies where the primary intent was neuroprotection where there is a reduction in the combined outcome of death or cerebral palsy (RR 0.85; 95% CI 0.74 to 0.98; \( P = 0.027 \); four trials; 4446 infants), and a borderline reduction in death or substantial gross motor dysfunction (RR 0.84; 95% CI 0.71 to 1.00; \( P = 0.05 \); three trials; 4387 infants). Antenatal magnesium sulphate therapy given to women at risk of preterm birth is neuroprotective against motor disorders for the very preterm fetus. Clinical practice guidelines have now been developed in several countries recommending magnesium sulphate for neuroprotection in women likely to deliver very preterm.

**Magnesium and cardiovascular disease**

**Klaus Kisters**

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Objective: An overview of magnesium metabolism and the role of magnesium in the development of primary hypertension and borderline hypertension is given. A role for lowered magnesium concentrations in vascular tone has been postulated in hypertensive disorder. Pathophysiological aspects for a magnesium deficiency in hypertension are discussed, e.g. calcium-magnesium-antagonism, sodium magnesium antiport, disturbed TRMP 6 and 7 channels, diminished membrane Na\(^+\), K\(^-\)-adenosine triphosphatase and Ca\(^++\) ATPase activities, and, as a corollary, increased Na\(^+\) and Ca\(^++\) concentrations. In essential hypertensives decreased serum/plasma, membrane and intracellular magnesium concentrations have often been described earlier by our group. In smooth muscle cells of spontaneously hypertensive rats we also found intracellular magnesium decreased. Material and Methods: Therefore we measured plasma and intracellular Mg\(^{2+}\) levels in erythrocytes in 18 untreated borderline hypertensive patients and in 35 untreated normotensive healthy subjects as controls. Results: In patients intracellular Mg\(^{2+}\) content was significantly lower (1.61 ± 0.09 mmol/L, mean ± SD), than in controls (1.84 ± 0.14 mmol/L, p<0.05). After 12-15 weeks of an oral supplementation with 240-480 mg Mg\(^{2+}\)/day, the erythrocyte Mg\(^{2+}\) content had increased significantly in the borderline hypertensive group (1.78 ± 0.11 mmol/L) (p<0.05). There was no significant difference between the normotensive and borderline hypertensive group in plasma Mg\(^{2+}\) concentrations (0.87 ± 0.13 versus 0.83 ± 0.17 mmol/L). Systolic and diastolic blood pressure values of the borderline hypertensive group also normalized after oral Mg\(^{2+}\) administration (before therapy: 147.6 ± 8.5/87.2 ± 4.4 mmHg, after therapy: 137.2 ± 4.6/83.8 ± 3.4 mmHg) (p<0.05). After 18-21 weeks of magnesium therapy in the hypertensives, blood pressure was 135.8 ± 4.8/82.9 ± 3.6 mmHg (p<0.05) and plasma magnesium was 0.91 ± 0.29 mmol/L. Conclusion: Magnesium plays an important role in the development of primary hypertension and borderline hypertension.

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role in the development of primary and borderline hypertension. A magnesium therapy is of benefit in this disorder. Long-lasting magnesium therapy should be preferred since the effects of a magnesium supplementation in hypertension occur mainly after a long-term treatment.

Expression of magnesium transporters in preeclamptic vs. healthy placental tissues
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Preeclampsia is one the leading causes of fetal and maternal morbidity and mortality around the world. Its causes remain elusive and magnesium deficiency is proposed to be relevant for the etiology of the disease. Supporting this, magnesium supplementation has proven to be the best choice for preventing eclampsia development. **Objective:** The aim of this study was to compare the expression of nine magnesium transporters (CNNM2, SLC41A1, SLC41A2, SLC41A3, MagT1, TRPM6, TRPM7, N33 and NIPA1) by qRT-PCR in placental tissues from preeclamptic vs. healthy pregnant women. **Material and Methods:** Twenty seven placental samples from both preeclamptic and healthy pregnant women were obtained from Mexico. Total RNA was converted to cDNA in order to evaluate the expression of 9 different magnesium transporter genes through qRT-PCR using SYBrGreen. Tuba1b gene was used to normalize gene expression. Four placental samples from Germany were evaluated for SLC41A1 protein by Western-blot. **Results:** All studied genes were identified in placenta. There were no differences in the expression of most genes between groups, with the exception of SLC41A1 gene which shows a 6 fold higher expression in preeclamptic placentas. The expression of NIPA1 and TRPM6 was almost undetectable. The SLC41A1 placental expression was confirmed through western-blot analysis in 4 healthy placental donors. **Conclusion:** As a conclusion the higher expression of SLC41A1 Na⁺/Mg²⁺ exchanger correlates with a higher transplacental transport of magnesium to the fetus. The latter causes an increase of sodium concentrations in the utero-placental arterial bed rising the local blood pressure a necessary condition for preeclampsia development.

Magnesium and Mg transporter genes in pregnancy induced hypertension
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**Objective:** Hypertension is a common disorder during pregnancy and may be present in up to 10% of all pregnancies. Blood pressure increase during pregnancy is a risk indicator of preeclampsia and eclampsia which are associated with major increases in maternal and foetal morbidity and mortality. Previous studies have evaluated the possibility to prevent the blood pressure increase during pregnancy using calcium or magnesium supplementation but the results are inconclusive. A study was undertaken to assess if magnesium supplementation during pregnancy would decrease the risk of blood pressure increase in a selected group at risk. **Material and Methods:** Pregnant subjects (n = 60) with a urinary calcium level above 7.5 mmol/L received a daily dose of 300 mg magnesium as citrate or placebo from the 20th week of pregnancy until delivery in a randomized, double-blind design. Blood pressure was measured throughout pregnancy and the expressions of several magnesium transporter genes were measured. **Results:** At 12 weeks there were significant relationships between the expression of TRPM6 and diastolic blood pressure (p = 0.016) and between diastolic blood pressure and the excretion of magnesium (p = 0.027). The expression of TRPM6 was higher in the pregnant subjects than in non-pregnant controls. In the magnesium supplemented group, the increase in diastolic blood pressure was lower than in the placebo group at week 35 and 37 (p = 0.022). The number of subjects with an increase above 10 mmHg diastolic pressure at week 37 was 11 out of 29 in the placebo group and 2 out of 22 in the magnesium group (p = 0.015). There were an inverse relationships between the change in diastolic blood pressure over pregnancy and the urinary excretion of magnesium (p = 0.005).
and between the expression of TRPM6 and the urinary excretion of magnesium (p = 0.015). Conclusion: Magnesium citrate supplementation prevented severe blood pressure increase in a group of pregnant subjects. The relations between blood pressure changes and magnesium and the expression of the magnesium transporter gene TRPM6 support the hypothesis that magnesium is of importance for pregnancy induced hypertension. Further studies to assess if magnesium supplementation also reduces the risk of preeclampsia are urgently required.

X-linked immunodeficiency with Mg$^{2+}$ defect, Epstein-Barr Virus (EBV) infection, and neoplasia (XMEN) disease, a novel primary immunodeficiency that unraveled new role for cellular Mg$^{2+}$

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X-linked immunodeficiency with Mg$^{2+}$ defect, Epstein-Barr Virus (EBV) infection, and neoplasia (XMEN) disease was initially characterized by us in two boys with chronic EBV infections and a 45-year-old male who deceased from an EBV-associated lymphoma. This primary immunodeficiency, which results from the loss of Magnesium transporter 1 (MAGT1), has a delayed T cell activation defect due to the absence of a TCR-gated Mg$^{2+}$ flux mediated by MAGT1 that is require for early activation of the PLC$\gamma$1. However, the consistent clinical phenotypes of this disease and the mechanism for its unique susceptibility to EBV infections were unclear. Now, we identified five additional XMEN patients from five different kindred, all exhibiting a decreased CD4:CD8 T cell ratio and chronic EBV infections. Four of these five patients have developed lymphomas. The susceptibility and morbidity of this disease to EBV-associated lymphomas was explained by cytotoxicity defects in patient NK cells and cytotoxic T lymphocytes (CTLs) associated with loss of expression of NKG2D, an activating receptor required for optimal cytotoxicity. Moreover, NKG2D expression can be rescued by Mg$^{2+}$ supplementation in patient CTLs and NK cell cultures in vitro. This increase in NKG2D expression was associated with restoration of basal intracellular Mg$^{2+}$ levels in patient lymphocytes. Given the recent demonstration of the significance of T cells and NKG2D for tumor immune-surveillance in a mouse model of EBV-driven lymphomas, our findings not only validate that this is also true in humans but also suggest that the loss of NKG2D expression could be a pre-diagnostic screening marker for XMEN disease. Moreover, we demonstrate for the first time that NKG2D expression is sensitive to Mg$^{2+}$ regulation, which has therapeutic implications for this disease.

Magnesium in oncology

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Magnesium is involved in crucial steps of carcinogenesis, tumour growth and anti-tumour therapies, however, the relationship between magnesium and cancer is not clear: basic and preclinical studies indicate that magnesium deficiency can have both anti- and pro-tumour effects. We will briefly review the state of the art and focus on the latest experimental evidence indicating that alteration in the expression and/or activity of magnesium channels is a frequent finding in cancer cells and human tumour tissues. The potential implications for developing novel diagnostic and therapeutic strategies will be discussed.

Endothelial cells and magnesium: implications in cardiovascular diseases

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There is no doubt that the functional and structural integrity of the endothelium is critical to maintain vascular homeostasis and prevent atherosclerosis. In the light of epidemiological and experimental studies, magnesium deficiency is emerging as an inducer of endothelial dysfunction. Studies on endothelial cells have shown that low extracellular Mg rapidly and transiently induces free radicals primarily through the membrane-associated nicotinamide dinucleotide phosphate oxidase.
enzyme complex. Reactive oxygen species then activate the transcription factor nuclear factor (NF)κB, which induces cytokines, growth factors, adhesion molecules and enzymes involved in inflammatory responses, thus generating a pro-atherogenic phenotype in endothelial cells. Through the action of free radicals, low extracellular magnesium also upregulates TRPM-7, a cation channel of the transient receptor potential channel family, which functions as a magnesium transporter. The role of TRPM-7 in modulating endothelial behavior has been revealed, in part, by its pharmacological and genetic inhibition. Our results indicate that TRPM-7 modulates endothelial behavior and that any condition leading to TRPM-7 upregulation might impair endothelial function. Our data on cultured endothelial cells reinforce the idea that maintaining magnesium homeostasis might be a helpful and inexpensive intervention to prevent and treat endothelial dysfunction and, consequently, atherosclerosis.

**Protective efficacy of Mg-supplementation against anti-HIV drug-induced cardiac toxicity**

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The protective efficacy of Mg-supplementation against AZT- (prototype NRTI anti-HIV agent) and ritonavir- (RTV, prototypical protease inhibitor) mediated endothelial cell (EC) and cardiac toxicity were studied. In cultured bovine ECs with normal Mg (0.8 mM), both AZT and RTV promoted iron-mediated EC lipid peroxidation and cytotoxicity. AZT or RTV alone caused dose-dependent (5-25 μM) EC oxidative stress (increased DCF fluorescence and GSSG content) within 6h and inhibited 40-50% NO release by 24h; significant losses of cell viability were evident by 48h. Both AZT and RTV induced increases in EC ROS and GSSG levels which were substantially suppressed by high extracellular Mg level (2 mM); concomitantly, NO synthesis and cell viability were restored up to 90%. We previously reported (Mak et al. 2009) that AZT administration to rats for 3 weeks resulted in systemic neutrophil activation, cardiac WBC cell infiltration (CD11b positivity) and loss of weight; most indices of systemic oxidative stress were attenuated up to 85% by 6-fold higher dietary Mg supplementation. We also examined oxidative stress, and cardiac toxicity induced by RTV up to 8 weeks in rats with or without dietary Mg supplementation. Blood neutrophils from 5 wk RTV-treated rats displayed a 3-fold higher superoxide activity, plasma 8-isoprostane rose 2.3-fold, but plasma nitrite decreased 30%. High Mg diet substantially (>75%) attenuated all oxidative indices and restored nitrite levels. Echocardiography at 5-8 weeks of RTV treatment showed significant decreases in cardiac LV shortening fraction and mitral valve E/A ratio accompanied by left ventricular posterior wall thinning in diastole and systole (10-12%), indicative of early dilated cardiomyopathy. Mg-supplementation attenuated RTV-induced declines in systolic and diastolic function (>70%) and lessened LVPWs wall thinning (by 75%). Histochernical staining revealed ventricular WBC (CD 11b+) infiltration at 5 wk, and fibrosis at 8 wk in RTV-treated hearts; both were diminished by Mg supplementation. Conclusion: This study demonstrates the benefits of Mg-supplementation against anti-HIV drug-induced endothelial and cardiac toxicity. (Supported by NIH R21 AT003993 and R21NR012649)

**The DASH diet and interactions with the Renin-Angiotensin System**

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The DASH (Dietary Approaches to Stop Hypertension) diet significantly lowers blood pressure (BP) and its use is strongly recommended for cardiovascular risk reduction. This dietary pattern emphasizes fruits, vegetables and low fat dairy products, is enriched with magnesium, potassium and calcium and is overall reduced in total and saturated fat. However, the effects of the DASH diet on specific BP
regulating mechanisms are not known. While the DASH diet has a number of components that may favorably affect BP, some evidence suggests that interactions of magnesium and/or potassium intake may be involved via interactions with the renin-angiotensin system. For example, increased potassium and magnesium intake lowers BP and short-term magnesium infusion increases plasma renin activity. In a series of studies we have shown that the DASH diet produces additive effects when combined with the ARB, losartan, affects the vascular response to angiotensin II (Ang II) and shifts the pressure-natriuresis curve to produce greater salt excretion. In addition, the DASH diet produces greater BP effects in individuals with certain alleles of the angiotensinogen and the B2-adrenergic receptor genes. In a recent study we tested whether the DASH diet alters target tissue responsiveness to Ang II in patients with isolated systolic hypertension. These results showed that the DASH diet increases plasma renin activity, renal blood flow, and the vascular response to Ang II, which is consistent with an effect of the diet on the tissue renin-angiotensin system. The factors responsible for these effects cannot be determined from these studies, but there is ample evidence that they derive from combined effects of the electrolytes and micronutrients contained in the DASH diet.

**Aldosterone activates ex vivo human polymorphonuclear leukocytes**

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Aldosterone (ALDO) is an important regulator of body sodium and has direct adverse effects on the vasculature. Administration of mineralocorticoids leads to lesions of the heart and circulating ALDO levels correlate with cardiac damage. Indeed clinical studies with mineralocorticoid receptor (MR) antagonists show decreased morbidity and mortality in patients with heart failure. Consistent with these observations, animal studies show that mineralocorticoid excess leads to myocardial inflammation and fibrosis. ALDO mediates its classical epithelial effect on the transport of sodium via activation of MR as a ligand-dependent transcription factor. However, there is increasing evidence that steroid hormones can likewise initiate rapid and nongenomic responses. ALDO has been shown to regulate signaling cascades, including c-Src, MAP kinase (MAPK) and the G protein-coupled receptor, GPR30 and lead to rapid increases in ERK 1/2 phosphorylation. However the mechanisms by which ALDO regulates vascular tissue are not entirely clear. An important role for MR in vascular inflammation has been proposed. Polymorphonuclear leukocytes (PMN) are critical players following an inflammatory response. We studied the role of ALDO on *ex vivo* PMN function by isolation of cells from circulating human blood following density gradient sedimentation with PolymorphPrep® from otherwise healthy subjects. Flow cytometric analyses showed greater than 97% of PMN were positive for myeloid-neutrophil markers, CD45, CD16 and CD66b. These cells express MR as confirmed by western blot, qRT-PCR and 3H-aldosterone binding analyses. Incubation of *ex vivo* PMN cells with ALDO (1-10 nM) showed a rise in superoxide production (*P*<0.01, *n*=9), an event that was blunted by pre-incubation with canrenoic acid (CA), a MR antagonist (*P*<0.03, *n*=6). Consistent with these results, we observed that 10 nM ALDO led to rapid increases in cytosolic Ca$^{2+}$ levels using FURA-2AM fluorescence and was associated with decreases in cellular Mg$^{2+}$ levels using MagFURA-2AM following 1.5 hr of ALDO. These events were associated with increases in VEGF secretion from PMN as determined by ELISA of supernatants (260.64 ± 44.6 vs. 134.03 ± 1.9 pg/mL, *P*<0.01 when compared to vehicle, *n*=8); thus suggesting that ALDO-activated PMN cells increase factors involved in migration and endothelial cell activation. Indeed, 10 nM ALDO stimulated PMN cell migration in a time-dependent manner by CyQUANT fluorescence that lasted up to 60 min and could be blocked by CA (*P*<0.01, *n*=3). We then studied the effect of ALDO on PMN degranulation following incubations with ALDO (10$^{-9}$ -10$^{-7}$ M) for 30 min and observed a significant increase in β-glucuronidase release (*P*<0.001, *n*=3) in cell-free supernatants and cell lysates measured by fluorescence detection. PMA and N-Formyl-Methionyl-Leucyl-Phenylalanine (fMLP) were used as positive controls for PMN activation. We also studied DMSO-differentiated HL-60 cells, a neutrophil-like human cell line. We detected the
presence of MR by western blot and qRT-PCR in these cells. Incubation with 10 nM ALDO led to increases in cellular Ca\textsuperscript{2+}, decreases in cellular Mg\textsuperscript{2+}, superoxide production (P<0.01, n = 3) and HL-60 neutrophil degranulation (P<0.02, n = 6) when compared to vehicle. In order to identify the mechanism by which this maybe occurring we studied the effects of striatin on ALDO function. Striatin has recently been shown to be associated with estrogen receptor-α (ER\textsubscript{α}) and MR, and mediate activation of nongenomic effects of ER\textsubscript{α} activation. We followed degranulation responses in HL-60 neutrophils by measuring the release of myeloperoxidase (MPO) and observed that 10\textsuperscript{-6} M ALDO was likewise associated with increased degranulation when compared to vehicle treated cells (P<0.02, n = 4). We observed that in HL-60 neutrophils, siRNA against striatin, when compared to scrambled treated cells, led to reduced MPO responses (P<0.03, n = 4) that were associated with significantly reduced superoxide release and reversal of the effects on cellular Mg\textsuperscript{2+}. Thus our results suggest that increases in aldosterone levels may activate neutrophils and as such may contribute to vascular inflammatory responses associated with MR activation in vivo.

Epidemiology of magnesium intake in relation to diabetes, metabolic syndrome and other chronic diseases

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This presentation systematically reviews and quantitatively summarizes the evidence generated from epidemiological studies relating magnesium intake to major chronic diseases such as diabetes, metabolic syndrome, hypertension and stroke. A body of literature indicates a pivotal role of magnesium in glucose homeostasis and insulin secretion and action. Accumulated epidemiological evidence supports that magnesium intake is significantly inversely associated with risk of type 2 diabetes in a dose-response manner. Another major role for magnesium is in the regulation of blood pressure. While data is not entirely consistent, magnesium intake appears to achieve a small but clinically significant reduction in blood pressure after quantitatively combining the available data. Also, a role for magnesium in metabolic syndrome has been noted in that increased magnesium intake may be related to decreased risk of metabolic syndrome. In addition, dietary magnesium is suggested to aid in the prevention of stroke. By pooling the epidemiological data, dietary magnesium intake is inversely associated with risk of stroke, specifically ischemic stroke. In summary, the most recent data from epidemiological studies suggest that adequate magnesium intake may be beneficial with respect to primary prevention of major chronic diseases. Because magnesium is an essential element that involved in numerous metabolisms without known serious adverse effects, magnesium-rich foods and magnesium supplements may be considered for primary prevention or complementary therapy for some chronic diseases.

Role of magnesium intake on chronic inflammation and diabetes

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Low-grade chronic inflammation, as reflected by elevated inflammatory markers, is one of the common antecedents underlying pathophysiology related to cardiometabolic disease, including type 2 diabetes. Emerging evidence from laboratory studies suggests that magnesium may exert potent immunomodulatory and anti-inflammatory functions. In epidemiological studies, magnesium intake has been inversely associated with elevated concentrations of high-sensitivity C-reactive protein (CRP) and other inflammatory cytokines. This inverse association was consistently observed in various populations, indicating that the metabolic effects of magnesium intake might be, at least in part, due to its effects on systemic inflammation. Some small clinical trials with short durations appeared to support the potential efficacy of magnesium supplementation in chronic inflammation, although this area still requires further investigation. Results from prospective studies of magnesium intake and risk of incident type 2 diabetes have been generally consistent; however, there are as yet no clinical trials examining the efficacy of magnesium supplementation or consumption of major
Magnesium-rich foods on the primary prevention of type 2 diabetes. The efficacy of oral magnesium supplementation as adjunct therapy in improving glycemic control among diabetic patients has been suggested in some small randomized clinical trials but its long-term benefits and safety for secondary prevention of diabetes remain to be determined in future large randomized controlled trials with long follow-up periods. This presentation provides an overview of the current evidence linking magnesium intake to chronic inflammation and type 2 diabetes from observational studies to intervention trials.

Magnesium, diabetes, and the cardiometabolic syndrome
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Cardiometabolic syndrome is a cluster of metabolic factors that increase the risk of developing cardiovascular disease and type 2 diabetes mellitus. The metabolic risk factors that cluster in the syndrome include insulin resistance, hypertension, impaired glucose tolerance, central obesity, and dyslipidemia. Other abnormalities, such as chronic proinflammatory and prothrombotic states and oxidative stress, have been added to the syndrome. Magnesium (Mg) plays a key role in regulating insulin action, glucose uptake and vascular tone. Other abnormalities, such as chronic proinflammatory and prothrombotic states and oxidative stress, have been added to the syndrome. Magnesium (Mg) plays a key role in regulating insulin action, glucose uptake and vascular tone. Other abnormalities, such as chronic proinflammatory and prothrombotic states and oxidative stress, have been added to the syndrome. Magnesium (Mg) plays a key role in regulating insulin action, glucose uptake and vascular tone. 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ABSTRACT

95\% CI 16.4-34.3) groups. Conclusions: Our results show that hypomagnesemia is associated with decrease of the 1st and 2nd phases of insulin secretion in non-diabetic subjects with hypomagnesemia.

Assessing magnesium in vivo: from brain to cells
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Assessing magnesium concentration in vivo is a challenging task. In this lecture I will provide some insight from past and present research on the limits and applications of the different approaches and techniques that can be employed to measure magnesium directly in human brain, or in other living tissues such as muscles as well as in cells. The possibility to assess the free cytosolic \( \text{Mg}^{2+} \) directly in the human brain by phosphorus magnetic resonance spectroscopy \((^{31}\text{P MRS})\) provided new hints on the \( \text{Mg}^{2+} \) homeostasis and on its involvement on the cellular bioenergetics. In addition, \(^{31}\text{P MRS} \) opened the chance to study the involvement of \( \text{Mg}^{2+} \) in different neurological pathologies, and particularly in those where the defective mitochondrial energy production represents the primary causative factor in pathogenesis. The results obtained, studying patients affected by different types of mitochondrial cytopathies, helped to clarify the functional relationship between the energy metabolism and free \( \text{Mg}^{2+} \), providing evidences that the cytosolic \( \text{Mg}^{2+} \) is a function of the energy charge of brain cells and a defective mitochondrial respiration causes a derangement of cytosolic \( \text{Mg}^{2+} \) homeostasis. More recently the development of a new class of fluorescent dyes (DCHQ) created the opportunity to assess the total magnesium (free and bound) in living cells providing a tool that can be employed also in brain cells. An increasing amount of studies are exploring the role of magnesium in astrocytes, particularly in response to ischemia. There are indeed compelling evidences about a modulation effect on astroglial currents exerted by magnesium, posing the question whether magnesium itself might modulate astroglial neuroprotective functions by regulating ionic channels expression. However the existence of the TRPM7 channel in astrocytes is under investigation representing an intriguing current challenge.

Magnesium in bipolar disorders-interactions with mood modulators
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Objectives: Magnesium and other bivalent cations misbalances are occurred in bipolar disorders (BD) patients. The aim of the study was the determination of magnesium plasma and intracellular concentration in adult patients type I bipolar disorders patients before any treatment and after different mood modulators therapy. Patients and methods: In the study were included only type I BD adult peoples aging 21-65 years hospitalized during the maniacal episode. The patients did not receive any treatment before hospitalization. The I\(^{st}\) group received sodium valproate 900 mg/day p.o. daily 4 weeks, the II\(^{nd}\) group received carbamazepine 600 mg/day p.o. daily 4 weeks and the III\(^{rd}\) group received quetiapine 600 mg/day daily 4 weeks. A group of 20 adult healthy subjects was the control group. The plasma levels of total magnesium, calcium, copper and zinc and erythrocyte magnesium were determined by atomic absorption spectrophotometry. In the III\(^{rd}\) group of patients, the clinical assessment was carried out using BPRS (Brief Psychiatric Rating Scale). Correlation between magnesium concentrations and BPRS values has been determined. Results: The intracellular magnesium level was significantly reduced in BD patients before treatment (46.2 \(\pm\) 1.03 mg/L in carbamazepine group vs. 59.15 \(\pm\) 2.01mg/L in control group p<0.05). In all groups the treatment increased the erythrocyte magnesium level (e.g. in sodium valproate group 45.01 mg/L before treatment vs. 52.02 mg/L after treatment p<0.05). No changes were found in plasma magnesium concentration (18.76 mg/L before treatment vs. 21.07 mg/L after quetiapine treatment). It was evidenced a positive correlation between the decrease of BPRS values and the increase of intracellular magnesium concentration and the plasma zinc level. Conclusions: The intracellular magnesium level is decreased...
ABSTRACT

Magnesium valproate in learning disabled children with interictal paroxysmal EEG patterns

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Introduction: Children with learning disorders (LD) are a heterogeneous population. Abnormal quantitative electroencephalogram (QEEG) recordings were found in 25-45% of reported cases of LD. Specifically, increased absolute (AP) and relative power (RP) in delta and theta bands with decreased RP in the alpha band have been observed in the QEEGs of children with LD. In addition, interictal paroxysmal pattern discharges, such as spikes, polyspikes, and sharp waves have been observed in conventional EEG. This activity is rarely seen in typically developing children. Studies measuring the effectiveness of antiepileptic treatment for reducing attention and learning deficits associated with LD in children who do not experience clinical seizures but do present EEG interictal paroxysmal patterns. These EEG segments were edited as in the normative database, and analyses were performed off-line. Fast Fourier Transforms and the crossspectral matrices were calculated each 0.39 Hz, and the AP and RP in each of four frequency bands (delta [0.5-3.5 Hz], theta [3.6-7.5 Hz], alpha [7.6-12.5 Hz], and beta [12.6-19 Hz]) were obtained for each referential lead. The ranges of these bands were selected according to normative data. QEEG: Current Source Frequency domain variable resolution electromagnetic tomography (FD-VARETA) was used to calculate the distributed sources from 0.78 to 19 Hz each 0.78 Hz. This technique was used to estimate the source generators of EEG data, which are restricted to cortical gray matter. In topographic maps, 3D color-coded images called brain electromagnetic tomography (BETs) are generated, in which the color code reflects the magnitude of the current at each point of the

type BP before treatment. Because all drugs used in the treatment significantly increased erythrocyte magnesium level we believe that changes in magnesium and zinc concentration play an important role in the therapeutic effects of mood modulators.
grid or voxel. In this manner, BETs for each EEG frequency band were obtained. Cognitive evaluations: a) The IQ of each subject was evaluated using the WISC-RM scale as well as both verbal and performance scores before and six months after initiating MgV or placebo treatment. b) Each child was administered a BTL for Hispanic children, designed to evaluate reading and pre-reading skills across eight different tasks. Tasks included comprehension, picture naming, sentence completion, ordering of words into syntactically correct sentences, phonological categorization (rhymes), and oral reading of texts, words, and pseudowords. Reaction times (RT) in milliseconds for both correct and incorrect answers were measured. Because the BTL and WISC-RM variable values did not follow a normal distribution and because the sample size was small, the Wilcoxon Test for intra-group comparisons and the Mann-Whitney U Test for inter-group analysis were selected for data analysis. Both within group (before vs. after treatment or placebo) and between group (differences in after-before values were used to compare the changes observed following MgV or placebo) comparisons were analyzed. Differences in number of EEG interictal paroxysmal patterns and EEG current sources obtained by FdVARETA for after-before treatment for each group were assessed by multivariate nonparametric permutation tests. Results: a) EEG: All children included in this study had interictal paroxysmal patterns. For each patient, the average number of discharges was determined before and after treatment by dividing the number of discharges recorded by the total length of the EEG recording. The two groups had a similar average number of interictal paroxysmal patterns at the beginning of the treatment. No statistical differences were found within groups between the beginning and the end of the treatment in the experimental and control groups. b) QEEG: Z scores were not significantly different for AP and RP between before-after and after-before for any group. However, current sources showed significant decreases in magnitude (p<0.001) in the experimental group after treatment. Specifically, the frequencies that showed significant decreases at theta band were 3.90, 4.29 and 5.07 Hz in the frontal regions and at 4.68 and 5.46 Hz in the parietal cortex. Also, significant increases (p<0.001) were observed at alpha band (10.92 and 12.87 Hz) in posterior temporal and occipital regions in the experimental group. In contrast, the control group showed no significant changes after receiving the placebo; c) BTL assessment: We found no significant differences in the non-parametric multivariate permutation tests between groups with the BTL variables before MgV or placebo. Comparison of the after-before difference values between the two groups showed significantly greater improvement of reaction time in the experimental group in rhyming (U = 18.000; Z = -1.954; p = 0.050) and ordering of words (U = 12.000; Z = -2.487; p = 0.012) than in the control group. Discussion and future directions: Even though LD NOS criteria persisted at the end of the study in all the evaluated children, our results showed greater improvements in performance in the experimental group than in the control group for Performance IQ score in the WISC-RM, RT in rhymes, and word ordering in the BTL. Anti-epileptic drugs (AEDs) have a variety of mechanisms of action which are reflected through different anticonvulsant activities and behavioral effects. Even though mechanisms of action of the AEDs are only partially known, and behavioral effects could be exerted by an unknown mechanism of action, MgV has been typified as an attenuation of glutamate excitatory neurotransmission agent with psychotropic profile and activating effects. The results in this study support the possible usefulness of MgV in treating children with LD NOS who present with EEG interictal paroxysmal patterns but not epilepsy. However, no changes were observed in the number of paroxysmal discharges in the EEG following a six-month treatment period. Current treatments for patients with epilepsy are recommended for several years; thus the results might be due to the short period of antiepileptic treatment in our study. By contrast, source EEG analysis showed significant changes after treatment in the experimental group but no changes in the control group. These changes included both increases in frequencies within the alpha band in posterior regions as well as current decreases at frequencies within the theta band in the left frontal and parietal cortices, and these findings support the notion that the EEG was more mature after treatment. These results may be interpreted as improvement in background activity associated with improved cognitive performance. An increase at 10.92 and 12.87 Hz in right parietal and occipital areas may be related to enhancement in attention and visual perception, as well as in semantic memory processes, as a previous study have found. An important limitation of the current study is the small sample number of interictal paroxysmal patterns at the beginning of the treatment. No statistical differences were found within groups between the beginning and the end of the treatment in the experimental and control groups.
size for both groups. Therefore, this investigation can be considered a pilot study that requires replication.

**Update on the relationship between magnesium and exercise**

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Magnesium is involved in numerous processes that affect muscle function including oxygen uptake, energy production, and electrolyte balance. Because of this involvement, exercise can affect the need and utilization of magnesium. It is not surprising, therefore, that the relationship between magnesium status and exercise has received and continues to receive research attention. This attention has been partially fueled by the thought that magnesium supplementation might improve athletic performance and prevent negative consequences of strenuous or exhaustive exercise. The studies of the relationship between magnesium and exercise have continually yielded four general findings. These are: (1) Magnesium deficiency impairs exercise performance and amplifies the negative consequences of strenuous exercise. (2) Magnesium requirements are increased by strenuous exercise because of increased urinary and sweat losses. (3) Magnesium supplementation or increased intake by physically active individuals with adequate magnesium status is not likely to enhance physical performance, but should improve performance of magnesium-deficient individuals. (4) Exercise induces a redistribution of magnesium in the body. The nature and extent of this redistribution depends upon magnesium status, time after exercise for determination, and exercise intensity, duration and conditioning. A corollary to findings 1 and 2 is that exercise that induces hypomagnesemia or a deficient magnesium status can result in increased inflammatory or oxidative stress and impaired immune response, and an increased risk for pathological consequences associated with these changes. Based on finding 2 above, the magnesium need for physically active individuals could be 10% to 20% higher than established dietary guidelines. A recent report indicated that neutral magnesium balance in healthy individuals with irregular physical activity occurred at about 165 mg/d and that 237 mg/d was the 95th percentile of the estimated average requirement for magnesium. This suggests that physically active individuals probably should strive for intakes over 260-285 mg/d.

**Magnesium, exercise and osteoporosis**

**Manuel Bicho**  
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Osteoporosis and cardiovascular diseases are major public health problems, leading to increased morbidity and mortality. Recent clinical, epidemiological and genetic studies indicate that there are common pathophysiological mechanisms underlying these diseases. Oxidative stress, dyslipidaemia, inflammation and hyperhomocysteinemia are associated to bone remodelling impairment and atherosclerosis process, explaining partially the independent of age coexistence of those diseases. Magnesium deficiency, a recognized factor common to those mechanisms, can act in part through disturbance of nitric oxide pathways in a dose dependent way. Physical activity can be a non pharmacological measure to prevent and ameliorate those conditions. It probably acts during all phases of osteoporosis natural history (including intrauterine life). The actual question is how it can be more effective and balanced by nutritional intake of magnesium, modulated by constitutional factors including the genetic ones.

**Magnesium status and performance**

**Maria J. Laires**, **Cristina P. Monteiro**, **Catarina N. Matias**, **Diana A. Santos**  

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Accumulating evidence supports that athletes should pay extra attention to magnesium status.
as performing exercise is highly dependent on the regulation of magnesium homeostasis. This cation plays significant roles in promoting strength and cardiorespiratory function in healthy persons and athletes due to the key role of magnesium in the energetic metabolism, transmembrane transport, muscle contraction, hydration and immune function. Magnesium has direct effect at cellular level on Na-K ATPase, Na-K-Cl co-transport, K channels, charge screening and permeability effects on membranes. The K-Cl co-transporter is a major determinant of cell dehydration. Exhaustive exercise and training can lead to a decrease of magnesium status. Surveys of athletes reveal that frequently these individuals fail to consume a diet that contains adequate amounts of minerals, including magnesium. The ratio between calcium and magnesium intake (Ca:Mg) is rarely calculated or reported. Magnesium deficit is associated with muscle weakness, cramps, and structural damage of muscle fibers and organelles, probably as a result of increased production of reactive oxygen species, lipid and protein damage, and impaired cation homeostasis and may result in substandard training and impaired performance. In combat sports, athletes are divided according to weight. In order to qualify for their respective weight category many athletes undergo impressive weight changes preceding the competition, many times associated with severe dehydration. Alterations of cellular hydration will influence membrane stretch, membrane bound signalling systems, cytoskeleton, protein phosphorylation, the ionic interior of cell as well as the extent of macromolecular crowding in the cytosol. Intracellular water decreases have been associated with strength reductions in athletes who decrease the intracellular water compartment. An increase in intracellular magnesium might attenuate those strength reductions in athletes. Team sports such basketball, volleyball and handball, have complex demands; it seems likely that strength and power are critical to athletes’ tasks and individual as well as team performance. These athletes are required to jump and handle the ball with expertise, which requires not only strength but coordination. These athletes consume diets with less magnesium than the recommended which may compromise the adequate cellular availability of magnesium. The observed direct correlations between Mg and muscle strength performance may result from the important role of magnesium in energetic metabolism, transmembrane transport and muscle contraction and relaxation. Whether magnesium supplementation will improve performance remains to be shown. After a review of the literature, we are going to present our recent data on magnesium status and performance in combat and team sports.

Magnesium deficiency increases Thy-1\(^+\) cells (oval cells) isolated from rat liver

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Objective: we have previously shown that Mg deficiency led to oxidative stress and apoptosis in rat livers and in rat and human hepatocytes. Considering that liver tissue can regenerate in some conditions, we have hypothesized that Mg concentration could modify the number of hepatic stem cells (oval cells in rat liver). In the present study we have isolated, counted and characterized liver stem cells (Thy-1+cells) from rats receiving different dietary intake of Mg. Material and Methods: Male rats were randomly divided into three groups (n = 3 rats/group) and were fed for 5 weeks with a normal semisynthetic diet that corresponds to 0.9 g/kg Mg (Std group), with a Mg-deficient diet that corresponds to 0.15 g/kg Mg (Def group) or with a Mg-supplemented diet that corresponds to 4.5 g/kg Mg (Suppl group). Thy-1+ (CD-90) cells were immunoselected from rat livers after cell dissociation (MACS technology, Miltenyi biotech). These cells were characterized by measuring the mRNA expression of different cellular markers and also cultured for up to ten days in RPMI medium. Results: We obtained a negative correlation between the intake of Mg in the different diets and the number of Thy-1+ cells present in rat liver; indeed, Mg deficiency led to a statistically significant increase in Thy-1+ cells (P<0.05). In these cells, the expression of CD-44 at the mRNA level was increased in Mg deficient group, as compared to the standard group, whereas CD-133, HNF1, c-met, AFP and CYP3A1 mRNA expressions were decreased. Moreover, when placed in culture, small colonies of cells which were adherent to the culture support appeared and proliferated over culture time. Conclusion: we
suggested that liver regeneration can occur in rat liver submitted to a deficient dietary Mg condition, as suggested by the increase in oval cells in rat liver and their growth when cultured.


Dual-color imaging of magnesium/calcium ion activities

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Objective: Ca$^{2+}$/Mg$^{2+}$ exchange is vital to the Ca$^{2+}$-regulated muscle contraction, and Mg$^{2+}$ promotes Ca$^{2+}$ influx in various tissues. The main objective of this work was to visualize Mg$^{2+}$/Ca$^{2+}$ activities in live cells and tissues by two-photon microscopy (TPM). Material and methods: We have developed a two-photon (TP) probe (FMg2 and FMg2-AM) for intracellular free Mg$^{2+}$ ([Mg$^{2+}$]). By using FMg2-AM and BCaM, a TP probe for near membrane Ca$^{2+}$ ([Ca$^{2+}$]$_{m}$), we have monitored Ca$^{2+}$/Mg$^{2+}$ activities in live cells and tissues by TPM. Results: FMg2 showed emission maximum at 555 nm, $K_{i}^{TP}$ = 7 ± 0.2 mM, and TP action cross section ($\Phi_{S,max}$) of 76 GM at 740 nm, while the corresponding values for BCaM were 470 nm, 89 $\mu$M, and 150 GM, respectively. This allowed dual-color imaging of [Ca$^{2+}$]$_{m}$ and [Mg$^{2+}$] by using 400-450 (BCaM) and 525-600 nm (FMg2-AM) as the detection windows. Indeed, the TPM images of the probe-labeled HepG2 cells co-labeled with BCaM and FMg2-AM clearly showed the distribution of [Ca$^{2+}$]$_{m}$ and [Mg$^{2+}$] (figure 1A-C). When the cells were stimulated with 5 $\mu$M calcimycin and 10 ng/mL of epidermal growth factor (EGF), a reagent that causes a PLC$\gamma_1$- dependent Ca$^{2+}$ influx, in the presence of 1.2 mM Mg$^{2+}$, the TP excited fluorescence (TPEF) intensity increased sharply in the plasma membrane until it reached the peak value and then decreased to the baseline level (figure 1D, green curve). A similar but slower change was observed in the cytoplasm (figure 1D, red curve), indicating that the EGF-induced transport of Ca$^{2+}$ occurs at a faster rate than that of Mg$^{2+}$. When the same experiment was conducted in a Mg$^{2+}$-depleted solution, the TPEF intensity increased in the plasma membrane (figure 1E, green curve) to a lesser extent and remained nearly the same in the cytoplasm (figure 1E, red curve). This indicates that the EGF-induced influx of Ca$^{2+}$ is decreased, while that of Mg$^{2+}$ is abrogated, by Mg$^{2+}$ depletion, which concurred with literature results. We then investigated the utility of this probe in tissue imaging. The TPM images collected from Ch1 and Ch2 revealed the [Ca$^{2+}$]$_{m}$ and [Mg$^{2+}$] distributions, which did not merge (images not shown). These results confirm that BCaM and FMg2 can independently detect [Ca$^{2+}$]$_{m}$ and [Mg$^{2+}$] in live cells and tissues with minimum interference from each other.

Conclusion: Combined with BCaM, FMg2 allows dual-color imaging of Ca$^{2+}$/Mg$^{2+}$ activities in live cells and [Mg$^{2+}$]/[Ca$^{2+}$]$_{m}$ distributions in live tissues at a depth of 100-200 $\mu$m without photobleaching artifacts.

Mg intracellular content and distribution map in drug-resistant and sensitive whole cells

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Magnesium plays crucial structural and regulatory roles within all cells. Despite the extensive amount of data about the biochemistry of Mg, a complete picture of its regulation and cellular homeostasis is lacking for both conceptual difficulties and technical limitations. Although recent efforts in applying new live imaging techniques to the field of magnesium research, an accurate characterization of Mg distribution and content in the cellular environment is still lacking. The acquisition of detailed information on intracellular processes requires high spatial resolution, quantitative data, and chemical information. In order to achieve this goal, X-ray micro and nano-probe analysis represents an ideal technique, thanks to recent improvements in third generation synchrotron X-ray sources and in X-ray focusing optics. In particular,
Figure 1. (JH Han, BR Cho. Dual-color imaging of Mg\textsuperscript{2+}/Ca\textsuperscript{2+} ion activities, p. 199) A-C) Dual-channel TPM images of HepG2 cells co-labeled with BCaM (1 \mu M) and FMg2-AM (2 \mu M) collected at 400-450 (BCaM, Ch1) and 525-600 nm (FMg2-AM, Ch2), respectively. TPM images were obtained in PBS buffer (A), 200 sec after stimulation with 5 \mu M calcimycin and 10 ng/mL EGF in the presence of 1.2 mM Mg\textsuperscript{2+} (B) or no Mg\textsuperscript{2+} (C). D, E) Time course of TPEF at designated positions A (green curve) and B (red curve) in (B) and (C), respectively, after stimulation. The TPEF intensities at A and B in (B) and (C) were measured before stimulation and normalized. Cells shown are representative images from replicate experiments (n = 5). Excitation wavelength: 740 nm. Scale bar: 15 \mu m.

X-ray Fluorescence Microscopy (XRFM) is a highly sensitive method for mapping elemental distributions in cells. XRFM can map the element content but not the concentration, which is a more relevant variable in a biological context. We tackled this issue by combining XRFM with Atomic Force Microscopy (AFM) that was used to obtain morphological information of the sample analyzed. This novel experimental approach gives also the advantage to combine both high-resolution elemental and morphological information in a single cell with a sub-micrometer spatial resolution. The aim of the present study is to compare the content and the distribution of Mg in a drug-resistant and -sensitive tumor cell line. The experimental model employed was a human colon carcinoma cell line (LoVo) sensitive and resistant to doxorubicin, one of the anticancer drugs mostly employed in the therapy of several solid tumors. Preliminary data has shown a massive increase of Mg in LoVo drug-resistant cells. Moreover, the map of intracellular Mg showed marked differences in the pattern distribution between sensitive and resistant cells.
Moderately elevated magnesium concentration prevents and reverses calcification in vascular smooth muscle cells \textit{in vitro}

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Objective: To determine the effect of moderately elevated magnesium concentrations on vascular calcification \textit{in vitro}. Material and Methods: Two in vitro models of vascular calcification were used; rat aortic rings (RAR) and human vascular smooth muscle cells (hVSMCs). Calcium deposition was determined by spectrophotometry of acid extract; mRNA expression of Cbfa1, Dlx5, MGP and OPG by RT-PCR. Data are expressed as mean ± SE. Results: Incubation of RAR with high-phosphate (2.8 mM) and normal magnesium (0.8 mM) induced calcification (0.2 ± 0.0 vs. 4.8 ± 0.5 μg calcium/mg protein). Moderately elevated magnesium levels (1.4 mM) however significantly (p<0.01) reduced calcification, to 1.3 ± 0.7 μg calcium/mg protein. The magnesium-induced decrease in calcification was associated with down-regulation of the calcification markers Cbfa-1 and Osterix. Similarly, inhibitory actions in calcification of 1.4 mM magnesium were observed using hVSMCs, even at phosphate concentration as high as 3.3 mM (6.0 ± 0.3 vs. 2.7 ± 0.5 μg calcium/mg protein, p<0.001). Cbfa-1 and Osterix were also down-regulated. The natural inhibitors of calcification MGP and OPG were up-regulated by magnesium 1.4 mM as compared to the high-phosphate/normal-magnesium group. In hVSMCs cultured with high-phosphate, 1.4 mM magnesium and an inhibitor of cellular magnesium transport (2-aminoethoxy-diphenylborate) the effects of magnesium on calcification and expression of osteogenic markers were no longer observed. Calcium deposition was not different from hVSMCs only with high-phosphate (6.03 ± 0.46 vs. 5.52 ± 0.58 μg calcium/mg protein, respectively) and expression of Cbfa-1, Dlx5, MGP and OPG was similar in both groups. To test the ability of magnesium to reverse calcification, hVSMCs were incubated with 3.3 mM phosphate to induce calcification. The delayed addition of 1.4mM magnesium at day 5 produced a decreased calcification at day 9 (from 5.9 ± 0.5 to 3.2 ± 0.6 μg calcium/mg protein; p<0.001), whereas it further increased in the control group without added magnesium (9.3 ± 0.9 μg calcium/mg protein). Conclusion: A moderate increase in magnesium not only prevented, but also reversed calcification \textit{in vitro}.

Treatment of STZ diabetic rats with bis(maltolato)oxovanadium (IV) as a glucose-lowering agent provokes tissue Mg depletion and alters Mg-related serum biochemical parameters

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Objective: The fact that Mg and V participate in glucose metabolism. Intervening in the same processes, led us to consider that these two elements could be related and be acting jointly in the metabolism of carbohydrates. This study investigates the changes in the metabolism of Mg, and related blood parameters, following treatment with vanadium in streptozotocin-diabetic rats. Materials and methods: During a period of five weeks, four groups were examined: control, diabetic, diabetic-treated with 1 mgV/day or 3 mg V/day. The vanadium was supplied in drinking water as bis(maltolato)oxovanadium(IV).
ABSTRACT

The magnesium was measured in food, faeces, urine, serum, muscle, kidney, liver, spleen, heart and femur. Albumin, uric acid, urea, total-cholesterol, LDL-cholesterol, triglycerides, α-amylase, aspartate aminotransferase and alkaline-phosphatase were determined in serum. Results: In the diabetic group, levels of Mg retained and Mg content in serum and femur and α-amylase activity fell, while levels of uric acid, urea, total-cholesterol, LDL-cholesterol, triglycerides and alkaline-phosphatase and aspartate-aminotransferase activity increased compared with the control group. In the diabetic group treated with 1mg V/day, levels of Mg retained, serum Mg, urea, triglycerides and alkaline-phosphatase activity remained unchanged, while levels of uric acid, total-cholesterol and LDL-cholesterol increased and the Mg content in femur and α-amylase and aspartate-aminotransferase activity decreased compared with the diabetic untreated group. In the diabetic rats treated with 3mg V/day, renal Mg losses increased and glycaemia, Mg content in serum, kidney and femur decreased. In addition, urea, LDL-cholesterol, aspartate-aminotransferase and alkaline-phosphatase activity decreased, in comparison with untreated diabetic rats.

Conclusion: Under our experimental conditions, the hypomagnesaemia and tissue depletion of Mg caused by the treatment could be responsible, in part, for the fact that although treatment with 3mg V/day normalised the glycaemia, it did not normalise the blood parameters that had been altered by diabetes.

Tissue content of Co in hypomagnesemic rats

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Objective: Magnesium deficiency is known to be associated with many alterations in neurologic, cardiovascular, renal, endocrine and other systems. The symptoms and signs of the magnesium deficiency have been traced, in large part, to complex electrolytic interactions secondary to magnesium deficit. There is accumulating evidence that the metabolism of several trace elements is altered in magnesium deficiency and that these nutrients might have specific roles in the pathogenesis and progress of the disease. Cobalt is an essential element for life in minute amounts. It is a key constituent of metalloproteins that occurs in humans. However, several studies had demonstrated that cobalt could induce a cytotoxic effect via the oxidative stress. This study investigates the absorption, retention and tissue distribution of Co in healthy and in Mg-deficient rats, in order to determine the possible existence of metabolic interactions between Mg and Co.

Methods: Two groups were used: Control (456.4 mg Mg and 0.024 mg Co/kg food) and Mg deficient (164.4 mg Mg and 0.023 mg Co/kg food). The experiment had duration of five weeks. We measured Co levels in serum, skeletal muscle, kidney, liver, adipose tissue, heart and femur. Total metal content was analyzed by ICP-MS. Results: Consumption of the Mg-deficient diet produces a significant reduction in urinary loses of Co and increased the retention of Co. However, the absorption of Co in Mg-deficient rats was similar to that observed in the controls. No significant changes were observed in Co serum levels. The Mg-deficient diet led to increases in kidney, liver and femur content of Co. Although no significant changes were observed in Co concentration in heart following consumption of the Mg-deficient diet, an upward tendency in these values was observed. Conclusion: The results show interactions between Mg and Co in renal system and tissues studied.

Mag-fura 2 characterization of SLC41A3 in HEK293 transgenic cell line

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Magnesium is the second most abundant cation within the cell playing an important role in most of the cellular physiological processes. Intracellular free Mg²⁺ concentration ranges
between 0.5 mM and 1.2 mM Mg\(^{2+}\) concentrations are maintained within this interval by action of specific magnesium transporters. The 41\(^{\text{st}}\) family of solute carrier superfamily became interesting after identification of SLC41A1 Na\(^+\)/Mg\(^{2+}\) exchanger. Apart of SLC41A1, there are also members A2 and A3 allocated to the same family. Not much is known about function of putative Mg\(^{2+}\) transporter SLC41A2 and literally there is no information available about member A3. Here we demonstrate ability of SLC41A3 to conduct Mg\(^{2+}\) transport when overexpressed in HEK293 cells. We also show ability of SLC41A3 to form protein complexes by use of blue native PAA electrophoresis and Western blot. In conclusion we propose to be a transport mechanism able specifically or unspecifically conduct transport of Mg\(^{2+}\) in cell. Its ability to form protein complexes of higher order it could be speculated that it might be able to interact with member A1 and/or A2 of the same family or with constituents of transport mechanisms transporting solutes other than Mg\(^{2+}\).

EGF stimulates Mg\(^{2+}\) influx in mammary epithelial cells

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Objective: To determine whether EGF regulates magnesium homeostasis in mammary epithelial cells and to identify the underlying molecular mechanisms. Materials and Methods: Mammary epithelial HC11 cells were loaded with specific fluorescent ion indicators and stimulated by EGF. Intracellular calcium and magnesium fluxes were analyzed by confocal live imaging. Results: EGF stimulation induces a rapid and sustained increase in intracellular magnesium, concomitantly with a rise in intracellular calcium. The increase in intracellular Mg\(^{2+}\) derives from an influx from the extracellular compartment, as it is inhibited in the absence of extracellular magnesium, and does not depend on Ca\(^{2+}\), as the calcium chelator BAPTA does not abolish the flux. On the contrary, the increase in intracellular Ca\(^{2+}\) derives from intracellular stores, as it occurs also in the absence of extracellular calcium, and is impaired in the absence of extracellular magnesium. The TRPM7 channel-specific inhibitor waixenicin A (kindly provided by the Laboratory of Marine Biological Chemistry, Hawaii Pacific University) does not inhibit the EGF-induced magnesium influx significantly. Conclusion: These findings suggest that intracellular magnesium homeostasis is regulated by EGF. This regulation may constitute an important event in the physiological response of HC11 cells to EGF. The molecular identity of the channel which mediates the EGF-induced magnesium influx remains to be determined. The lack of inhibition by waixenicin A seems to rule out the TRPM channels. Further studies are required to ascertain which magnesium channel is involved in this pathway and to identify the downstream molecular players involved in the signal transduction which ultimately leads to cell proliferation.

Preliminary study of transdermal permeation of magnesium cream formulations across skin

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Objective: The purpose of this study was to compare the passive permeation across human skin of magnesium (Mg) from pharmaceutical grade Mg chloride (MgCl\(_2\)) formulated in cream to that of pharmaceutical grade MgCl\(_2\) in solution. Material and methods: The permeation was performed using human cadaver skin (Pel-freez, USA). The skin was soaked in the receptor medium (phosphate buffer saline 7.4) to equilibrate and then cut into appropriate size for the study. The transdermal Diffusion Cell Drive Console (Logan System FDC-6) was used to study the permeation. The transdermal permeation efficiency of Mg from MgCl\(_2\) cream I and MgCl\(_2\) cream II was studied across skin compared to positive control MgCl\(_2\) solution and negative control phosphate buffer solution. The cream or MgCl\(_2\) solution equivalent to 2.76 mg of Mg were applied per 2.52 cm\(^2\) of skin and mounted in diffusion cell. Samples were collected after 1, 2, 3, 4, 5 and 24 h and analyzed using atomic
absorption spectroscopy at 285 nm. The experiments were performed in triplicates. The results were analyzed using unpaired t-test. Results: The cumulative Mg permeation from Mg cream I, Mg cream II, MgCl₂ solution, and phosphate buffer across human skin after 24 h were found to be 29.79 ± 13.92, 24.53 ± 9.98, 6.18 ± 1.36, and 5.62 ± 1.83 µg/2.52 cm² respectively. Both creams showed statistically significant (p<0.05) Mg permeation compared with the two control solutions; Mg cream I showed greater Mg permeation than Mg cream II, but the difference was not statistically significant. The MgCl₂ solution showed a similar result to that of phosphate buffer. Conclusion: A formulated Mg cream was able to successfully deliver the Mg of pharmaceutical grade MgCl₂ across human skin. Transdermal Mg may play an important role treating symptoms of sub-optimal Mg status. However, further in vitro and animal studies are warranted to establish the efficacy of formulations.

Immunological effects in rats with a varying dietary magnesium intake
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Objective: Magnesium (Mg) deficiency is a common nutritional disorder that is linked to an inflammatory state characterized by increased plasma acute phase protein and proinflammatory cytokine concentrations. In this study, we analyzed the systemic inflammatory response to evaluate the immunological effects in rats with a low and high dietary magnesium intake. Material and methods: We had 45 male Hoffman rats for the experiment. They were randomly divided in 3 groups of 15 rats each. The groups received a dietary Magnesium intake of 30 (group A), 5,000 (group B), 700 (group C) mg per kilogram of food, with deionized water for 78 days. We sacrificed 5 rats of each group, after every 26 days. Then hemoglobin, white and red cells blood count, platelets and the C-reactive protein (CRP) were measured. Also, we analyzed the histological changes in the brain, liver, heart and kidney of each rat. Results: The Mg-deficient (group A) animals had a slower weight gain than the controls fed on the Mg sufficient diet (group B and group C). Paired t-test analysis showed that the difference in the body weights was statistically significant (P<0.05). The total circulating leucocyte pool increased progressively in rats on the Mg-deficient diet from the first week to the last week. In comparison to the rats fed in the controlled diet, rats fed to the Mg-deficient diet for 45 days had leukocytosis plus a 45% higher CRP blood level and a 30% higher liver, brain and renal damage (ANOVA, P<0.05). Our histological studies revealed more inflammatory damage in Mg-deficient rats with a longer days with the Mg-deficient diet (P<0.05), and occurred primarily during the 30 and 45 days especially in the liver (perivascular areas) were we found hepatitis (60%), multifocal coagulated necrosis (80%) and diffuse coagulated necrosis (20%). Conclusion: Our observation suggests that Mg deficiency induces an acute-phase inflammatory response that is followed by a chronic phase inflammation. We found prolonged levels of C-reactive protein, sustained neutrophilia at 30 and 45 days of Mg deprivation, and the number and size of the liver, brain and renal lesions that increased statistically significant during the last period of the experiment in a manner that closely paralleled the enhanced peripheral leukocytosis. In conclusion, we show that Mg deficiency, independently of any other changes in nutrient intake, modulates the concentration of inflammatory systemic cells and time-dependently affect and infiltrate the liver, brain and kidneys.

Magnesium and vitamin D in the prevention and therapy of hypertension
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Vitamin D and/or magnesium deficiency are independent risk factors for hypertension. Epidemiological and clinical studies have long shown an association between inadequate exposure to sunlight, vitamin D deficiency and hypertension or increased plasma-renin activity. This is additionally underlined by the fact that mean blood pressure values are lower in summer than in winter. Persons with vitamin D insufficiency (25(OH)D <30 ng/mL) have a
3.2-fold higher risk of developing hypertension than persons with a good vitamin D status. A recently published systematic review and meta-analysis came to the conclusion that vitamin D produces a fall in systolic blood pressure of $-6.18$ mmHg and a nonsignificant fall in diastolic blood pressure of $-2.56$ mmHg in hypertensive patients [1]. Animal studies have shown that vitamin D deficiency increases blood pressure through an interaction with the renin-angiotensin system. In genetically altered mice (so-called vitamin D receptor null mice), which cannot synthesize vitamin D, it was observed that renin expression, the activity of the renin-angiotensin system, and the production of angiotensin II were drastically increased. The mice developed hypertension, cardiac hypertrophy, and edema. These observations correlate with those made in normal mice, in which inhibition of vitamin D biosynthesis led to a rise in renin expression, whereas the injection of $1,25(OH)_2D$ suppressed renin expression [2-4]. Other mechanisms contributing to the antihypertensive effect of vitamin D are the direct effects of $1,25(OH)_2D$ on endothelial function, parathyroid hormone secretion and insulin sensitivity (figure 2). Vitamin D and magnesium have a mutually enhancing effect on endothelial function and vascular reactivity and on many metabolic processes (e.g. insulin metabolism). The antihypertensive effect of magnesium has been demonstrated in numerous interventional studies. Although administration of vitamin D and magnesium alone to patients with hypertension (severity II or III) is not likely to normalize blood pressure according to the WHO criteria, supplementation of vitamin D and magnesium monitored by laboratory-diagnostic tests may nevertheless allow attempts to reduce the dosage of other antihypertensive substances (e.g. diuretics, ACE inhibitors). This could certainly reduce many side effects of the antihypertensive drugs used (e.g. disturbances of glucose tolerance).


Deep ocean mineral (DOM) water accelerates recovery from fatigue after a prolonged bout of exercise in men

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Objective: Seawater is a natural source of minerals and trace elements characterized...
by high magnesium concentration. Here, we conducted a randomized, double-blind, placebo-controlled crossover study to evaluate the ergogenic value of desalinated deep ocean water (DOW).

Material and Methods: Twelve volunteers (aged 24 ± 0.76 years) performed a treadmill running at 40% maximal oxygen consumption (VO_{2\text{max}}) (30°C) until a weight drop to 3%. During recovery, drink containing DOW or pure water (Placebo) supplied every 30 min for 4 times to reach the total amount of 1.5 fold of their weight loss. Physical performance, biomarkers for muscle damage and oxidative damage, and stress hormone levels were measured during a 48-h recovery (4 h, 24 h, and 48 h) against pre-exercise level. Results: The exercise challenge caused a protracted decreased aerobic capacity (VO_{2\text{max}}) during the recovery period. In a contrary, aerobic capacity slightly increased above pre-exercise level and well above placebo level when consuming DOW drink (P<0.05). Additionally, consuming DOW drink increased explosive power at 4 h and 24 h above pre-exercise and placebo levels (P<0.05). Increases in plasma creatine kinase (CK) and myoglobin, indicatives of muscle damage, were completely eliminated when DOW drink was consumed (P<0.05). Exercise-induced increases in TBARS were also attenuated when DOW is consumed (P<0.05). No significant difference was found in stress hormone response during recovery (IL-6, cortisol, testosterone, and erythropoietin) between both trials. Conclusion: Our data provide evidence suggesting that DOW contains soluble elements which is essential for maintaining normal physical performance. This benefit is associated with preserving muscle integrity against physical challenge.

Deep ocean minerals exhibit lipid lowering properties via modulation of the AMPK-ACC pathway in hypercholesterolemic rabbits

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Objective: The deep-sea water from bedrock 662 meters under the sea level is rich in minerals such as Ca, Mg, Na, K, Fe and others. The present study was conducted to explore if deep ocean minerals (DOM) from deep-sea water could reduce serum lipid levels and prevent the development of atherosclerosis in a hypercholesterolemic rabbit model. Material and Methods: Rabbits were fed with 0.5% high cholesterol diet with concomitant daily oral ingestion of 0.1 × DOM (0.26 mL/kg), 1 × DOM (2.6 mL/kg) and 2 × DOM (5.2 mL/kg) for 8 weeks. The blood was withdrawn at 0, 2, 4, 8 weeks from ear vein for biochemical analysis. The livers were harvested at the end of 8 weeks to determine the fatty liver formation and lipid deposition. The aortas were also retrieved for staining with Sudan IV to depict the atherosclerotic plaque. Results: Our results demonstrated that 1 × DOM obviously decreased the serum levels of cholesterol, reduced lipid accumulation in liver tissues, and limited aortic fatty streaks at the end of 8 weeks of high fat diet feeding. Mechanistically, DOM activates AMPK (5'-adenosine monophosphate-activated protein kinase) followed by inhibiting the ACC (acetyl-CoA carboxylase) phosphorylation. DOM is evidently effective in reducing blood total cholesterol, oil droplet, and also decreasing fatty streak lesions in hypercholesterolemic animals. Conclusion: Our results suggest that DOM could be further developed as a potential lipid-lowering agent and a natural health food to prevent atherosclerosis.

Influence of metformin treatment on urinary magnesium and zinc loss in NIDDM adult patients

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Objectives. Metforminul is one of the most widely used drugs in the treatment of diabetes. Some cation disbalances were observed in the body of NIDDM (non-insulin dependent diabetes mellitus) patients. In this study was determined the urinary elimination of magnesium and zinc

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in adult NIDDM patients and the metformin therapy influence on these losses. Patients and methods. In the study were included 57 adult NIDDM subjects. The total urine from 24 h was collected and the magnesium and zinc concentration was determined. The determination was performed before the treatment and after 90 daily treatments with 3 g p.o. metformin. Non-including criteria were: kidney diseases, intake of food supplements containing magnesium and other bivalent cations, diuretic drugs use. A group of 25 healthy adult subjects (both genders) was the control group. The urine levels of total magnesium and zinc were determined by atomic absorption spectrophotometry. The obtained data were statistically interpreted. Results: The obtained results showed that the urinary loss of magnesium and zinc was higher in NIDDM patients compared to control group (237.28 ± 34.51 mg magnesium/24 h in NIDDM group vs. 126.25 ± 35.22 mg/24 h p<0.01 and 1347.54 ± 158.24 μg/24 h zinc in NIDDM patients vs. 851.65 ± 209 μg/24 h p<0.01. The metformin treatment significantly reduced the magnesium urinary loss in NIDDM patients (from 237.28 ± 34.51 mg magnesium/24 h to 188 ± 17 mg/24 h p<0.05. The urinary zinc elimination was not changed. Conclusions. Metformin decreases urinary magnesium while reducing blood glucose. It is possible that the reduction of the magnesium loss and the increase of concentration of this cation in the body contribute to metformin anti-diabetic effect. The metformin action was different in the case of magnesium renal loss compared with the zinc urinary loss.

Recent findings indicating that chronic latent magnesium deficiency contributes to chronic disease in humans

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Objective: Determine whether chronic latent magnesium deficiency (CLMD), indicated by serum magnesium <0.75 mmol/L or magnesium intakes less than the United States EAR (Estimated Average Requirement of 265 mg/day) affects indicators of inflammatory stress and bone health. Material and Methods: Two community-based studies were performed in which magnesium status was determined. In the first study, 100 adults, 22 males and 78 females older than 51 years were randomly assigned to two groups matched by gender, age and sleep quality. One group was given a 300 mg/day magnesium supplement as magnesium citrate, and the other a sodium citrate placebo for seven weeks. Three-day food diaries and serum magnesium concentrations were determined at the beginning and five and seven weeks after supplementation initiation. In the second study, 224 healthy postmenopausal women with similar femoral neck T scores and body mass index (BMI) were assigned to two groups; one was given a supplemental 2 mg copper and 12 mg zinc/day and the other a maize starch placebo for two years. Five-day food diaries were obtained annually. Whole body bone mineral contents, densities, and T scores were determined biannually by dual-energy X-ray absorptiometry. Results: In study one, 37 of 100 participants had serum magnesium concentrations <0.75 mmol/L, which indicated magnesium deficiency. Food diaries indicated that 58% of participants were consuming less than the EAR, which was associated with a significantly higher BMI and plasma C-reactive protein concentration (an indicator of inflammatory stress). In study two, magnesium intakes <237 mg/day (38% of participants) did not affect changes in bone status indicators induced by copper and zinc supplementation. However, women with intakes <237 mg/day exhibited markedly lower whole-body mineral contents, densities and T scores than those with intakes ≥237 mg/day. Conclusion: CLMD is associated with increased inflammatory stress and decreased bone health indicators.

Low serum magnesium levels are associated with prehypertension in healthy subjects

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Objective. To evaluate the relationship between low serum magnesium levels and preHTN in
healthy subjects. Material and methods. A total of 290 apparently healthy subjects, men and non-pregnant women, 18 to 65 years of age, were enrolled in a population-based cross-sectional study and allocated into groups with hypomagnesemia and normomagnesemia. Diabetes, hypertension, pregnancy, and intake of magnesium supplements were exclusion criteria. Hypomagnesemia was defined by serum magnesium <1.8 mg/dL levels. Prehypertension was defined by the presence of systolic blood pressure 12 to 130 mmHg and diastolic blood pressure 80 to 89 mmHg. Results. A total of 199 (68.6%) women and 91 (31.4%) men were enrolled. Hypomagnesemia was identified in 92 (46.2%) women and 53 (58.2%) men, p = 0.03. Individually with hypomagnesemia showed higher body mass index, waist circumference, systolic and diastolic blood pressure, fasting insulin levels, uric acid concentration, and Ca/Mg ratio as compared with normomagnesemic individuals. The frequency of prehypertension was significantly higher in the hypomagnesemic individuals than in the control group of normomagnesemic individuals. The systolic (r = -0.152, p = 0.01) and diastolic (r = -0.179, p = 0.002) blood pressure were inverse and significantly related with serum magnesium levels. Women with hypomagnesemia showed higher systolic and diastolic blood pressure, serum triglycerides and uric acid levels, and lower alcohol intake as compared with men with hypomagnesemia. In the group of normomagnesemic individuals, women exhibited higher systolic blood pressure and uric acid levels, and lower frequency of smoking and alcohol intake than men. In the overall population, the crude OR showed a significant association between hypomagnesemia and pre-HTN (OR 2.42; 95%CI 1.1-5.2, p = 0.01); in the multivariate logistic conditional forward analysis, adjusted by BMI, waist circumference, fasting insulin, and uric acid, hypomagnesemia and preHTN remained significantly associated (OR 1.05; 95%CI 1.001-1.092, p = 0.02).

Conclusion: Hypomagnesemia is strongly associated with preHTN in apparently healthy subjects.

Magnesium extracellular efflux induced by cAMP in HL60 cells assessed by a new hydroxyquinoline fluorescent chemosensor

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This work aimed at monitoring the intracellular magnesium movements upon cAMP stimulation by using DCHQ5, the phenyl-derivative of hydroxyquinolines fluorescent probe family, capable to assess intracellular total magnesium [1]. The homeostasis of intracellular magnesium is still not completely defined. Several studies documented the occurrence of fluxes of magnesium across the plasma membrane within minutes from the application of metabolic or hormonal stimuli. These fluxes, however, result in limited variation of free Mg2+ intracellular concentration and large changes in total Mg content. In this perspective, DCHQ5 probe, which is stable up to 30 minutes of incubation and highly retained within loaded cells, results to be the proper tool for monitoring the magnesium fluxes [1]. It has been reported that a stimulation with cAMP caused a movement of total magnesium within 10 minute after treatment in cardiomyocytes, suggesting that this stimuli caused a magnesium efflux in excitable cells [2]. In this study we tested this hypothesis in HL60 leukemic cells, not excitable but highly proliferating cell model. We evaluated Mg flux by DCHQ5 using spectrofluorimetric and cytofluorimetric assays. We observed a drastic decrease of intracellular total magnesium in the first three minutes. We also verified that at least 10% of the total intracellular amount of magnesium moved in the supernatant of stimulated cells, confirming that cAMP induced an
extracellular Mg efflux. On the other hand the evaluation of free intracellular magnesium by Mag-Fluo-4 probe revealed no variation of this fraction. These results highlight the capability of DCHQ5 to quantitatively assess the intracellular total magnesium as well as its capability of monitoring magnesium cellular fluxes. Furthermore, we showed that cAMP stimulation caused a movement of total magnesium also in non-excitable and highly proliferative cell model.


Magnesium deficiency suppresses cell cycle progression of human osteosarcoma cells

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Growing evidence indicates a correlation between magnesium availability and cell proliferation. In particular, lack of magnesium suppresses cell growth though the molecular mechanism is far to well defined. It is reported that cell proliferation is attenuated by a decrease in extracellular magnesium in capillary endothelial cells, in breast epithelial MCF7 cells and in leukemic HL-60 cells [1]. Here we investigated the effects of extracellular magnesium deficiency on cell cycle progression and expression of cell cycle regulators in the osteosarcoma cell lines SaOS-2 and U2OS. We observed that in synchronized cells caused by serum starved method, over 80% cells were distributed in G1 phase. Cell proliferation and percentage of the cells in S phase in the presence of MgCl₂ were higher than those in the absence of MgCl₂, suggesting that magnesium is involved in the cell cycle progression from G1 to S phase. After serum addition, the expression levels of p21 protein were constant in the absence of MgCl₂, as well as for its transcriptional activator p53 protein. For the first time, we demonstrated that magnesium deficiency suppressed cell cycle progression from G1 to S phase also human osteosarcoma cells. The effect of magnesium deficiency on the expression levels of p21 and p53 was not markedly evident; therefore, we might conclude that Mg acts upstream the p-53 cascade.