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## Critical issues on magnesium status and magnesium assessment, from cell to clinic

André Mazur

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[sdrmsociety@gmail.com](mailto:sdrmsociety@gmail.com)

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### Official Journal:

**Magnesium Research**





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Dear Colleague,

Welcome to the

**XV International Magnesium Symposium  
“MAGNESIUM IN HEALTH AND DISEASE”  
Bethesda, NIH – USA, March 20-22, 2019**

After the success of the latest XIV International Magnesium Symposium held in Rome June 23-24 2016, we will have our meeting in the very heart of science: NIH in Bethesda.

As usual, we collected the latest scientific data on Magnesium Research spanning from molecular mechanisms to epidemiology and clinical data. Indeed this time we bring together the forefront of molecular biology of magnesium transport and analysis and crucial nutritional aspects, as well as biological and clinical research on magnesium in diseases.

We hope that the contributions will stimulate questions, inspire discussions, hypotheses and trigger new projects and collaborations.

We invite you to share your experience, participate to discussions, bring new ideas or suggestions in a heterogeneous, multifaceted, informal atmosphere surrounded by the inspiring environment of NIH and attractive area of Washington DC.

Dr FI Wolf

Dr MJ Lenardo





SCIENTIFIC  
PROGRAM





## WEDNESDAY, MARCH 20<sup>th</sup>, MORNING

09:00 - 10:00 **Registrations**

10:00 - 10:15 **Welcome Remarks**

**Michael J. Lenardo** (Bethesda, MD, USA)

**Federica I. Wolf** (Roma, Italy)

### OPENING LECTURE

10:15 - 11:00

**Cation trafficking is essential for both nucleic acid synthesis and degradation**

**Wei Yang**

Laboratory of Molecular Biology, NIDDK,  
National Institutes of Health, Bethesda, MD, USA

## SESSION 1 Magnesium Homeostasis and Status

### Chairmen

**Andrzej Mazur** (Clermont-Ferrand, France)

**Federica I. Wolf** (Roma, Italy)

11:00 - 11:30

**Intestine, the entrance door of magnesium into the body**

**Vladimir Chubanov**

Walther-Straub Institute of Pharmacology and Toxicology,  
Ludwig Maximilians University, Munich, Germany

11:30 - 12:00

**Kidney, the key organ of magnesium homeostasis**

**Jeroen H.F. de Baaij**

Department of Physiology, Radboud University Medical Center,  
Nijmegen, The Netherlands

12:00 - 12:30

**Factors affecting magnesium status, requirements, and recommended dietary intakes**

**Forrest Nielsen**

Research Nutritionist Consultant – Grand Forks, ND, USA

12:30 - 13:00

**Oral Communications**

12:30 - 12:40

**OC01**

**Measures of muscle magnesium status from <sup>31</sup>P magnetic resonance spectroscopy are associated with age, sex, and knee extension strength**

**Donnie Cameron**

National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

12:40 - 12:50

**OC02**

**SGLT2 inhibition in the management of intractable hypomagnesemia**

**Evan C. Ray**

University of Pittsburgh, Renal-Electrolyte Division, PA, USA

12:50 - 13:00

**OC03**

**HUVEC in simulated microgravity: interplay between magnesium homeostasis and cytoskeleton**

**Laura Locatelli**

Dept Biomedical and Clinical Sciences L. Sacco, Università di Milano, Italy

13:00 - 14:00

**Welcome Buffet Lunch**



## WEDNESDAY, MARCH 20<sup>th</sup>, AFTERNOON

### SESSION 2 Magnesium in Cardio-metabolic Diseases

**Chairmen** **Andrea Rosanoff** (Pahoa, HI, USA)  
**Rhian M Touyz** (Glasgow, UK)

**14:00 - 14:30** **Magnesium and cardiovascular biology in health and disease**  
**Rhian M Touyz**  
*Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK*

**14:30 - 15:00** **Calcium: magnesium ratios, vitamin D synthesis and metabolism, and mortality**  
**Qi Dai**  
*Department of Medicine, Division of Epidemiology, Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, TN, USA*

**15:00 - 15:30** **TRPM7 and cardiovascular inflammation**  
**Francisco Rios**  
*Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK*

**15:30 - 16:00** **Coffee/Tea Break around *Posters***

**16:00 - 17:00** ***Oral Communications***

**16:00 - 16:10** **OC04**  
***Intra-hepatic cortisol production increases under Mg<sup>2+</sup>-deficiency conditions: implications for hepatic inflammation and hormonal dysmetabolism***  
**Andrea M.P. Romani**  
*Dept. of Biochemistry and Biophysics, Case Western Reserve University, Cleveland, OH, USA*

**16:10 - 16:20** **OC05**  
***Acute and chronic exercise down-regulate magnesium transporter genes in rats and diabetes***  
**Fu-Chou Cheng**  
*Dept. of Medical Research, Taichung Veterans General Hospital, Taiwan*

**16:20 - 16:30** **OC06**  
***Vascular effects of VEGF and EGF are mediated through TRPM7***  
**Zhi-Guo Zou**  
*Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK*



## WEDNESDAY, MARCH 20<sup>th</sup>, AFTERNOON

### SESSION 2 Magnesium in Cardio-metabolic Diseases

- 16:30 - 16:40**    **OC07**  
*Comparative effects of sodium–glucose cotransporter 2 inhibitors on serum magnesium levels in patients with type 2 diabetes: a network meta-analysis of randomized controlled trials*  
**Yiqing Song**  
Department of Epidemiology, Richard M. Fairbanks School of Public Health, Indiana University, Indianapolis, IN, USA
- 16:40 - 16:50**    **OC08**  
*Magnesium supplementation improves diabetic mitochondrial and cardiac diastolic function*  
**Samuel C. Dudley Jr**  
Division of Cardiology, Department of Medicine, University of Minnesota at Twin Cities, Minneapolis, MN, USA
- 16:50 - 17:00**    **OC09**  
*Effect of magnesium bicarbonate-rich water on high normal and hypertension grade 1 blood pressure: a double-blind, placebo-controlled, two-center trial*  
**Mašenjka Katić**  
NutriProject Consultancy, Zagreb, Croatia



## THURSDAY, MARCH 21<sup>st</sup>, MORNING

### SESSION 3 Magnesium in Epidemiology and Nutrition

**Chairmen** **Rebecca Costello** (Bethesda, MD, USA)  
**Qi Dai** (Nashville, TN, USA)

**09:00 - 09:30** **Magnesium metabolism and requirements in adolescents**  
**Connie M. Weaver**  
*Department of Nutrition Science, Purdue University,  
West Lafayette, IN, USA*

**09:30 - 10:00** **Magnesium and athletic performance**  
**Stella Lucia Volpe**  
*Department of Nutrition Sciences, Drexel University, Philadelphia,  
PA, USA*

**10:00 - 10:40** **Oral Communications**

**10:00 - 10:10** **OC10**  
**Global human Mg nutrition: need and supply**  
**Andrea Rosanoff**  
*Center for Magnesium Education & Research LLC, Pahoa,  
Hawaii, HI, USA*

**10:10 - 10:20** **OC11**  
**Associations of dietary and urinary magnesium, calcium  
and their ratio with blood pressure: intermap**  
**Linda Van Horn**  
*Department of Preventive Medicine, Northwestern University,  
Chicago, IL, USA*

**10:20 - 10:30** **OC12**  
**Magnesium – impact of food processing on the bioavailability  
of this shortfall nutrient**  
**Roger Clemens**  
*USC School of Pharmacy, Los Angeles, CA, USA*

**10:30 - 10:40** **OC13**  
**Observational data from a database of 23 thousand russian  
women, pregnant or with sex hormone-related conditions:  
epidemiology, diagnosis and clinical significance  
of hypomagnesemia**  
**Sofya P. Konchits**  
*Sanofi-Aventis group, Moscow, Russia*

**10:40 - 11:00** **Coffee Break around Posters**



## THURSDAY, MARCH 21<sup>st</sup>, MORNING

### SESSION 4 **Round Table: Magnesium Distribution, Biophysical status and Assessment**

*Chairmen* **Andrzej Mazur** (Clermont-Ferrand, *France*)  
**Federica I. Wolf** (Roma, *Italy*)

- 11:00 - 11:15** **Introduction: critical issues on magnesium status and magnesium assessment, from cell to clinic**  
**Andrzej Mazur**  
*Human Nutrition Unit, INRA and University of Clermont Auvergne, Clermont-Ferrand, France*
- 11:15 - 11:30** **The importance of “being” (and measuring) magnesium**  
**Stefano Iotti**  
*Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy*
- 11:30 - 11:45** **New small molecule and hybrid fluorescent sensors for the study of intracellular magnesium distribution and mobilization**  
**Daniela Buccella**  
*Department of Chemistry, New York University, New York, NY, USA*
- 11:45 - 12:00** **Magnesium clinical detection: results of the Kuwait clinical trial**  
**William B. Weglicki**  
*Department of Biochemistry and Molecular Medicine, The George Washington University School of Medicine and Health Sciences, Washington DC, USA*
- 12:00 - 12:15** **Importance of ionized magnesium measurements in various clinical settings**  
**Bogdan Milojkovic**  
*Nova Biomedical, Waltham, MA, USA*
- 12:15 - 12:45** **General discussion and conclusion**
- 12:45 - 13:30** **Buffet Lunch**



**THURSDAY, MARCH 21<sup>st</sup>, AFTERNOON****SESSION 5 Molecular Biology of Magnesium I**

**Chairmen** **Vladimir Chubanov** (Munich, *Germany*)  
**Andrea Fleig** (Honolulu, HI, *USA*)

- 13:30 - 14:00** **Magnesium channels and transporters, an overview**  
**Andrea Fleig**  
*Center for Biomedical Research, The Queen's Medical Center and University of Hawaii Cancer Center, Honolulu, HI, USA*
- 14:00 - 14:30** **TRPM7-channel-kinase activity in cellular signalling and *in vivo* immune reactions**  
**Susanna Zierler**  
*Walther Straub Institute of Pharmacology and Toxicology, Faculty of Medicine, Ludwig Maximilians University, Munich, Germany*
- 14:30 - 15:00** **TRPM7-dependent gene transcription**  
**Thomas Gudermann**  
*Walther Straub Institute of Pharmacology and Toxicology, Faculty of Medicine, Ludwig Maximilians University, Munich, Germany*
- 15:00 - 15:30** **Coffee/Tea Break around *Posters***
- 15:30 - 16:00** **The PRL phosphatases-CNNMs complex, a magnesium-dependent autoregulate system in the maintenance of intracellular magnesium homeostasis and cellular bioenergetics**  
**Michel L. Tremblay**  
*Department of Biochemistry, McGill University, Montreal, Quebec, Canada*
- 16:00 - 16:30** **Magnesium transporters of SLC41 family and Mrs2**  
**Gerhard Sponder**  
*Institute of Veterinary Physiology, Veterinary Medicine, Free University of Berlin, Germany*
- 16:30 - 17:00** ***Oral Communications***
- 16:30 - 16:40** **OC14**  
**Osteogenic signaling pathways stimulated by magnesium**  
**Charles Sfeir**  
*The Center for Craniofacial Regeneration, University of Pittsburgh, PA, USA*
- 16:40 - 16:50** **OC15**  
**Magnesium and its transporters: impact on osteogenic differentiation of human bone mesenchymal stem cells**  
**Sara Castiglioni**  
*Dept. of Biomedical and Clinical Sciences L. Sacco, University of Milano, Italy*
- 16:50 - 17:00** **OC16**  
**Regulation of and by the murine TRPM7 channel-kinase**  
**J. Ashot Kozak**  
*Department of Neuroscience, Cell Biology and Physiology, Wright State University, Dayton, OH, USA*



## FRIDAY, MARCH 22<sup>nd</sup>, MORNING

### SESSION 6 Molecular Biology of Magnesium II

**Chairmen** **Andrea Fleig** (Honolulu, HI, USA)  
**Michael J. Lenardo** (Bethesda, MD, USA)

**08:30 - 09:00** **Deletions or mutations in the MAGT1 gene cause defects in N-linked glycosylation of cellular glycoproteins and are responsible for glycosylation disorders with alternative phenotypes**  
**Reid Gilmore**

*Department of Biochemistry and Molecular Pharmacology,  
University of Massachusetts Medical School, MA, USA*

**09:00 - 09:30** **Mg<sup>2+</sup> regulation of the immune response**  
**Michael J. Lenardo**

*Laboratory of Immune System Biology National  
Institute of Allergy and Infectious Diseases  
National Institutes of Health, Bethesda, MD, USA*

**09:30 - 09:40** **Oral Communication**

**09:30 - 09:40** **OC17**  
**Cadmium exposure enhances TRPM7 expression and intracellular magnesium leading to EMT in mammary epithelial cells**  
**Mathieu Gautier**

*Laboratory of Cellular and Molecular Physiology,  
University of Picardie Jules Verne, Amiens, France*





**FRIDAY, MARCH 22<sup>nd</sup>, MORNING****SESSION 7 Magnesium in Neuropathology and Stress**

**Chairmen** **Louise Dye** (Leeds, UK)  
**Gisèle Pickering** (Clermont-Ferrand, France)

**09:40 - 10:10** **Magnesium and vitamin B6 combination on severe stress in healthy adults**  
**Gisèle Pickering**  
*Clinical Pharmacology Department, CHU, Université Clermont Auvergne, Clermont-Ferrand, France*

**10:10 - 10:40** **Magnesium in stress and anxiety: lessons from preclinical and clinical studies**  
**Louise Dye**  
*Human Appetite Research Unit - School of Psychology - University of Leeds, UK*

**10:40 - 11:10** **Mg<sup>2+</sup> as a second messenger in early developmental stages of neurons**  
**Kotaro Oka**  
*Center for Biosciences and Informatics, School of Fundamental Science and Technology, Graduate School of Science and Technology, Keio University, Yokohama, Japan*

**11:10 - 11:40 Oral Communications**

**11:10 - 11:20** **OC18**  
***The anticancer EGFR-TKI, erlotinib, causes cutaneous, cardiac and hypomagnesemic side effects that were inhibited by aprepitant, a substance P receptor-blocker***  
**William B. Weglicki**  
*Department of Biochemistry and Molecular Medicine, The George Washington University School of Medicine and Health Sciences, Washington DC, USA*

**11:20 - 11:30** **OC19**  
***Magnesium pidolate vs magnesium sulphate: which salt performs better to protect the brain?***  
**Romeo Valentina**  
*Dept. of Biomedical and Clinical Sciences L. Sacco, University of Milano, Italy*

**11:30 - 11:40** **OC20**  
***Magnesium acetyltaurate: a prospective drug candidate for retinal neuroprotection in glaucoma***  
**Igor Iezhitsa**  
*Center for Neuroscience Research, Faculty of Medicine, University Teknologi, Selangor, Malaysia*

**11:40 - 12:10** **Coffee and Light Lunch around [Posters](#)**



## FRIDAY, MARCH 22<sup>nd</sup>, AFTERNOON

### SESSION 8 Magnesium in Chronic Diseases and Aging

**Chairmen** **Andrzej Mazur** (Clermont-Ferrand, *France*)  
**Halima Ouadid-Ahidouch** (Amiens, *France*)

**12:10 - 12:40** **Magnesium, skeletal muscle health, sarcopenia, osteoporosis and fracture risk**

**Ailsa A. Welch**

*Department of Epidemiology & Public Health, Norwich Medical School, University of East Anglia, Norwich, UK*

**12:40 - 13:10** **The janus of magnesium in the bone**

**Jeanette AM Maier**

*Dept. of Biomedical and Clinical Sciences L. Sacco, University of Milano, Italy*

**13:10 - 13:30** **Oral Communications**

**13:10 - 13:20** **OC21**

**Dietary magnesium alleviates murine colitis through upregulation of TRPM6 and modulation of intestinal microbiota**

**Valentina Trapani**

*General Pathology, Faculty of Medicine A. Gemelli, Università del Sacro Cuore, Roma, Italy*

**13:20 - 13:30** **OC22**

**Combination antiretroviral therapy (cART) altered redox/nitrosative genes, enhanced systemic, renal and cardiac dysfunction in HIV-1 transgenic rats: protection by Mg-supplementation**

**I. Tong Mak**

*Department of Biochemistry and Molecular Medicine, The George Washington University School of Medicine and Health Sciences, Washington DC, USA*

**13:30 - 13:45** **Award Nomination and Closing Ceremony**

**Chairmen** **Michael J. Lenardo** (Bethesda, MD, USA)  
**Federica I. Wolf** (Roma, Italy)





POSTERS

# POSTERS

## P01

### Monitoring the serum Ca/Mg ratio in response to magnesium supplementation

*Rebecca Costello<sup>1</sup>, Forrest Nielsen<sup>2</sup>, Andrea Rosanoff<sup>3</sup>, Michael Plesset<sup>3</sup> and Ronald Elin<sup>4</sup>*

<sup>1</sup> NIH Office of Dietary Supplements, Bethesda, MD, USA;

<sup>2</sup> USDA Grand Forks, ND, USA;

<sup>3</sup> Center for Magnesium Education and Research, Pahoia, HI, USA;

<sup>4</sup> University of Louisville, KY, USA

## P02

### Magnesium and depression in patients at risk of suicide

*Emily Young<sup>1</sup>, Andrea Rosanoff<sup>2</sup>, Ph.D., Rebecca Costello<sup>2</sup>, Ph. D., Michael R. Plesset<sup>2</sup>, Bernadette P. Marriott, Ph. D.<sup>1</sup>*

<sup>1</sup> Medical University of South Carolina, Charleston, SC, USA;

<sup>2</sup> Center for Magnesium Education and Research, Pahoia, HI, USA

## P03

### Overview of the NIH research portfolio on magnesium

*Rebecca B. Costello and Karen Regan*

Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA

## P04

### Necessity of determining ionized magnesium in relation to total magnesium in blood

*Branimir Radosavljević<sup>1</sup>, Svetlana Ignjatović<sup>2</sup>*

<sup>1</sup> Institute of Chemistry in Medicine, Faculty of Medicine, University of Belgrade, Belgrade, RS;

<sup>2</sup> Center for Medical Biochemistry, Clinical Center of Serbia and Faculty of Pharmacy, University of Belgrade, Belgrade, RS

## P05

### Magnesium and insulin stimulate glucose uptake in differentiated bovine adipocytes

*S. Jurek<sup>1</sup>, M.A. Sandhu<sup>1,2</sup>, S. Trappe<sup>1</sup>, M. Kolisek<sup>1,3</sup>, G. Sponder<sup>1</sup> and J.R. Aschenbach<sup>1</sup>*

<sup>1</sup> Freie Universität Berlin;

<sup>2</sup> PMAS-Arid Agriculture University, Rawalpindi, Pakistan;

<sup>3</sup> Comenius University Bratislava, Slovakia

## P06

### Magnesium in hypertensive heart disease

*Klaus Kisters, Uwe Gröber*

Med. Clinic I, St. Anna-Hospital, Herne, University of Bochum & ESH Excellence Centre, Bochum, Germany

## P07

### TRPM7 is a potential pharmacological target in progressive kidney fibrosis

*Sayuri Suzuki<sup>1</sup>, Reinhold Penner<sup>1,2</sup>, Andrea Fleig<sup>1,2</sup>*

<sup>1</sup> Queen's Center for Biomedical Research, The Queen's Medical Center, Honolulu, HI, USA;

<sup>2</sup> Cancer Biology Program, University of Hawaii Cancer Center, Honolulu, HI, USA

## P08

### Incidence of hypomagnesemia and hypermagnesemia in a university hospital

*A. Catalano, D. Chilà, F. Bellone, N. Morabito, S. Loddo, S. Benvenga, F. Corica*

Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

# POSTERS

## P09

### Stressed cerebral organoids: can magnesium help?

*Alessandra Cazzaniga, Valentina Romeo, Laura Locatelli, Roberta Scrimieri, Sara Castiglioni, Jeanette A.M. Maier*  
Dipartimento di Scienze Biomediche e Cliniche L. Sacco, Università di Milano, Milano I-20157, Italy

## P10

### Magnesium deficiency syndrome

*Corneliu D. Zeana, MD, PhD*

Angiomedica Clinic, Bucharest, Romania

## P11

### Involvement of CaSR, claudin-14 and 16 in the development of hypermagnesiuria associated with tubulo-interstitial nephropathy

*Taisuke Shimizu<sup>1</sup>, Kaori Takayanagi<sup>1,2</sup>, Akira Kurosawa, Masaaki Terao<sup>1</sup>, Hiroaki Hara<sup>1</sup>, Takatsugu Iwashita<sup>1</sup>, Maiko Yamasaki<sup>1</sup>, Yumiko Nakamura<sup>1</sup>, Mariko Sato<sup>1</sup>, Yu Shimozato<sup>1</sup>, Akira Ikari<sup>3</sup>, Hajime Hasegawa<sup>1</sup>*

<sup>1</sup>Department of Nephrology and Hypertension, Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan;

<sup>2</sup>Ishikawa Kinenkai, Kawagoe Ekimae Clinic, Saitama, Japan;

<sup>3</sup>Laboratory of Biochemistry, Gifu Pharmaceutical University, Gifu, Japan

## P12

### A focus on magnesium in epilepsy research and treatment – a review

*Marija Stanojević<sup>1,2</sup>, Srdjan Lopacic<sup>1</sup>, Jelena Nesovic Ostojic<sup>1</sup>, Svetolik Spasic<sup>1</sup>, Sanjin Kovacevic<sup>1</sup>, Vladimir Nedeljkov<sup>1</sup>, Milica Prostran<sup>3</sup>*

<sup>1</sup>Institute for Pathological Physiology, Faculty of Medicine, University of Belgrade, Belgrade, RS;

<sup>2</sup>Department of Medical Neurobiology, Institute for Medical Research, Israel-Canada, Hebrew University – Hadassah School of Medicine, Jerusalem, IL;

<sup>3</sup>Institute for Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, RS

## P13

### Magnesium (Mg) deficiency enhances oxidative stress and migratory potential of colon cancer cells

*Gopal Kumar<sup>1,2</sup>, Swati Madankumar<sup>2</sup>, Xiangying Xue<sup>2</sup>, Prodyot Chatterjee<sup>2</sup> and Christine N Metz<sup>1,2</sup>*

<sup>1</sup>Elmezzi Graduate School of Molecular Medicine, Manhasset, NY, USA

<sup>2</sup>The Center for Biomedical Sciences, Feinstein Institute for Medical Research, Manhasset, NY, USA

## P14

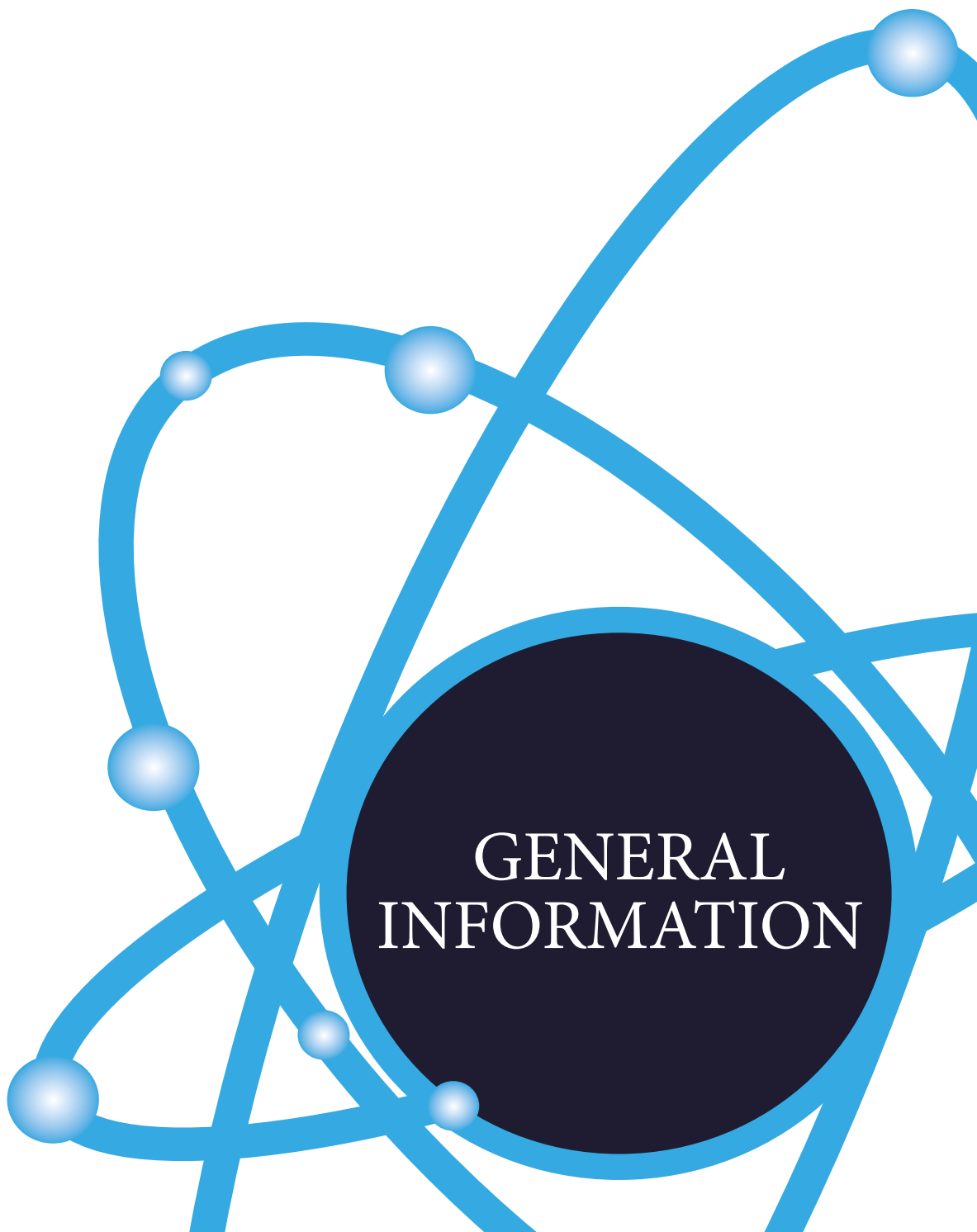
### Cetuximab Impacts on magnesium uptake in the colon

*Giuseppe Pietropaolo<sup>1</sup>, Daniela Pugliese<sup>2</sup>, Francesca Luongo<sup>1</sup>, Federica I. Wolf<sup>1</sup> and Valentina Trapani<sup>1</sup>*

<sup>1</sup>Istituto di Patologia Generale, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario “Agostino Gemelli” IRCCS, Roma, IT

<sup>2</sup>IBD Unit, Presidio Columbus Fondazione Policlinico Universitario “Agostino Gemelli” IRCCS, Università Cattolica del Sacro Cuore, Roma, IT





GENERAL  
INFORMATION



## Symposium Venue



*National Library of Medicine*

### *National Library of Medicine*

National Institutes of Health

Building 38A

8600 Rockville Pike

Bethesda, MD 20894

**Registrations:** All IMS 2019 fees shown are in US DOLLARS (US\$) and in EURO (€) - You are free to choose your currency.

Registration categories	Amount
Delegate as SDRM member (*)	350 US\$ or 301 €
Delegate (non member)	500 US\$ or 431 €
Student (**)	125 US\$ or 108 €

(\*) As an SDRM member (€ 40 regular membership - € 25 for students under 30 years old), you are entitled to:  
 - reduced registration fees to SDRM sponsored and organized events,  
 - access to the website reserved area with free download of proceedings and selected presentations from SDRM events.

(\*\*) As a student, you are kindly requested to show your student card.



Delegates' registration fee includes:

- **Congress kit (badge, pen, program/abstract book and bag)**
- **Access to the scientific sessions**
- **Access to the commercial exhibition**
- **Luncheons on Wednesday March 20<sup>th</sup> and Thursday March 21<sup>st</sup>**
- **Coffee/Tea Break around Posters on Wednesday March 20<sup>th</sup> and on Thursday March 21<sup>st</sup>**
- **Coffee and Light Lunch around Posters on Friday March 22<sup>nd</sup>**
- **Certificate of attendance**

The Symposium Cocktail Reception is not included in the Registration fee.

### **Certificate of Attendance**

A certificate of attendance will be issued to all registered participants at the end of the Symposium upon request at the Secretariat Desk.

Please note that the certificate of attendance will be issued upon receipt of your completed symposium survey. Your feedback will be highly appreciated.



## Symposium Cocktail Reception



*Old Ebbitt Grill*

**Thursday, March 21<sup>st</sup> 2019**

**19:30 - 21:30**

### **Old Ebbitt Grill**

*Established in 1856, Old Ebbitt Grill is Washington's oldest saloon, just steps from The White House and museums downtown. It was a favorite of Presidents Grant, Cleveland, Harding, and Theodore Roosevelt and is still a popular meeting spot for political insiders, journalists, celebrities and theater-goers.*

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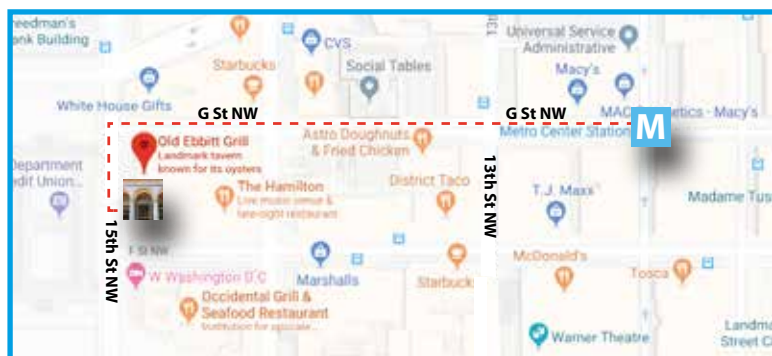
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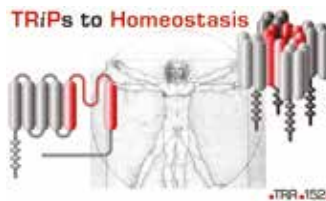


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**ABSTRACTS**  
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Speakers







# Cation trafficking is essential for both nucleic acid synthesis and degradation

Wei Yang

*Laboratory of Molecular Biology, NIDDK, National Institutes of Health, USA*

Enzyme catalysis has been studied for over a century. How it actually occurs has not been visualized until recently. By combining *in crystallo* catalysis with X-ray diffraction analysis of reaction intermediates, we have observed the processes of DNA synthesis and RNA degradation at unprecedented atomic details. Contrary to the canonical view that enzyme-substrate, transition state, and enzyme-product states have identical atomic composition and catalysis occurs by a two-Mg<sup>2+</sup>-ion mechanism with rearranging of electrons, protons and electrostatic charges, we have discovered that it is essential for enzyme-substrate complexes to capture a third Mg<sup>2+</sup> ion en route to product formation. Unlike the canonical two metal ions, which are coordinated by DNA polymerases and nucleases, this third metal ion is free of enzyme coordination and is liganded by nucleic acid substrate/product only. Its location between the  $\alpha$ - and  $\beta$ -phosphates of dNTP in the case of DNA synthesis suggests that the third metal ion may drive phosphoryl-transfer by breaking the existing phosphodiester bond in dNTP. In contrast, during RNA degradation, the transiently bound third Mg<sup>2+</sup> ion appears to activate the nucleophilic water. Experimental data indicate that binding of the third metal ion may be the rate-limiting step in DNA synthesis, and the free energy associated with metal-ion binding may overcome the activation barrier to the DNA synthesis reaction.

**References:** 1. Gao, Y. & Yang, W. (2016) Capture of a third Mg<sup>2+</sup> is essential for catalyzing DNA synthesis, *Science*, 352, 1334-1337. 2. Samara, N. & Yang, W. (2018) Cation trafficking propels RNA hydrolysis, *NSMB*, 25, 715-721. 3. Nakamura, T., Zhao, Y., Yamagata, Y., Hua, Y.J. & Yang, W. (2012) Watching DNA polymerase make a phosphodiester bond. *Nature*, 487, 196-201.

## Biographical Sketch

Wei Yang, Ph. D.  
Distinguished Investigator  
Lab. Mol. Biol. NIDDK  
National Institutes of Health - Bethesda, MD, Bethesda, USA  
Contact: [weiy@niddk.nih.gov](mailto:weiy@niddk.nih.gov)



# Intestine, the entrance door of magnesium into the body

## Vladimir Chubanov

*Walther-Straub Institute of Pharmacology and Toxicology, LMU Munich, Munich, Germany*

Mg<sup>2+</sup> regulates many physiological processes and signalling pathways. However, little is known about the mechanisms underlying the organismal balance of Mg<sup>2+</sup>. Approximately 10 plasma membrane Mg<sup>2+</sup> channels have been proposed including TRPM6 and TRPM7. TRPM6 and TRPM7 are bifunctional proteins comprising a TRP channel segment linked to an  $\alpha$ -type protein kinase. Recently, we have uncovered the molecular and organismal roles of TRPM6 in mice. We showed that TRPM6 activity in the placenta and yolk sac is essential for embryonic development. In adult mice, TRPM6 is required in the intestine to maintain organismal Mg<sup>2+</sup> balance, but is dispensable in the kidney. In our follow-up studies, we aimed to define a role of TRPM7 in adult mice. The function of TRPM7 was assessed by single channel analysis of TRPM7, phenotyping of TRPM7-deficient cells in conjunction with metabolic profiling of mice carrying intestine- and kidneys-restricted null mutations in *Trpm7*, and animals with a global 'kinase-dead' point mutation in the gene. Overall, our study offers a new paradigm for the physiological role of TRPM7.

### Biographical Sketch

Group Leader, Walther-Straub Institute of Pharmacology and Toxicology, LMU Munich, Germany. Studies of my group are focused on TRPM6 and TRPM7, bifunctional proteins comprising a channel segment linked to an  $\alpha$ -type protein kinase. Loss-of-function mutations in the human *TRPM6* gene cause an autosomal recessive disorder, hypomagnesemia 1, intestinal (HOMG1) also called hypomagnesemia with secondary hypocalcemia (HSH). Mechanistically, the regulation of Mg<sup>2+</sup> homeostasis by these two channel kinases and the pathophysiological consequences of TRPM6 mutations are only poorly understood. Therefore, the major goal of our studies is to attain mechanistic knowledge about the physiological and pathophysiological roles of kinase-coupled channels using gene-modified mice and primary cells derived from TRPM6- and TRPM7-deficient animals.  
Contact: [vladimir.chubanov@lrz.uni-muenchen.de](mailto:vladimir.chubanov@lrz.uni-muenchen.de)



Institute for Molecular Life Sciences  
Radboudumc

# Kidney, the key organ of magnesium homeostasis

Jeroen H.F. de Baaij

*Department of Physiology, Radboud University Medical Center, Nijmegen, The Netherlands*

**Purpose:** Magnesium ( $Mg^{2+}$ ) is an essential ion to the human body, playing an instrumental role in supporting and sustaining health and life. As the second most abundant intracellular cation after potassium, it is involved in over 600 enzymatic reactions including energy metabolism and protein synthesis. Although  $Mg^{2+}$  availability has been proven to be disturbed during several clinical situations, serum  $Mg^{2+}$  values are not generally determined in patients.

**Materials and methods:** This presentation aims to provide an overview of the regulation of  $Mg^{2+}$  in human health and disease.

**Results:** Over the last decade, several hereditary forms of renal causes of hypomagnesemia have been deciphered, including mutations in transient receptor potential melastatin type 6 (TRPM6), claudin 16, and cyclin M2 (CNNM2). These disease were instrumental in gaining knowledge about the reabsorption of  $Mg^{2+}$  in the kidney. This presentation provides an extensive and comprehensive overview of  $Mg^{2+}$  reabsorption in the kidney and disturbances which may result in hypomagnesemia. This includes hypomagnesemia that is the consequence of the use of certain types of drugs, such as diuretics, epidermal growth factor receptor inhibitors and proton pump inhibitors.

**Conclusion:** Studying hereditary hypomagnesemia aids to increase our understanding of renal  $Mg^{2+}$  handling.

## Biographical Sketch

Jeroen de Baaij is a physiologist interested in the molecular and genetic origin of renal electrolyte disorders, including hypomagnesemia. His research focusses on the identification and characterization of new genes involved in renal magnesium reabsorption. He identifies the genetic origin of disease in patients with magnesium disturbances by next generation sequencing techniques. Using a wide range of molecular, cellular and physiological techniques, he examines new genes or new regulatory pathways in magnesium (re)absorption in the kidney. Supported by NWO Rubicon and Veni Grants and a Dutch Kidney Foundation Kolff Grant, his team is also examining the role of magnesium in the prevention of cardiovascular complications and in the development of the type 2 diabetes mellitus.

Contact: [Jeroen.deBaaij@radboudumc.nl](mailto:Jeroen.deBaaij@radboudumc.nl)



## Factors affecting magnesium status, requirements, and recommended dietary intakes

**Forrest Nielsen**

*Research Nutritionist Consultant - Grand Forks, USA*

Determination of the public health concern about magnesium (Mg) in health and disease has been confounded by the lack of practical measures of status, and thus resulting in difficulty in determining nutritional requirements. Some attempts at associating Mg with a chronic disease have used the current United States Dietary Reference Intakes (DRIs) as a status assessment measure. Use of these DRIs is problematic because recent evidence suggests that they should be updated and based on body weight. Based on this evidence, suggestions for the Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) for a 70 kg individual are 175 and 250 mg/day, respectively. However, numerous dietary and physiological factors can affect the need for Mg, and thus affect the use of the current or suggested DRIs to assess Mg status. Calcium intakes above the normal requirements might decrease Mg balance and exacerbate signs of Mg deficiency. Magnesium deficiency apparently occurs often in obesity because of increased need to counteract inflammatory stress induced by adipose tissue dysfunction. Deficiency in anti-oxidant nutrients such as vitamin E and selenium can exacerbate a response to low dietary intake. Dietary modifiers of Mg absorption and excretion affect balance and thus the need for Mg. Factors decreasing Mg balance include low dietary protein and non-fermentable fiber, while factors that can increase balance include fructose and fermentable fiber and fructose-containing oligosaccharides. Establishing DRIs to assess the Mg status of an individual or a population group needs to consider their physiological characteristics and dietary habits and be aware the DRIs may need updating.

### Biographical Sketch

Research Nutritionist Consultant, Research Nutritionist and former Center Director, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota, USA. Principal Investigator for 14 outpatient and in-house metabolic unit human studies determining the deficiency signs and nutritional importance of boron, copper, iron, magnesium, and zinc. Magnesium studies focused on determining sub-clinical deficiency signs, status indicators, and nutritional requirements. Results still used to make recommendations for daily dietary intakes. Contact: [FrostyNielsen@yahoo.com](mailto:FrostyNielsen@yahoo.com)



# Magnesium and cardiovascular biology in health and disease

**Rhian M Touyz**

*Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK*

Magnesium is a vital and abundant intracellular divalent cation involved in all major cellular and physiological processes, primarily through its nucleotide-binding properties and its regulation of enzymatic activity. Derangements in magnesium homeostasis, which have the potential to contribute to birth defects and multiple clinical pathologies, including arrhythmias, diabetes and cardiovascular disease, are likely under-detected and under-reported. Physiologically  $Mg^{2+}$  regulates cell growth, apoptosis, migration and contraction/dilation and is especially important in the normal contractile functioning of the heart and vessels. Pathologically, perturbations in  $[Mg^{2+}]_i$  and alterations in magnesium transporters influence cellular inflammation, fibrosis, cytoskeletal disorganization, immune dysregulation and contraction/dilation. Clinically  $Mg^{2+}$  deficiency and mutations in magnesium transporters have been implicated in various disorders including neurodegenerative diseases, immune-deficiency, pre-eclampsia, stroke, metabolic and cardiovascular diseases. Factors contributing to hypomagnesemia include drugs (diuretics, EGFR inhibitors, calcineurin inhibitors, laxatives and proton pump inhibitors), diet, kidney disease and malabsorption syndromes. The role for magnesium as a therapeutic agent is being tested in numerous clinical trials. This presentation will present new insights into the molecular biology and transmembrane transport of magnesium and will discuss clinical implications of magnesium in health and disease with a focus on vascular biology and cardiovascular disease.

## Biographical Sketch

Director of the Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK. She has received numerous academic and research awards from the American Society of Hypertension, the Canadian Society of Hypertension, the Quebec Society of Hypertension, the American Physiological Society and the American Heart Association (AHA). Her main focus of research relates to molecular, cellular and vascular mechanisms of hypertension. Prof Touyz is a clinician-scientist and her areas of research include clinical and experimental hypertension, signal transduction, oxidative stress, ion transport, vascular biology, adipose tissue biology and diabetes. She has a particular interest in translational and clinical research. Specific areas of research include 1) NADPH oxidases, oxidative stress and redox signaling; 2) vascular biology of angiotensin II and aldosterone; 3) TRPM6/7 and ion transport in the vasculature and 4) tyrosine kinases, inflammation and vascular remodeling.  
Contact: [Rhian.Touyz@glasgow.ac.uk](mailto:Rhian.Touyz@glasgow.ac.uk)



# Calcium: magnesium ratios, vitamin D synthesis and metabolism, and mortality

Qi Dai

Qi Dai<sup>1</sup>, Xiangzhu Zhu<sup>1</sup>, Yiqing Song<sup>2</sup>, Rebecca B Costello<sup>3</sup>, Andrea Rosanoff<sup>3</sup>, Lei Fan<sup>4</sup>, Martha J. Shrubsole<sup>1</sup>

<sup>1</sup> Department of Medicine, Division of Epidemiology, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, USA

<sup>2</sup> Department of Epidemiology, Richard M. Fairbanks School of Public Health, Indiana University-Purdue University Indianapolis, USA

<sup>3</sup> Center for Magnesium Education and Research, Pahoehoe, HI

<sup>4</sup> Master of Public Health Program, Vanderbilt University School of Medicine, Nashville, USA

**Purpose:** For patients with “magnesium (Mg)-dependent vitamin-D-resistant rickets”, characterized by reduced 1,25 (OH)<sub>2</sub>D, intramuscular infusion with vitamin D up to 600,000 IU alone did not lead to any improvements in vitamin D deficiency. However, Mg supplementation substantially reversed this resistance to vitamin D treatment. Furthermore, we reported from observational studies in the general US population that Mg intake significantly interacted with vitamin D intake in affecting vitamin D status as well as interacted with circulating 25(OH)D in risk of cardiovascular disease(CVD) mortality. We test in a randomized trial the hypothesis that Mg supplementation differently affects vitamin D metabolism dependent on baseline 25(OH)D levels.

**Materials and methods:** The current study is a double-blind 2x2 factorial randomized controlled trial conducted in Vanderbilt University Medical Center. A precision-based dosing strategy of Mg supplementation was used to reduce the Ca:Mg ratios in the diet to around 2.3. Changes in plasma 25(OH)D<sub>3</sub>, 25(OH)D<sub>2</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>2</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub> were measured by LCMS.

**Results:** We found that the effects of Mg treatment on plasma concentrations of 25(OH)D<sub>3</sub>, 25(OH)D<sub>2</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub> were significantly different by baseline concentrations of 25(OH)D, and statistically significant interactions persisted after Bonferroni corrections. Mg supplementation increased 25(OH)D<sub>3</sub> when baseline 25(OH)D levels were close to 30 ng/ml, but decreased it when baseline 25(OH)D was higher (from about 30 to 50 ng/ml). Magnesium treatment significantly affected 24,25(OH)<sub>2</sub>D<sub>3</sub> when baseline 25(OH)D levels were at 50 ng/ml, but not at 30 ng/ml. On the other hand, magnesium treatment increased 25(OH)D<sub>2</sub> as baseline 25(OH)D increased.

**Conclusion:** Our findings suggest that reducing Ca:Mg ratio to around 2.3 may be important for optimizing 25(OH)D status. For example, a U-shaped relationship between plasma 25(OH)D concentration and risk of incident CVD and levels of 25(OH)D around 30 ng/ml was linked to the lowest risk of incident CVD.

## Biographical Sketch

Dr. Dai has extensive expertise in nutritional and molecular epidemiologic studies. Dr. Dai's research focus is to identify and investigate nutrient-nutrient and gene-nutrition interactions in the etiology of cancer and other chronic diseases and to subsequently develop precision-based prevention strategies. In particular, Dr. Dai has studied nutrient-nutrient (i.e. magnesium with calcium or vitamin D) and nutrient (i.e. magnesium with calcium or vitamin D)-gene interactions in the etiology and prevention of colorectal neoplasia and other chronic diseases.

Contact: [qi.dai@Vanderbilt.Edu](mailto:qi.dai@Vanderbilt.Edu)



# Chanzyme TRPM7 protects against cardiovascular inflammation and fibrosis

Francisco Rios

Francisco J. Rios<sup>1</sup>, Zhi-guo Zou<sup>1</sup>, Ryszard Nosalski<sup>1</sup>, Livia L. Camargo<sup>1</sup>, Silvia Lacchini<sup>2</sup>, Alexey G Ryazanov<sup>3</sup>, Lillia Ryazanova<sup>4</sup>, Sarah McGrath<sup>5</sup>, Tomasz J. Guzik<sup>1</sup>, Carl S. Goodyear<sup>5</sup>, Augusto C. Montezano<sup>1</sup>, and Rhian M. Touyz<sup>1</sup>

<sup>1</sup> Institute of Cardiovascular and Medical Sciences, BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, United Kingdom

<sup>2</sup> Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, São Paulo, Brazil

<sup>3</sup> Department of Pharmacology, Rutgers Robert Wood Johnson Medical School, USA

<sup>4</sup> Lewis Sigler Institute of Integrative Genomics, Princeton University, Princeton, USA

<sup>5</sup> Centre of Immunobiology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom

**Purpose:** We previously demonstrated that TRPM7 is regulated by vasoactive mediators and plays a protective role in hypertension. Here we hypothesized that TRPM7-kinase is cardiovascular protective through effects on pro-inflammatory and pro-fibrotic processes.

**Material and methods:** Wild-type (WT) and heterozygote mutant mice for TRPM7-kinase (M7+/-) were studied. Vascular function and inflammatory cell migration were studied in vivo by intravital microscopy. Cardiac function was assessed by echocardiogram. Immune cells were investigated by flow cytometry. Fibrosis was investigated by sirius-red staining. Bone-marrow derived macrophages (BMDM) and cardiac fibroblasts (CF) were obtained from WT and M7+/-.

**Results:** Leukocytes from M7+/- showed reduced velocity (47%), increased adhesion (222%) and transmigration (480%) in small arteries. Vessels from M7+/- showed increased VCAM-1(33-fold), iNOS (12-fold), and IL-12 (6.8-fold) vs WT. M7+/- presented increased plasma levels of the cardiac fibrosis marker galectin-3 (Gal-3) (2.5±0.3ng/mL vs WT:1.5±0.2ng/mL). Hearts from M7+/- showed increased Gal-3 (16.6±3.6 vs WT 9.2±1.2 cells/field), collagen area (6.7% vs WT 2.6%), protein expression for fibronectin (280%), TGFβ (125%), p-Smad3 (66%), and reduced E/A ratio 30%. Increased expression of fibronectin (75%), TGFβ (80%), and macrophages (50%) was observed in spleens from M7+/- mice. Hearts from M7+/- had reduced total [Mg<sup>2+</sup>] (1.2±0.06 vs WT 1.6±0.14 mmol/mg), increased inflammatory infiltration CD45+ cells (6±0.6% vs WT 4±0.4%). Cardiac macrophages from M7+/- presented 45% reduction of intracellular Mg<sup>2+</sup> [Mg<sup>2+</sup>]<sub>i</sub> and high CD206 expression (30% vs WT 14%), which are important in fibrosis. [Mg<sup>2+</sup>]<sub>i</sub> was also reduced in circulating monocytes (50%) from M7+/- . BMDM from M7+/- had increased Gal-3, determined by WB (1.7-fold) and ELISA (2.6±0.05 vs WT 2.1±0.09ng/mL), IL-10 (807±92 vs WT 305±37 pg/mL), IL-6 (84±8 vs WT 13±5 pg/mL). A similar profile was observed in resident peritoneal macrophages. Treatment of CF with macrophage supernatant from M7+/- exhibited increased expression of fibronectin (43%) and PCNA (36%). These effects were reduced by Mg<sup>2+</sup> treatment.

**Conclusion:** TRPM7-kinase plays a cardiovascular protective role by preventing inflammation and fibrosis. These processes are Mg<sup>2+</sup> -dependent.

## Biographical Sketch

Dr Rios obtained his PhD in 2009 (Immunology) from the University of Sao Paulo (Brazil) and Karolinska Institute (Sweden). Following a post-doctoral fellowship in Sao Paulo, he moved to Glasgow in 2013 as a Research Fellow at the Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK. He has published more than 40 peer-reviewed papers and presented numerous talks at national and international conferences. His research focuses on the role of TRPM7 on the inflammatory response with a particular interest on how TRPM7 influences immune cell activation and effects on cardiovascular diseases.

Contact: [Francisco.Rios@glasgow.ac.uk](mailto:Francisco.Rios@glasgow.ac.uk)





# Magnesium metabolism and requirements in adolescents

## Connie M. Weaver

*Connie M. Weaver, Sisi Cao, Joanna K. Hodges*

*Department of Nutrition Science, Purdue University, West Lafayette, IN*

**Purpose:** Magnesium requirements have not been updated in the Dietary Reference Intakes (DRI) since 1978. Recommendations were set based on the factorial method to replace losses. The functions of magnesium are broad ranging because it is a cofactor in 300 enzymes, has an important role in energy transfer and vitamin D status, and plays an important structural role with 60% residing in bone. Surveys conducted over 30 years indicate rising calcium-to-magnesium food-intake ratios in the United States. These ratios have not been previously measured in adolescents. The interaction of magnesium with other electrolytes, such as calcium and sodium, is an important consideration for intake recommendations across the age spectrum.

**Methods:** Magnesium metabolism will be reviewed, especially as influenced by other electrolytes. Intakes of magnesium in adolescents compared to recommendations will be reviewed.

**Results:** Average intakes in adolescents are suboptimal relative to current recommendations. It is estimated that for males and females aged 14-18 years, over 70% and 90% failed to meet the Estimated Average Requirement (EAR) for magnesium, respectively. Magnesium deficiency can result from inadequate intake, excess excretion or altered homeostasis. Magnesium excretion varies by subgroup. Black adolescent females excrete less urinary magnesium compared to whites. Magnesium retention is similar between races but higher with the low-sodium diet. High calcium intake, on the other hand, does not alter magnesium balance in adolescent girls. Adequate magnesium intakes may protect against inadequacy of other electrolytes and add protection against excess salt intake.

**Conclusions:** The gaps in evidence needed to revise magnesium recommendations are great. Too few resources have been invested in magnesium research. The new DRI category of Chronic Disease Risk Reduction offers an opportunity to refine public health recommendations on magnesium, especially for adolescents. Ratios of magnesium to sodium and calcium may be more important than intake of a single mineral.

### Biographical Sketch

Connie M. Weaver, Ph.D., is a Distinguished Professor at Purdue University in the Department of Nutrition Science in West Lafayette, Indiana. She is an elected member of The National Academies of Science, Engineering, and Medicine since 2010 and a member of the Food and Nutrition Board. She is a member of the FDA Science Advisory Board and the NIH Advisory Committee on Research on Women's Health. She is founder and director of the Women's Global Health Institute (WGHI) at Purdue University. She is Deputy Director of the National Institutes of Health funded Indiana Clinical and Translational Science Institute since 2008. Her research interests include mineral bioavailability, calcium metabolism, and bone and cardiovascular health. She is on the Board of Trustees of the International Life Sciences Institute and Showalter Biomedical Research Committee. Dr. Weaver was appointed to the 2005 Dietary Guidelines Advisory Committee for Americans. She has published over 400 research articles to date. Dr. Weaver received a Bachelor of Science and Master of Science in food science and human nutrition from Oregon State University. She received a Ph.D. in food science and human nutrition from Florida State University and holds minors in chemistry and plant physiology.  
Contact: [weavercm@purdue.edu](mailto:weavercm@purdue.edu)



# Magnesium and athletic performance

## Stella Lucia Volpe

*Department of Nutrition Sciences, Drexel University, Philadelphia, USA*

It has been well-established that magnesium is a required micronutrient for over 300 reactions in the body. Many of these metabolic reactions are related to energy production and expenditure. Despite its relationship to energy production and energy expenditure, there are a paucity of data on the effects of magnesium on athletic performance. For this presentation, research on the following will be discussed: 1) Assessment of magnesium intake in various athletes; and, 2) Effects of magnesium supplementation on athletic performance.

### Biographical Sketch

Stella Lucia Volpe, PhD, RDN, ACSM-CEP, FACSM, is Professor and Chair of the Department of Nutrition Science at Drexel University, Philadelphia, PA. Her degrees are in both Nutrition and Exercise Physiology; she also is an American College of Sports Medicine (ACSM) Certified Clinical Exercise Physiologist® and a Registered Dietitian Nutritionist. Dr. Volpe's research focuses on obesity and diabetes prevention using traditional interventions, mineral supplementation, and by altering the environment to result in greater physical activity and healthy eating. She also conducts research on athletes of all levels, from recreational to professional. Prior to beginning her faculty appointment at Drexel University, Dr. Volpe was on faculty at the University of Pennsylvania, and previous to that, she was on faculty at the University of Massachusetts Amherst. Dr. Volpe is Editor-in-Chief of *Current Nutrition Reports*. She is an associate editor of *ACSM's Health & Fitness Journal*® and the *Translational Journal of ACSM*. Contact: [SLV43@drexel.edu](mailto:SLV43@drexel.edu)



## Introduction: critical issues on magnesium status and magnesium assessment, from cell to clinic

**Andrzej Mazur**

*Human Nutrition Unit, INRA and University of Clermont Auvergne, Clermont-Ferrand, France*

This speech will introduce a roundtable on the assessment of magnesium status, focusing on current knowledge and future trends. Suboptimal magnesium intake in a large part of the population in western countries supports an increased risk of latent magnesium deficiency. This mild and chronic deficiency is linked to a wide spectrum of health disorders that can be considered nonspecific and can lead to progressive and severe health deterioration, especially cardiometabolic diseases. In turn, several pathophysiological conditions e.g. metabolic syndrome, obesity, type 2 diabetes, stress and dysfunctions related to aging can contribute and aggravate chronic magnesium deficiency. The diagnosis of latent deficiency is difficult because of nonspecific clinical symptoms and magnesemia often within reference intervals. Current clinical laboratory tests are almost limited to total serum magnesium. Less frequently ionized serum magnesium, red blood cell magnesium and urinary excretion of magnesium are measured. However, the assessment of magnesium status to diagnose latent deficiency remains problematic because only subtle changes occur in these parameters and there is a large inter-individual variability. Magnesium is primarily an intracellular cation and is found mainly in bone and soft tissues. Therefore, it is difficult to develop a rapid and robust test representative of intracellular magnesium or body magnesium in humans. The purpose of the presentation and the roundtable is to discuss progress in various areas (chemistry, molecular biology, mass spectrometry), offering great opportunities for the development of new methods for evaluating magnesium status, e.g. use of stable isotopes of magnesium, new dyes for intracellular magnesium or magnesium metabolism related gene expression measurements. In addition, it can be considered that magnesium deficiency diagnosis and deficiency risk patterns can be obtained from combined data composed of questionnaires, laboratory tests and physiological measurements.

### Biographical Sketch

Director of the Human Nutrition Unit, INRA and University of Clermont Auvergne, Clermont-Ferrand, France, editor-in-chief of the "Magnesium Research"- Official Journal of the International Society for the Development of Research on Magnesium. His lab is involved in research intended to provide knowledge of the mechanisms by which nutrients, especially micronutrients, contribute to the prevention of age-related diseases. His research on magnesium has been focused on its role in inflammation and the CVD risk as well as on magnesium status assessment. Contact: [andre.mazur@inra.fr](mailto:andre.mazur@inra.fr)



# The importance of “being” (and measuring) magnesium

## Stefano Iotti

*Department of Pharmacy and Biotechnology (FABIT), University of Bologna, Bologna, IT*  
*National Institute of Biostructures and Biosystems- Rome-Italy*

**Purpose:** In my speech, I talk about the involvement of magnesium in the fundamental biochemical processes of cell life and how different approaches and techniques for detecting and quantifying magnesium in cells allows to unveil new aspects on its metabolic role.

**Materials and methods:** Different techniques capable of assessing Mg in living tissues and cells will be presented:

- a) studies employing <sup>31</sup>P-NMR in vivo to study the Mg homeostasis in vivo in the human brain and skeletal muscle;
- b) the application of a novel class of fluorescent dyes capable to assess the total intracellular Mg concentration;
- c) innovative approaches to quantify and mapping Mg in single cells by synchrotron-based x\*-ray microscopy techniques.

**Results:** Several studies will presented showing the possibilities offered by the different approaches presented to untangle at least in part the complex role of Mg in different fundamental metabolic processes of living organisms

**Conclusion:** The involvement of Mg in the main metabolic pathway is well known. However, the complete comprehension of the molecular mechanisms governing the Mg homeostasis is still far away.

### Biographical Sketch

Prof. Stefano Iotti, currently works at Department of Pharmacy and Biotechnology of the University of Bologna coordinating the research group of Molecular Imaging, Biosensors and Cell Biology. He has contributed to the development of in vivo NMR spectroscopy in basic research and in diagnostic applications. His scientific activity ranged from organic and physical chemistry to biochemical thermodynamics. He contributed to the development of a novel approach to simplify the treatment of the thermodynamics of complex systems. At present the research activity is devoted to the study of magnesium homeostasis in cell culture combining synchrotron X-ray fluorescence and the use of a novel class of fluorescent chemo-sensors.

Contact: [stefano.iotti@unibo.it](mailto:stefano.iotti@unibo.it)



NEW YORK UNIVERSITY

# New small molecule and hybrid fluorescent sensors for the study of intracellular magnesium distribution and mobilization

Daniela Buccella

Michael Brady<sup>1</sup>, Kevan Chu<sup>1</sup>, Jessica J. Gruskos<sup>1</sup>, Mu-Chun Liu<sup>1</sup>, Guangqian Zhang<sup>1</sup> and Daniela Buccella<sup>1</sup>

<sup>1</sup> Department of Chemistry, New York University, New York, USA

**Purpose:** To develop fluorescent indicators for organelle-specific visualization and quantitation of cellular Mg<sup>2+</sup>

**Materials and methods:** Fluorescent sensors were synthesized and characterized by a combination of NMR, MS and UV-Vis absorption and fluorescence emission techniques. Metal binding profile in vitro was studied in aqueous PIPES buffer at pH 7. Magnesium detection in various cells lines was demonstrated by widefield microscopy. Organelle targeting was assessed by fluorescence colocalization with organelle stains in microscopy images.

**Results:** Ratiometric fluorescent sensors functionalized with lipophilic cations for mitochondria targeting, or a chlorohexyl moiety for *in cellulo* covalent attachment to HaloTag-fused proteins in specific organelles, were synthesized and characterized by various spectroscopic techniques. The sensors display low millimolar dissociation constants for Mg<sup>2+</sup> suitable for detection of intracellular free Mg<sup>2+</sup>. Phosphonium-functionalized sensors displayed preferential accumulation in mitochondria, whereas protein-conjugated sensors could be directed to various organelles such as the nucleus and Golgi. A fluorogenic two-step protein bioconjugation protocol, incorporating a tetrazine quencher, was developed for the purpose of increasing the signal-to-background ratio in microscopy imaging using the hybrid sensors. Through this protocol, local activation of the fluorescence signal can be achieved within organelles of interest with ~10 fold enhancement upon protein conjugation. A simpler, one-step bioconjugation protocol was also developed and demonstrated. The hybrid sensors combine the fast ratiometric response characteristic of small-molecule fluorescent sensors with the ease of subcellular localization of genetically encoded proteins. Furthermore, the hybrid design enhances significantly the intracellular retention of the sensors, enabling observation of Mg<sup>2+</sup> changes in time course experiments lasting more than 18 h; these could not be performed with current small molecule sensors due to their rapid extrusion from the cell. The new sensors and bioconjugation strategies display general applicability, and Mg<sup>2+</sup> changes in various cellular process could be monitored.

**Conclusion:** Small-molecule fluorescent indicators can be functionalized with organelle-targeting moieties to direct their subcellular localization. A general strategy for local activation of the fluorescence via fluorogenic bioorthogonal reactions within organelles enables constraining the signal to compartments of interest. Hybrid sensors also enable long term imaging that is limited with small molecule counterparts. The new sensors and staining methodologies enable imaging of intracellular metal mobilization with subcellular resolution and low background.

## Biographical Sketch

Daniela Buccella, Associate Professor in the Department of Chemistry at New York University in New York, USA. She received a PhD in Chemistry from Columbia University, and completed NIH-sponsored postdoctoral training at the Massachusetts Institute of Technology. Her research group at NYU focuses on the development of new molecular probes and imaging strategies for the study of cellular metal homeostasis and chromatin remodeling by metalloenzymes. She has extensive experience in the development of fluorescent indicators for cellular detection of magnesium. Contact: [dbuccella@nyu.edu](mailto:dbuccella@nyu.edu)





## Clinical detection of cellular magnesium: the Kuwait clinical trial

**William B. Weglicki**

*W B Weglicki, I T Mak, N Zghoul and B Silver*

*The George Washington University School of Medicine, Dept. of Biochemistry & Molecular Medicine;  
Dasman Diabetes Institute (DDI), and IntraCellular Diagnostics Inc.*

**Purpose:** To assess cellular and circulating levels of magnesium in hypomagnesemic type 2 diabetic patients before and after supplementation with oral magnesium tablets, and the effects on parameters of oxidative stress and inflammation.

**Materials and Methods:** This single arm study of 47 hypomagnesemic diabetic patients was performed over a three month period, and was supported by a grant from the Kuwait Foundation for Advancement of the Sciences. Patients were screened for exclusion criteria before signing consent forms. The study was registered in [clinicaltrials.gov/NCT01980459](https://clinicaltrials.gov/NCT01980459). Participating patients (32% non-obese and 68% obese) were supplemented with 336 mg of Mg (as MagTab SR tablets from Niche Pharmaceutical Co., TX). The clinical staff of DDI were trained to obtain and perform slide fixation of the sublingual mucosal cells for elemental X-ray analysis (EXA Test, Intra-Cellular Diagnostics, Inc., Medford, OR). Serum magnesium levels were obtained monthly along with CRP, TNF $\alpha$ , HbA1c, 8-isoprostane, and the clinical assessment of BP, EKG and other parameters for safety at the DDI clinical / research laboratory site. The low normal serum Mg level at the DDI was 0.74mmol/L (1.8 mg %). The serum Mg of patients initially averaged 0.71 mmol/L and, rose to 0.73 mmol/L ( $p < 0.001$ ) after three months. The matched cellular Mg (normal range 34-42 mEq/L) rose from 33.1 to 35.5 mEq/L ( $p < 0.01$ ); interestingly, the cellular potassium (reference range 80-240 mEq/L) rose from 144 to 185 mEq/L, ( $p < 0.01$ ), both in normal range. The TNF $\alpha$  levels decreased from 9.1 to 6.1 ( $p < 0.001$ ) and 8-Isoprostane levels decreased from 91.7 to 71.7 pg/mL ( $p < 0.05$ ). The CRP levels and HbA1c levels did not decrease significantly over the three month period.

**Conclusion:** Treatment of hypomagnesemic type 2 diabetic patients with 336mg/day of Mg lactate tablets significantly increased the cellular and serum Mg levels to normal range along with significant elevations of normal K cellular levels; significant decreases were found for TNF $\alpha$  and 8-isoprostane levels.

### Biographical Sketch

Professor of Biochemistry and Molecular Medicine, and Medicine (Cardiology), the George Washington University School of Medicine and Health Sciences.

His earlier research on the role of oxidative stress/free radicals in cardiovascular ischemia/reperfusion injury led to the ESR spin trapping/identification of some of the injurious elevated ROS species, and the characterization of cardio-protective drugs with antioxidant potency. Later studies of the cardiomyopathy due to magnesium deficiency (MgD) identified: elevations of superoxide and nitric oxide in rodent models, and the role of substance P-induced neurogenic inflammation in the pathobiology due to MgD.

Contact: [wweg@gwu.edu](mailto:wweg@gwu.edu)



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## Importance of ionized magnesium measurements in various clinical settings

**Bogdan Milojkovic**

*Nova Biomedical, Waltham, MA, USA*

**Purpose:** In normal adults serum magnesium is present in three forms: 20% is protein bound, 65% is in ionised form and the rest is complexed with various anions such as phosphate and citrate. Magnesium testing nowadays is mainly performed by the central laboratory measuring serum magnesium. Central laboratory methods measure the total magnesium (tMg) present in serum and do not distinguish between the three forms, mentioned above. As such the levels of ionized magnesium (iMg), the physiologically active component, in blood or serum are often not known.

There is evidence to show that monitoring and managing ionized dysmagnesemia improves outcomes compared to using total serum magnesium. For example, evidence shows that targeting magnesium correction therapy on the basis of iMg instead of tMg improves outcomes in critically ill patients. iMg can also be useful in unmasking unrecognized hyper- and hypo-magnesemic and false hyper-magnesemic hemodialysis patients. Ionized dysmagnesemia has been reported in various other clinical conditions.

However, until recently iMg testing has not been available as a routine marker and as such is not being monitored routinely in patients. Today iMg is available as a marker on a low maintenance point of care blood gas analysers together with other relevant parameters. This provides an opportunity to elucidate further the role of iMg as a one of the key parameters in managing patients in different clinical settings.

The aim of my presentation is to give an overview and elucidate potential importance of ionized magnesium testing in different clinical conditions.

### Biographical Sketch

Bogdan followed academic research pathway after graduating from School of Medicine in Belgrade, Serbia. He's done postdoctoral research at Yale University (USA), ERASMUS Medical Center (Netherlands) and Dundee University. He then transitioned to clinical research working for NIHR Clinical Research Networks managing studies in stroke and dementia. His next assignments took him to pharmaceutical industry where he worked for Allergan and Novo Nordisk. Last 5 years he spent in diagnostics industry working for Roche diagnostics and now leading EU Medical and Scientific Affairs team for Nova Biomedical.

Contact: [Bmilojkovic@novabio.com](mailto:Bmilojkovic@novabio.com)



# Magnesium channels and transporters, an overview

## Andrea Fleig

Center for Biomedical Research, The Queen's Medical Center and University of Hawaii Cancer Center, Honolulu, USA

**Purpose:** The last two decades have seen the identification of several protein targets implicated in cellular and systemic magnesium homeostasis [1, 2]. Some of these targets have been validated functionally to act as bona fide magnesium channels/transporters. Others await functional confirmation, and still others cannot be regarded as direct magnesium transporters anymore. This presentation will provide a brief history of magnesium channels and transporters and a brief overview on the latest consensus.

1. Kolisek M, Sponder G, Pilchova I, et al (2018) Magnesium Extravaganza: A Critical Compendium of Current Research into Cellular Mg<sup>2+</sup> Transporters Other than TRPM6/7. *Rev Physiol Biochem Pharmacol*. [https://doi.org/10.1007/112\\_2018\\_15](https://doi.org/10.1007/112_2018_15)
2. Schäffers OJM, Hoenderop JGJ, Bindels RJM, de Baaij JHF (2018) The rise and fall of novel renal magnesium transporters. *Am J Physiol Renal Physiol* 314:F1027–F1033. <https://doi.org/10.1152/ajprenal.00634.2017>

### Biographical Sketch

Associate Director of Biomedical Research, Center for Biomedical Research, The Queen's Medical Center, Honolulu, HI, USA.

Her research focuses on cellular signaling events as they relate to health and disease by elucidating ion channel function and its relationship to cell signaling. Mediated by very fruitful national and international collaborations, this work has led to the original identification and biophysical as well as functional characterization of several novel ion channels, including the channel-kinase TRPM7. TRPM7 has been identified as a central mechanism for magnesium homeostasis, and possibly trace metal regulation, in mammals. Her current work has expanded into medical drug development focusing on ion channel targets with the vision to translate her research into patient care.

Contact: [afleig@hawaii.edu](mailto:afleig@hawaii.edu)





# TRPM7-channel-kinase activity in cellular signalling and *in vivo* immune reactions

## Susanna Zierler

Walther Straub Institute of Pharmacology and Toxicology, Faculty of Medicine, LMU Munich, Munich, Germany

**Purpose:** The dual-function transient-receptor-potential protein, TRPM7, combines a Ca<sup>2+</sup>- and Mg<sup>2+</sup>-permeable channel with a serine/threonine kinase. It has been shown to be essential for immune cell proliferation and development. However, the precise mechanisms how TRPM7 is involved in immune regulation remain elusive.

**Materials and methods:** Using different genetic mouse models, we decipher an essential role for this unique channel-kinase in cellular signaling and *in vivo* immune function.

**Results:** Investigating the role of TRPM7-kinase in balancing immune system homeostasis, we found that its disruption in mice triggers altered susceptibility to allergic reactions. Our findings highlight a regulatory role of Mg<sup>2+</sup> and TRPM7-kinase activity on mast cell degranulation and immunological reactivity [1]. Previously it was shown that conditional knock out of *Trpm7* in the T cell lineage disrupts thymopoiesis, and results in altered cytokine expression profiles. Using a homozygous kinase-dead mouse model with a single point mutation at the active site of the kinase, we demonstrated that TRPM7-kinase activity is not essential for thymopoiesis. Importantly, we found that the enzymatic activity of TRPM7 is required for intraepithelial T cell homeostasis. Kinase-dead mice lack gut intraepithelial T cells due to an intrinsic defect in CD103 transcriptional regulation. TRPM7-kinase activity controls TGF- $\beta$ -induced CD103 expression as well as proinflammatory T<sub>H</sub>17 cell differentiation, but is dispensable for anti-inflammatory, regulatory T cell (T<sub>reg</sub>) differentiation. Notably, we identified the C-terminal SXS-region of SMAD2 as novel substrate of the TRPM7-kinase. Moreover, we found that the TRPM7-kinase activity promotes gut colonization by T cells in acute graft-versus-host-disease [2].

**Conclusion:** Thus, our results unravel a fundamental role of the TRPM7-kinase in immune cell function and suggest a therapeutic potential of kinase inhibitors in averting allergic reactions and acute graft-versus-host-disease. Reinstatement of immune homeostasis via TRPM7-kinase blockade might also be beneficial for the treatment of autoimmune diseases.

[1] Zierler S., Sumoza-Toledo A., Suzuki S., O'Duill F., Ryazanova L. V., Penner R., Ryazanov A. G., and Fleig A. TRPM7 kinase activity regulates murine mast cell degranulation. *The Journal of Physiology*, 594, 2957-70, 2016

[2] Romagnani A., Vettore V., Rezzonico-Jost T., Hampe S., Rottoli E., Nadolni W., Perotti M., Meier M.A., Hermanns C., Geiger S., Wennemuth G., Recordati C., Matsushita M., Muehlich S., Proietti S., Chubanov V., Gudermann T., Grassi F., Zierler S. TRPM7 kinase activity is essential for T cell colonization and alloreactivity in the gut. *Nature Communications*, 8:1917. doi: 10.1038/s41467-017-01960-z, 2017.

### Biographical Sketch

Group leader at the Walther Straub Institute of Pharmacology and Toxicology, Faculty of Medicine, LMU Munich, Germany.

The aim of her research is to gain a better understanding of the importance and regulation of cation channels at the cellular level as well as in the context of *in vivo* immune reactions. This should lead to the identification of novel pharmacological targets against pro-inflammatory diseases, leukemia and allergies. Particular interest lies on the melastatin-like 'transient receptor potential' TRP divalent cation channel TRPM7, and its enzymatic domain, which encodes for a serine/threonine kinase.

Contact: [susanna.zierler@lrz.uni-muenchen.de](mailto:susanna.zierler@lrz.uni-muenchen.de)



## TRPM7-dependent gene transcription

**Thomas Gudermann**

*Thomas Gudermann<sup>1</sup>, Sandra Voringe<sup>1</sup>, Tobias Siekmann<sup>1</sup>, Susanna Zierler<sup>1</sup>, Vladimir Chubanov<sup>1</sup>, Susanne Muehlich<sup>1,2</sup>*

*<sup>1</sup> Walther Straub Institute of Pharmacology and Toxicology, Faculty of Medicine, LMU Munich, Germany*

*<sup>2</sup> Department of Chemistry and Pharmacy, Molecular and Clinical Pharmacy, University of Erlangen, Germany*

TRPM7 is a unique member of the transient receptor potential channel TRP superfamily. At its cytosolic C-terminal end TRPM7 contains a serine/threonine protein kinase domain. The relationship between TRPM7 channel and kinase activity is only partially understood. The TRPM7 channel domain modulates kinase activity through changes of the intracellular Mg<sup>2+</sup> concentration. Genetic inactivation of the *trpm7* gene at different developmental stages in mice underscored a major impact of TRPM7 on the induction of developmental gene programs. Mechanistically, at least two different general modes of gene regulation by TRPM7 have been worked out: The kinase domain has been reported to be cleaved and to translocate to the nucleus to modify the epigenetic chromatin phosphorylation landscape thereby affecting gene expression profiles. An additional mechanism has recently been uncovered by us in T lymphocytes where a transcription factor, Smad2, is phosphorylated by TRPM7 kinase to control TGF- $\beta$  induced gene expression. Here, we report a novel general mechanism of gene regulation by TRPM7. TRPM7 blockade results in growth arrest of carcinoma cells in vitro and of xenografts in vivo. Therefore, the TRPM7 channel may represent a promising new oncology target at the plasma membrane.

### Biographical Sketch

Director of Walther Straub Institute of Pharmacology and Toxicology, LMU Munich, Germany;

Vice Dean of the Faculty of Medicine, LMU Munich, Germany

He is a member of the German National Academy of Sciences and has received various awards from national and international scientific societies, i.e. German Society of Endocrinology, Society of Toxicology. He is currently heading a large center grant of the German Research Foundation (DFG), Transregional Collaborative Research Center 152, focusing on the maintenance of body homeostasis by Transient Receptor Potential (TRP) ion channels. In addition, he is speaker of a research consortium, Research Training Group 2338, dealing with the identification of molecular targets in lung toxicology, also funded by the German Research Foundation (DFG).

His main focus of research relates to the physiological and pathophysiological role of TRP channels for mineral homeostasis, the role of G protein-coupled receptors in mechanobiology, and the identification of new therapeutic targets in lung toxicology.

Contact: [thomas.gudermann@lrz.uni-muenchen.de](mailto:thomas.gudermann@lrz.uni-muenchen.de)



## The PRL phosphatases-CNNMs complex, a magnesium-dependent autoregulate system in the maintenance of intracellular magnesium homeostasis and cellular bioenergetics

Michel L. Tremblay

Serge Hardy<sup>1</sup>, Noriko Uetani<sup>1</sup>, Elie Kostantin<sup>1,2</sup>, Michel L. Tremblay<sup>1,2</sup>

<sup>1</sup> Goodman Cancer Research Centre, McGill University, Montreal, Quebec, Canada

<sup>2</sup> Department of Biochemistry, McGill University, Montreal, Quebec, Canada

**Purpose:** The Phosphatase of Regenerative Liver (PRL) enzymes (PRL-1,-2,-3; gene name PTP4A1, PTP4A2, PTP4A3) are members of the protein tyrosine phosphatase family. These enzymes have been identified as key contributors to tumor progression and metastasis in several human cancers, yet the molecular basis of their pro-oncogenic property is unclear. To better understand their function and mechanism of action we investigated their contribution to magnesium homeostasis and its consequences on cellular metabolism.

**Materials and methods:** We employed several approaches from: mass spectrometry studies, biochemical and cell based assays, and knockout mouse models to characterize the function of the mammalian PRLs-CNNMs complex.

**Results:** Using mass spectrometry studies, we identified the CNNM magnesium transporter as key binding partners of PRLs in an evolutionarily conserved complex that regulates the intracellular magnesium concentration. Importantly, either the abolishment of complex formation or PRL-2 knockdown resulted in reduced magnesium transport.

As cellular magnesium transport is regulated by intrinsic mechanisms controlling various magnesium transporters and magnesium uptake in cells, we also aimed to examine the involvement of PRLs in the adaptive response related to changes in magnesium availability. Using various cell lines cultured under low magnesium conditions, we observed a decrease in intracellular magnesium levels correlating with a marked increase of PRL-1 and -2 (PRL-1/2) expression. Although the expression levels of the magnesium transporter CNNM3 remained unchanged after magnesium depletion, we found that the interaction between endogenous PRL-1/2 and CNNM3 was also markedly increased, indicating a pivotal regulatory role for PRL-1/2 in the complex to compensate for decreased magnesium availability.

In addition, we uncovered that this rapid change of PRL expression, modulated by intracellular magnesium levels, employs a mechanism involving its 5' untranslated mRNA region. Mutations or targeting by CRISPR/Cas9 of a conserved upstream open reading frame present in the mRNA leader derepresses PRL protein synthesis and attenuate the translational response to magnesium levels. Mechanistically, magnesium depletion reduces intracellular ATP levels but upregulates PRL protein expression via activation of the AMPK/mTORC2 pathway, which controls cellular energy status. Hence, altered PRL-2 expression leads to metabolic reprogramming of the cells.

**Conclusion:** These findings uncover a magnesium sensitive mechanism controlling PRL expression, which plays a role in cellular bioenergetics.

### Biographical Sketch

Distinguished James McGill Professor in the Department of Biochemistry at McGill University in Montreal, Canada. He funded and directed the Rosalind and Morris Goodman Cancer Research Centre from 2000 to 2012. A fellow of the Royal Society of Canada, Dr. Tremblay's laboratory focuses on characterizing the function and regulation of several members of the Protein Tyrosine Phosphatase (PTP) gene family using both biochemical and genetic approaches. He has several translational research programs towards clinical applications of PTP inhibitors in the fields of immunotherapy and cancer. Within international collaborations, he has published key findings on the interaction of the CNNMs and PTP4As gene sub-families and their relationships between magnesium and cancer.

Contact: [michel.tremblay@mcgill.ca](mailto:michel.tremblay@mcgill.ca)



# Magnesium transporters of SLC41 family and Mrs2

## Gerhard Sponder

*Institute of Veterinary Physiology, Veterinary Medicine, Free University of Berlin, Berlin, Germany*

The effective regulation of cellular Mg<sup>2+</sup> homeostasis requires mechanisms that mediate influx and extrusion of the ion across the plasma membrane as well as mechanisms conveying intracellular Mg<sup>2+</sup> storage, distribution between organelles and mobilization.

The solute carrier family (SLC) 41 comprises three members (A1-A3) which exhibit distant homology to the bacterial Mg<sup>2+</sup>-transporter MgtE. SLC41A1 is ubiquitously expressed in mammalian tissues with the highest levels in heart, testis, adrenal and thyroid glands, prostate and ovaries (1). The protein is localized in the plasma membrane where it mediates the extrusion of Mg<sup>2+</sup>. This efflux mechanism is strongly dependent on extracellular Na<sup>+</sup> implying that the protein acts as a Na<sup>+</sup>/Mg<sup>2+</sup> exchanger (2).

Knowledge about the role of SLC41A2 in cellular Mg<sup>2+</sup> homeostasis is still rudimentary. The protein seems to be expressed in most human tissues. However, it is unclear if the protein resides in the cytoplasmic membrane or in intracellular compartments (3).

SLC41A3 has initially been described as a Mg<sup>2+</sup> channel of the plasma membrane. Recent evidence, however, points to a role of this protein as an antiporter localized in the inner mitochondrial membrane mediating the efflux of Mg<sup>2+</sup>. Similar to SLC41A1, the transport activity of SLC41A3 is dependent on Na<sup>+</sup> (4).

SLC41A3 might therefore act as functional counterpart to the mitochondrial Mg<sup>2+</sup> channel Mrs2 mediating the high-capacity uptake of Mg<sup>2+</sup> into mitochondria. This channel was one of the first identified eukaryotic Mg<sup>2+</sup> channels and has been extensively studied over the years. It is a distant homolog of CorA, the main Mg<sup>2+</sup> uptake system in bacteria. Transport via Mrs2 is driven by the strong, inside-negative mitochondrial membrane potential (5). Knock-down of Mrs2 in HEK293 cells eventually leads to cell death. Furthermore, a mutation in Mrs2 has been demonstrated to be causative for the severe and progressive breakdown of myelin in the central nervous system of rats (6).

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(2) Kolisek M, Nestler A, Vormann J, Schweigel-Rontgen M. Human gene SLC41A1 encodes for the Na<sup>+</sup>/Mg<sup>2+</sup> exchanger. *Am J Physiol Cell Physiol.* 2012;302(1):C318-26.

(3) Sahn J, Scharenberg AM. The SLC41 family of MgtE-like magnesium transporters. *Mol Aspects Med.* 2013;34(2-3):620-8.

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(5) Kolisek M, Zsurka G, Samaj J, Weghuber J, Schweyen RJ, Schweigel M. (2003) Mrs2p is an essential component of the major electrophoretic Mg<sup>2+</sup> influx system in mitochondria. *EMBO J.* 22(6):1235-44.

(6) Kuramoto T, Kuwamura M, Tokuda S, Izawa T, Nakane Y, Kitada K, Akao M, Guénet JL, Serikawa T. (2011) A mutation in the gene encoding mitochondrial Mg<sup>2+</sup> channel MRS2 results in demyelination in the rat. *PLoS Genet.* 7(1):e1001262.

### Biographical Sketch

Gerhard Sponder is postdoctoral researcher at the Institute of Veterinary Physiology at Free University Berlin, Germany. From the beginning of his scientific career, he has focused on cellular Mg<sup>2+</sup> homeostasis, in particular on the functional and structural characterization of the channel Mrs2 that forms the main Mg<sup>2+</sup> influx system in mitochondria. Later, his research focused on the physiological relevance of solute carrier 41A1 which represents the main Mg<sup>2+</sup>-efflux system of mammalian cells and its dysregulation is associated with diseases such as diabetes mellitus, hypertension, and neurodegenerative disorders. Also in dairy cows and horses he investigates Mg<sup>2+</sup> transport, especially under metabolically challenging situations like the onset of lactation and equine metabolic syndrome. Contact: [gerhard.sponder@fu-berlin.de](mailto:gerhard.sponder@fu-berlin.de)



## Deletions or mutations in the MAGT1 gene cause defects in N-linked glycosylation of cellular glycoproteins and are responsible for glycosylation disorders with alternative phenotypes

Reid Gilmore

Natalia A. Cherepanova<sup>1</sup>, Eline Blommaert<sup>2</sup>, Romain Pe'anne<sup>2</sup>, Sergey Venev<sup>3</sup>, Gert Matthijs<sup>2</sup>, Reid Gilmore<sup>1</sup>

<sup>1</sup> Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, USA

<sup>2</sup> Laboratory of Molecular Diagnosis, Centre for Human Genetics, KU Leuven, Belgium

<sup>3</sup> Program in Bioinformatics and Integrative Biology, University of Massachusetts Medical School, USA

**Purpose:** MAGT1 and its homologue TUSC3 are alternative subunits of the ER localized STT3B oligosaccharyltransferase complex. MAGT1/TUSC3 are also thought to be required for magnesium homeostasis, perhaps serving as magnesium transporters. Mutations in human STT3B cause a severe form of congenital disorders of glycosylation (STT3B-CDG). Mutations in MagT1 cause XMEN disease, a less severe immunodeficiency disease linked to defective magnesium homeostasis. We sought to determine whether MagT1 is necessary for STT3B-dependent N-linked glycosylation, and to explain why previously characterized MagT1 mutations do not cause CDG.

**Materials and methods:** The role of the MagT1 protein in N-linked glycosylation was addressed by a SILAC-based quantitative glycoproteomics procedure using HEK293-derived knockout (KO) cell lines (STT3A-KO, STT3B-KO, MagT1-KO/TUSC3-KO). Expression of MagT1, TUSC3 and STT3B was evaluated in fibroblasts derived from patients with MagT1 mutations that presented with either XMEN disease or CDG. Radiolabeling of selected glycoproteins in fibroblasts and HEK293 derived cell lines was performed to detect defects in N-glycosylation caused by MagT1 deletions or mutations.

**Results:** The quantitative glycoproteomics results indicated that STT3B and MagT1 are necessary for N-glycosylation of a subset of acceptor sites including (a) extreme C-terminal sites in secretory glycoproteins and (b) sites in luminal loops of multi-spanning membrane proteins including metabolite and ion transporters. Analysis of fibroblasts from MagT1 patients revealed that certain MagT1 alleles are unstable and are functionally repressed by incorporation of TUSC3 into the STT3B complex. Fibroblasts from MagT1-XMEN and MagT1-CDG patients display defects in N-glycosylation of STT3B dependent substrates.

**Conclusion:** Either MagT1 or TUSC3 are required for full activity of the STT3B oligosaccharyltransferase. We propose that MagT1 involvement in magnesium homeostasis is by an indirect mechanism involving protein hypoglycosylation. The alternative diseases caused by MagT1 mutations (XMEN vs. CDG) are caused by the extent to which TUSC3 replaces defective Magt1 alleles in the STT3B complex.

### Biographical Sketch

Professor and Vice-Chair of Biochemistry and Molecular Pharmacology at the University of Massachusetts Medical School. Reid Gilmore's lab has been investigating the biosynthesis and translocation of secretory proteins or integration of membrane proteins into the endoplasmic reticulum (ER) for more than 30 years. Asparagine-linked glycosylation of proteins by the oligosaccharyltransferase is an essential pathway in metazoan organisms because N-linked glycans are important for protein folding, stability, trafficking and function of proteins synthesized in the ER. Defects in N-linked glycosylation, including mutations in oligosaccharyltransferase subunits, cause a family of human diseases known as congenital disorders of glycosylation (CDG).

Contact: [reid.gilmore@umassmed.edu](mailto:reid.gilmore@umassmed.edu)

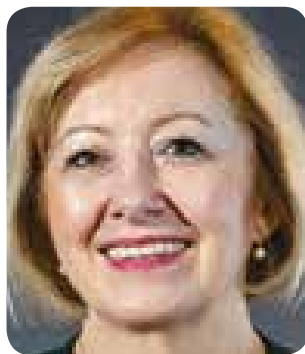


## Mg<sup>2+</sup> regulation of the immune response

Michael J. Lenardo

### Biographical Sketch

Michael Lenardo was born in Chicago, Illinois on December 1, 1955. He attended the Johns Hopkins University and graduated with a Bachelor of Arts in Natural Sciences in 1977. He then attended Washington University in St. Louis, Mo. and obtained his Doctor of Medicine (M.D.) in 1981. He carried out clinical and research training at the University of Iowa from 1981-1985. He was then a Research Fellow at the Whitehead Institute for Biomedical Research at Massachusetts Institute of Technology with an adjunct appointment at Harvard Medical School. During this time, he carried out molecular biology research under the mentorship of Nobel laureates David Baltimore and Philip Sharp. He was then appointed Section Chief in the National Institute of Allergy and Infectious Diseases, National Institutes of Health from 1989 to the present, directing research on T-lymphocyte regulation, HIV-1, and genetic diseases of the immune system. He has served on the editorial boards for the European Journal of Immunology, the Journal of Experimental Medicine, Science magazine, and Biology Direct. He is an Adjunct Professor of Pathology at the University of Pennsylvania School of Medicine, and a Visiting Fellow at Cambridge University. He has founded or co-founded several joint research programs including the NIH-Oxford-Cambridge Biomedical Research Scholars, the NIH-University of Pennsylvania Immunology Program, the NIH-Marshall Scholars, the NIH-Rhodes Scholars, the National M.D./Ph.D. partnership program, and the NIH-Institut Pasteur Infectious Disease and Immunology Program. Dr. Lenardo has published over 200 scholarly works and holds a number of medical patents. He discovered the proapoptotic mechanism of immune regulation and his work has defined several genetic diseases of the immune system including the Autoimmune Lymphoproliferative Syndrome, Caspase-8 deficiency syndrome, and X-linked magnesium deficiency with EBV and neoplasia (XMEN) disease. He is currently the Director of the Clinical Genomics Program and Chief of the Molecular Development of the Immune System Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Among his honors and awards, he is Officer of the Most Excellent Order of the British Empire (O.B.E.), conferred by Queen Elizabeth II, March, 2006 and a Fellow of the American Association for the Advancement of Science and the American Academy of Arts and Sciences. He is married to Lesley-Anne Furlong, M.D. and has two sons, Brian and Timothy.  
Contact: [lenardo@nih.gov](mailto:lenardo@nih.gov)



# Effect of magnesium and vitamin B6 combination on severe stress in healthy adults

## Gisèle Pickering

Etienne Pouteau<sup>1</sup>, Marmar Kabir-Ahmadi<sup>2</sup>, Lionel Noah<sup>1</sup>, Andre Mazur<sup>3</sup>, Louise Dye<sup>4</sup>,  
Juliane Hellhammer<sup>5</sup>, Gisèle Pickering<sup>6</sup>, Claude Dubray<sup>6</sup>

<sup>1</sup> Nutritional, Sanofi, Gentilly, France

<sup>2</sup> Statistics, Sanofi, Gentilly, France

<sup>3</sup> Unité de Nutrition Humaine, INRA, Université Clermont Auvergne, Clermont-Ferrand, France

<sup>4</sup> Nutrition and Behaviour Group, School of Psychology, University of Leeds, Leeds, United Kingdom

<sup>5</sup> Contract Research and Saliva Lab, Daacro, Trier, Germany

<sup>6</sup> Clinical Pharmacology Department, CHU, Université Clermont Auvergne, Clermont-Ferrand, France

**Objectives:** Animal and clinical studies suggest complementary effects of Magnesium (Mg) and vitamin B6 (B6) on stress. The aim of the current study, sponsored by Sanofi, was to determine the benefit of Mg-B6 combination compared to Mg alone in stressed healthy adults with suboptimal serum Mg concentrations using a validated measure of perceived stress.

**Methods:** A Phase IV, 8-week randomized, investigator-blind trial (EudraCT 2015-003749-24) was conducted in adults with a Depression Anxiety Stress Scale-42 stress subscale score (DASS-42SS) >18 and serum Mg 0.5-0.85mmol/L (below mean value for healthy adults), randomized 1:1 to oral Mg-B6 (Magne B6®, Mg 300mg; B6 30mg, QD) or oral Mg alone (Magnespasmyl®, Mg 300mg, QD). The following outcomes were analyzed: change in DASS-42 from baseline (BL) to week 8 (wk8) (primary) and week 4 (wk4); incidence of adverse events (AEs).

**Results:** In the intention-to-treat analysis (n=264 subjects), both treatments substantially reduced DASS-42SS equally from BL to wk8 (Mg-B6, -12.44 points 95%CI -13.83, -11.05; Mg, -11.72 points 95%CI -13.10, -10.33; p>0.05). An interaction (p=0.0097) between BL stress level and treatment warranted subgroup analysis (per analysis plan); adults with BL severe/extremely severe stress (SESS) (DASS-42SS ≥25; n=162) had 24% greater improvement with Mg-B6 vs Mg at wk8 (3.16 points, 95%CI 0.50, 5.82; p=0.0203); there was no difference between arms in the BL normal/moderate stress subgroup (DASS-42SS <25; n=102; p>0.05). Per-protocol analysis and change from BL to wk4 resulted in similar greater improvement in Mg-B6 versus Mg in the SESS subgroup (p<0.05). Overall, 12.1% of Mg-B6-treated and 17.4% of Mg-treated subjects experienced AEs potentially related to the treatment.

**Conclusions:** These results suggest that oral Mg administration alleviated stress in healthy adults bearing low magnesemia. Supplementation with Mg-B6 combination was not superior to Mg supplementation alone in the whole population studied. Finally, with regard to the level of stress, this study provides clinical evidence supporting the benefit of Mg combined with vitamin B6 over Mg alone in subjects with severe/extremely severe stress.

### Biographical Sketch

Professor Gisèle Pickering (MD, PhD, DPharm) is Professor of Medicine and Clinical Pharmacology at the University Hospital of Clermont-Ferrand, France. She coordinates the Inserm CIC 1405 Clinical Research Centre and is a permanent member of the Inserm 1407 Laboratory of Fundamental and Clinical Pharmacology of Pain. Her main topics of research concern the mechanism of action of analgesics, the impact of pain on cognitive-emotional processes and pain management in older persons. She regularly contributes to peer-reviewed publications on Pharmacology and pain, to international meetings and belongs to national and international Pain, Pharmacology and Geriatrics Societies. She is the French Councillor at the European Pain Society (EFIC). She is the author of over 150 publications and the editor of several books. Her interest in Magnesium in the context of Pain has been translational for the last 15 years, and her group published on the interest of Mg on NMDA receptors from animal models of pain to patients suffering from chronic neuropathic pain. She presently coordinates clinical trials with Magnesium in cancer patients, neuropathic pain, and in fibromyalgia, with a special focus on pain relief and quality of life. Her presentation at the SDRM Society meeting in Bethesda will refer to the benefits of Magnesium alone and combined with Vit B6 on stress.  
Contact: [gisele.pickering@uca.fr](mailto:gisele.pickering@uca.fr)



UNIVERSITY OF LEEDS

## Magnesium in stress and anxiety: lessons from preclinical and clinical studies

### Louise Dye

*Human Appetite Research Unit- School of Psychology -University of Leeds, UK*

This presentation will examine the effects of magnesium in stress and anxiety, drawing on evidence from animal and human studies. Pigs are a good model for human stress since they show susceptibility and behavioural responses to stress. On a commercial farm, pigs experience stressful events throughout their lifecycle. Stress and aggressive behaviours are not only detrimental to the pigs and their welfare but also for performance and production as increased stress/aggression can increase susceptibility to disease. Our systematic review examined studies in which magnesium was given to pigs to examine the effects on measures of stress and aggressive behaviour. As an example, a recent randomised controlled trial of the effects of magnesium with and without phytase on stress related behaviours in pigs experiencing an environmental stressor namely the mixing of pens after weaning will be described and the effects on indicators of stress such as ear, tail and body scores will be presented. Our systematic review of magnesium in humans examined the available evidence for the efficacy of Mg supplementation, alone or in combination, to alleviate subjective measures of anxiety and stress. The strength of the evidence for effects of magnesium on stress related symptoms from human and pig studies is suggestive of a beneficial effect of magnesium on stress related outcomes. However, the quality and consistency of the human studies in particular, precludes firm conclusions. For illustration, the level of stress reported at recruitment in a recent human clinical trial and the comorbidities in that sample which has important implications for future studies will be discussed.

#### Biographical Sketch

Louise Dye is Professor of Nutrition and Behaviour in the Nutrition and Behaviour Group, in the Human Appetite Research Unit in the School of Psychology, University of Leeds. Louise is N8 Chair in Theme 3 (Improved Nutrition and Consumer Behaviour) and Academic Lead for the University of Leeds of the HEFCE catalyst funded N8 Agrifood Programme. Louise is a Chartered Health Psychologist and British Psychological Society member. She began her career in Human Psychopharmacology and has over 30 years' experience in the assessment of nutritional and pharmacological intervention on cognitive function and wellbeing. She is Associate Editor of Nutritional Neuroscience and the European Journal of Nutrition. Her research interests include functional foods for wellbeing, stress management, mental health and cognitive performance/decline. She has published a number of influential systematic reviews including on the effects of magnesium in stress and anxiety and studies on the effects of different food components to maintain mental performance under conditions of stress. She is a member of the Scientific Advisory Board of ILSI Europe and has served on 5 of their expert groups.  
Contact: [L.Dye@leeds.ac.uk](mailto:L.Dye@leeds.ac.uk)





Keio University  
Faculty of Science and Technology

## Mg<sup>2+</sup> as a second messenger in early developmental stages of neurons

Kotaro Oka

Center for Biosciences and Informatics, School of Fundamental Science and Technology,  
Graduate School of Science and Technology, Keio University, Yokohama, Japan  
Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Taiwan

**Purpose:** Mg<sup>2+</sup> is recognized as a multi-target analogue regulator that performs many roles, such as circadian timekeeping, due to the following properties; (i) it influences wide-ranging biological processes, (ii) its concentration is tightly controlled within a narrow sub-millimolar range, and (iii) its intracellular dynamics are slow and long-lasting; its regulatory manner is not all-or-none in contrast to the switch-like signal transduction by the well-established second messenger Ca<sup>2+</sup>. Recent studies, however, have reported another role for Mg<sup>2+</sup> as a second messenger in immune cells. These multifaceted characteristics of Mg<sup>2+</sup> raise the question of how Mg<sup>2+</sup> processes information and how common its role is as a signaling molecule. We, therefore, investigated the role of Mg<sup>2+</sup> as a second messenger in early developmental stages of neurons with several fluorescent imaging techniques.

**Materials and methods:** We focused on the effects of  $\gamma$ -aminobutyric acid (GABA) and its developmental transition. We show that in neurons, GABA signaling, whose action is excitatory, triggers Mg<sup>2+</sup> release from mitochondria specifically at early developmental stages with several fluorescent Mg indicators. We also demonstrate that released Mg<sup>2+</sup> stimulates the CREB and mTOR signaling pathways with FRET-based sensors. Finally, the Mg<sup>2+</sup> function for neural network maturation was investigated as the fractional connectivity by Ca<sup>2+</sup> and FM dye imagings.

**Results:** We found that cytosolic Mg<sup>2+</sup> fluctuations within physiological ranges is enough to crucially regulate ERK, CREB, and mTOR activities; quantitative analysis of the Mg<sup>2+</sup> concentration-response curves demonstrates that cytosolic Mg<sup>2+</sup> regulates the signaling activities of ERK negatively, CREB positively, and mTOR sigmoidally. Together, intracellular Mg<sup>2+</sup> physiologically integrates and coordinates cellular information and Mg<sup>2+</sup> is a novel signal transducer for organizing neural networks.

**Conclusion:** We succeed to demonstrate the Mg<sup>2+</sup> as a second messenger in neurons. Mg<sup>2+</sup> has a important role for facilitating structural and functional maturation of neural networks.

### Biographical Sketch

Professor, Keio University; Visiting Adjunct Professor, Kaohsiung Medical University  
His researches are related to intracellular information processing by using fluorescent and other imaging techniques. He has succeeded to simultaneously visualize the cAMP/cGMP behavior in growth cones (Kobayashi et al. *Sci. Rep.* 2015), ATP and mitochondrial activity in neuronal axons (Suzuki et al. *Sci. Rep.* 2018), and Ca<sup>2+</sup> and cGMP mobilization in sensory neurons of *C.elegans* (Shidara et al. *J. Neurosci.* 2017). Furthermore, for last 20 years, he has been also investigated Mg<sup>2+</sup> mobilization and signal transduction in neurons by developing fluorescent imaging techniques. He demonstrated mitochondrial as intracellular Mg<sup>2+</sup> source, Mg<sup>2+</sup> mobilization by neuronal excitation (Yamanaka et al. *Neurosci.*, 2015), and also nitric mono-oxide (NO) stimulation (Yamanaka et al. *FEBS Lett.*, 2013). Recently he found GABA application induced Mg<sup>2+</sup> mobilization in early stages of developmental neurons and it regulates several kinase activities (Yamanaka et al. *Curr. Biol.* 2018).  
Contact: [oka@bio.keio.ac.jp](mailto:oka@bio.keio.ac.jp)



# Magnesium, skeletal muscle health, sarcopenia, osteoporosis and fracture risk

## Ailsa A Welch

Welch A.A. & Hayhoe R.P.G<sup>1</sup>

<sup>1</sup> Department of Epidemiology & Public Health, Norwich Medical School, University of East Anglia, Norwich, UK

**Purpose:** Sarcopenia and frailty are becoming increasingly recognised conditions in our ageing society, leading to poor quality of life, falls and fractures. Sarcopenia is the presence of low skeletal muscle mass and function. To date, the majority of nutritional interventions and research have focused on protein as the solution to these conditions. However, dietary magnesium is integral to skeletal muscle physiology and has the anti-inflammatory potential to impact on progression of skeletal muscle loss and function during aging.

**Materials and methods:** Epidemiological studies across population cohorts in the UK (TwinsUK, The EPIC-Norfolk Study and the UK Biobank) relating magnesium intake or circulating concentrations in blood to osteoporosis, risk of fracture, low skeletal muscle mass and function have been recently published (Hayhoe et al., 2015, Hayhoe et al., 2018, Welch et al., 2016, Welch et al., 2017).

**Results:** We found that dietary magnesium is related to skeletal muscle mass (as fat free mass) in men and women of different life stages. Moreover, our findings were stronger than those with protein intake. Dietary magnesium was also very strongly related to leg explosive power in women, with interquintile differences of 25%. In women dietary magnesium also attenuated the associations between C-Reactive Protein and skeletal muscle mass and function. Magnesium was also related to bone density.

**Conclusion:** Dietary magnesium is likely important for musculoskeletal health, particularly in middle and older age.

**References:** HAYHOE, R. P., et al & WELCH, A. A. 2015. Am J Clin Nutr, 102, 376-84. HAYHOE, R. P. G., et al & WELCH, A. A. 2018. Clin Nutr. 2018 WELCH, A. A., et al MACGREGOR, A. 2016. J Bone Miner Res, 31, 317-25. WELCH, A. A., SKINNER, J. & HICKSON, M. 2017. Nutrients, 9.

### Biographical Sketch

Ailsa Welch is a professor of Nutritional Epidemiology at the University of East Anglia Medical School. She researches the protective factors in diet for aging particularly for cardiovascular and musculoskeletal health (sarcopenia, skeletal muscle mass and function), osteoporosis and fracture risk. She also researches into cancer, in developing dietary assessment methods, quantifying measurement error, and evaluating public health improvement interventions. Ailsa aims to understand the effects of micronutrients (vitamins & minerals), diet quality (dietary patterns, fatty acids & acid-base load) on aging. Understanding how diet improves health during aging is important as the age profile of populations is increasing with consequences for the burden on health and social care. Micronutrient malnutrition also co-exists alongside obesity and chronic disease. Ailsa has 193 peer-reviewed publications, an H Index of 66 (Scopus), and is a top 1% world researcher (Thompson Reuters, 2014). Ailsa chairs the Nutrition & Lifestyle Forum of the UK National Osteoporosis Society and is a member of their Scientific Advisory Committee. She also chairs an initiative 'Optimising Nutrition and Hydration in Care Homes' and is the Public Health Nutrition scientific theme lead for the UK Nutrition Society. Ailsa is a State Registered Dietitian. Ailsa previously led development of dietary assessment methods for the EPIC-Study at the University of Cambridge, compiled UK food composition databases and researched in Somalia and Nigeria. Contact: [A.Welch@uea.ac.uk](mailto:A.Welch@uea.ac.uk)



## The janus of magnesium in the bone

### Jeanette AM Maier

*Dept. of Biomedical and Clinical Sciences L. Sacco, University of Milano, Italy*

A tight control of magnesium homeostasis is important for bone health. Indeed, experimental and epidemiological studies have demonstrated that both low and high magnesium exert harmful effects on the bones. Magnesium deficiency leads to osteopenia indirectly by altering the secretion and the activity of parathyroid hormone as well as the metabolism of vitamin D, and also by promoting low grade inflammation. Moreover, low magnesium directly impacts on crystal formation and shapes the behaviour of bone cells. While it reversibly accelerates mesenchymal stem cell differentiation into osteoblasts, low magnesium inhibits osteoblast function and stimulates osteoclast activity.

Evidence has been provided about harmful effects on osseous metabolism and parathyroid gland function by elevated magnesium. Accordingly, high bone Mg inhibits the formation of hydroxyapatite crystals by competing with calcium and by binding to pyrophosphate forming an insoluble salt. *In vitro* high Mg retards the osteogenic differentiation of bone mesenchymal stem cells, inhibits osteoblastic mineralizing activity and potentiates osteoclastic differentiation.

Overall, an optimal range of Mg concentrations seems to be required to ensure bone homeostasis.

#### Biographical Sketch

MD, Professor of General Pathology at the University of Milan. She was trained in the USA on the pathophysiology of the endothelium, with a focus on aging and senescence. In the last 15 years she investigated the response of vascular cells to magnesium and to the silencing of its transporters. Lately, attention has been devoted also to the role of magnesium in bone homeostasis.

Contact: [jeanette.maier@unimi.it](mailto:jeanette.maier@unimi.it)



ABSTRACTS  
Oral  
Communications

## Measures of muscle magnesium status from <sup>31</sup>P magnetic resonance spectroscopy are associated with age, sex, and knee extension strength

Donnie Cameron<sup>1,2</sup>, Ailsa Welch<sup>2</sup>, Fatemeh Adelnia<sup>1</sup>, Christopher M. Bergeron<sup>1</sup>, David A. Reiter<sup>1,3</sup>, Nicholas A. Brennan<sup>1</sup>, Kenneth W. Fishbein<sup>1</sup>, Richard G. Spencer<sup>1</sup>, Luigi Ferrucci<sup>1</sup>

<sup>1</sup> National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

<sup>2</sup> Norwich Medical School, University of East Anglia, Norwich, UK

<sup>3</sup> Emory University School of Medicine, Atlanta, GA, USA

**Purpose:** Magnesium status is usually assessed by serum levels, but these represent only a fraction of whole-body magnesium, and it is unclear whether they reflect magnesium's physiologic activity. Given that magnesium is a critical cofactor in muscle crossbridge cycling, we used phosphorus magnetic resonance spectroscopy (<sup>31</sup>P-MRS) to examine skeletal muscle magnesium status, studying its relationship with age and muscle function in a group of healthy men and women dispersed over a wide age-range.

**Materials and methods:** We obtained <sup>31</sup>P-MRS spectra in 459 participants (215 male, median [range] age=73 [24–98]yrs) of the Baltimore Longitudinal Study on Aging, estimating muscle [Mg<sup>2+</sup>] in the left thigh using a quadratic equation incorporating the observed chemical shift of  $\beta$ -ATP and the intracellular pH. Additionally, we measured: serum [Mg<sup>2+</sup>] using a conventional clinical colorimetric assay; and maximum knee-extension torque in the left leg, which was assessed using an isokinetic dynamometer, with isometric contractions being performed at 70° flexion.

**Results:** Muscle [Mg<sup>2+</sup>] was inversely associated with serum [Mg<sup>2+</sup>], as shown in Figure 1, and with age, with  $\beta = -0.31$ ,  $p < 0.001$ ,  $R^2 = 0.09$ . The association with age remained significant after segregating by sex, as illustrated in Figure 2, and there was a statistically-significant difference in muscle [Mg<sup>2+</sup>] between male and female participants ( $p < 0.001$ ). Serum [Mg<sup>2+</sup>] was not significantly associated with age, and there were no sex differences. Finally, muscle [Mg<sup>2+</sup>] was associated with knee-extension torque in male and female subgroups ( $\beta = 0.18$ ,  $p = 0.01$ ,  $R^2 = 0.06$ ; and  $\beta = 0.35$ ,  $R^2 = 0.12$ ,  $p < 0.001$ , respectively, adjusting for BMI), while serum [Mg<sup>2+</sup>] showed no association.

**Conclusion:** We found that magnesium in muscle was more-strongly associated with age and muscle function than was serum magnesium. Characterizing muscle changes with age is important for understanding sarcopenia and frailty, and our <sup>31</sup>P-MRS measures of muscle magnesium demonstrate such differences, permitting future testing of dietary, exercise, and pharmaceutical interventions in aging.

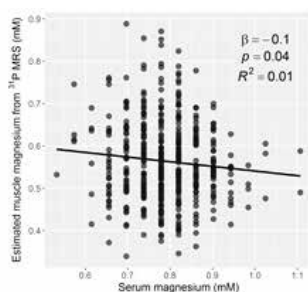


Fig.1

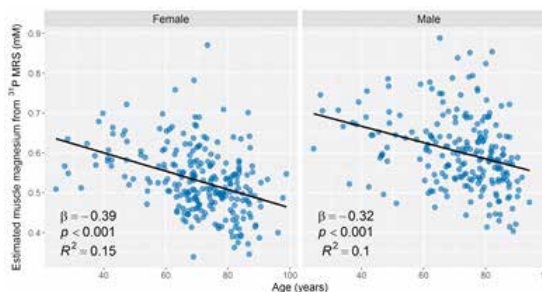


Fig.2

## SGLT2 inhibition in the management of intractable hypomagnesemia

Evan C. Ray,<sup>1</sup> Cary R. Boyd-Shiwarski<sup>1</sup>, Pengfei Liu<sup>2</sup>, Danica Novacic<sup>3</sup>, David Cassiman<sup>4</sup>

<sup>1</sup> University of Pittsburgh, Pittsburgh, Pennsylvania, USA

<sup>2</sup> Baylor College of Medicine Houston, Texas, USA

<sup>3</sup> Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA

<sup>4</sup> Catholic University of Leuven, Leuven, Belgium

**Purpose:** Hypomagnesemia can be challenging to treat, with conventional therapies often failing to normalize serum magnesium levels or alleviate symptoms. Glucose intolerance frequently accompanies hypomagnesemia. Reasoning that renal tubular reabsorption of magnesium could be enhanced by diverting sodium reabsorption from the proximal tubule to more distal nephron segments, we evaluated the ability of type 2 sodium glucose co-transporter (SGLT2) inhibitors to increase serum magnesium levels in patients with urinary wasting of magnesium and diabetes mellitus.

**Materials and methods:** Three patients were selected with urinary magnesium wasting and intractable hypomagnesemia. Two of these experienced hypomagnesemia as a consequence of HNF1B deletion; the third was idiopathic. Each patient was started on a different SGLT2 inhibitor: canafli flozen, empagliflozen, or dapagliflozen. Serum electrolyte levels were monitored regularly and compared to historical values. Twenty-four hour urine collections were performed and used to assess the changes in fractional excretion of magnesium before and after initiation of the SGLT2 inhibitor.

**Results:** All three patients demonstrated improved serum magnesium levels, with a mean increase of  $0.36 \pm 0.12$  mg/dL ( $0.15 \pm 0.05$  mmol/L). In one patient who previously require two daily infusions of intravenous magnesium, levels normalized and infusions were held. Serum magnesium levels after SGLT2 inhibitor initiation were higher than they had been in the context of intravenous infusion. All three patients experienced higher magnesium levels than at any point in their medical history. Two patients described improvements in hypomagnesemia-associated symptoms. All three patients tolerated the therapy well. Fractional excretion of magnesium declined in both patients with HNF1B mutations, but did not improve in the third patient.

**Conclusion:** These findings demonstrate that SGLT2 inhibitors are effective in at least some patients with previously intractable symptomatic hypomagnesemia. Increased renal tubular reabsorption of magnesium may contribute to this phenomenon in at least some patients.

## HUVEC in simulated microgravity: interplay between magnesium homeostasis and cytoskeleton

Laura Locatelli, Alessandra Cazzaniga, Valentina Romeo, Roberta Scrimieri, Sara Castiglioni and Jeanette A.M. Maier

*Dept. Biomedical and Clinical Sciences L. Sacco, Università di Milano, Via G.B. Grassi 74, Milano, Italy*

**Purpose:** Endothelial cells (ECs), not just an inert barrier that line blood vessels, maintain the homeostasis of tissues through the interaction with the environment and perception of different stimuli, among which shear stress and gravity. Nowadays it is well known that ECs in space sense microgravity, i.e. the reduction of gravity, and engage adaptive responses to cope with such a stressful condition. However, these adaptations cause a series of disorders in astronauts, among which cardiovascular deconditioning.

Magnesium (Mg), the most abundant intracellular bivalent cation, is fundamental in regulating ECs functions. Since little is known about its homeostasis in microgravity, we investigate this topic in ECs grown in simulated microgravity.

**Materials and methods:** To simulate microgravity, human ECs from the umbilical vein (HUVEC) were grown in the Rotating Wall Vessels (RWV) developed by NASA. The levels of two magnesium transporters in ECs, i.e. magnesium transporter MagT1 and Transient Receptor Potential Melastatin 7 (TRPM7) channel, were evaluated by Western Blot and the total amount of intracellular Mg was evaluated using the fluorescent chemosensor DCHQ5.

**Results:** After 4 and 10 days in the RWV HUVEC downregulate TRPM7 and upregulate MagT1, suggesting a compensatory mechanism to maintain the homeostasis of Mg. On the contrary, the total amount of intracellular Mg were decreased in HUVEC grown in microgravity compared to cells in 1G conditions. Preliminary results suggest that the dysregulation of cytoskeleton, an immediate and constant event following the exposure to microgravity, could play a role in the regulation of endothelial adaptive response as well as magnesium homeostasis in simulated microgravity.

**Conclusion:** We show that after 4 and 10 days in the RWV HUVEC decrease TRPM7 levels and increase MagT1 but this is not sufficient to prevent Mg decrease after 10 days in simulated microgravity. These events seem to be linked the disorganization of the cytoskeleton.

## Intra-hepatic cortisol production increases under Mg<sup>2+</sup>-deficiency conditions: implications for hepatic inflammation and hormonal dysmetabolism

Andrea M.P. Romani

*Dept. Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, USA*

**Purpose:** Decreased tissue and plasma Mg<sup>2+</sup> levels have been observed in patients affected by metabolic syndrome. Yet, it is undefined whether Mg<sup>2+</sup> decrease is cause or consequence of the onset of the pathology and its complications.

**Materials and methods:** Male Sprague-Dawley rats, and C7Balb Mice were exposed for 2 weeks to 40% magnesium deficient diet. Liver tissue and isolated hepatocytes were assessed for Mg<sup>2+</sup> content by atomic Absorbance spectrophotometry. Protein content and mRNA expression were assessed by Western Blot Analysis and qRT-PCR, respectively. Similar experiments and assessments were carried out in HepG2 cells cultured in 40% magnesium deficient medium.

**Results:** Decreased intrahepatic Mg<sup>2+</sup> content increases reticular G6P entry and oxidation by H6PD, which enhances the conversion of cortisone to active cortisol by the 11 $\beta$ -HSD1. The increased cortisol production promotes gluconeogenesis and intrahepatic fatty acid synthesis and triglycerides deposition, while decreasing IRS1 mediated insulin responsiveness. These metabolic changes are paralleled by decreased IRS1/PI3K/Akt expression and function, partially compensated by increased IRS2 expression. Returning hepatic Mg<sup>2+</sup> content to physiological levels reverses the observed modifications in cortisol production, and related changes in expression and activity of H6P, 11 $\beta$ -HSD1, gluconeogenic and fatty acid cortisol-responsive genes. Investigation into the mechanism responsible for 11 $\beta$ -HSD1 increased expression points to Mg<sup>2+</sup>-dependent increased NF $\kappa$ B translocation to the nucleus and enhanced TNF $\alpha$  expression.

**Conclusion:** Taken together, these results suggest that Mg<sup>2+</sup> deficiency may precede the onset of metabolic syndrome, setting the conditions for increased intra-hepatic inflammation, production of cortisol, and decreased insulin responsiveness by acting at multiple levels including NF $\kappa$ B translocation, IRS expression, H6PD and 11 $\beta$ -HSD1 expression and activity, the latter being supported by a steady entry of G6P into the ER. The altered hormonal balance can play a major role in the onset and progression of the metabolic syndrome and its complications.



## Acute and chronic exercise down-regulate magnesium transporter genes in rats and diabetes

Hsuan-Ying Chen<sup>1,2</sup>, Jason Chien<sup>1,2</sup>, Fu-Chou Cheng<sup>1,2</sup>

<sup>1</sup> *Stem Cell Center, Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan*

<sup>2</sup> *Taiwan Nutritional Magnesium Association, Taichung, Taiwan*

**Purpose:** Exercise is an effective therapy in diabetes care as it helps to regulate glucose and magnesium (Mg) homeostasis. Nevertheless, the mechanisms by which exercise exerts effects on Mg<sup>2+</sup> transportation remain unclear.

**Materials and methods:** This study investigated the expression of genes encoding for Mg<sup>2+</sup> transporters (GMTs) in rats and diabetics. The effects of Mg on the dynamic changes of glucose levels in muscle, blood and brain of rats were determined. Sprague-Dawley rats were pretreated with saline or magnesium sulfate (MgSO<sub>4</sub>, 90 mg/kg, i.p.) 30 min before treadmill exercise. A total of fifteen adult patients with type 2 diabetes were recruited and underwent a three-month indoor bicycle exercise program. Expressions of five GMTs (CNNM2, TRPM6, TRPM7, SLC41A1, and SLC41A3) were determined in blood samples of rats and diabetes.

**Results:** In rats, these glucose levels were significantly enhanced to approximately two-fold in the Mg group in the muscle, blood, and brain during acute exercise when compared to the control group. In addition, CNNM2, MagT1 and SLC41A1 genes expression significantly down-regulated in both blood and kidney tissues after exercise. Relevant anthropometric values and biochemical parameters were also determined in diabetes. Fasting blood glucose, glycosylated hemoglobin (HbA1c), waist circumference (WC), and Mg levels decreased after the exercise program. Expression of all five GMTs was down-regulated after exercise, but only CNNM2, TRPM6, and TRPM7 showed lower expression compared with that before the exercise program ( $P < 0.05$ ). HbA1c was inversely correlated with expression of SLC41A3 ( $P = 0.03$ ), and WC was inversely correlated with expression of TRPM6 and TRPM7 after the exercise program ( $P < 0.05$ ).

**Conclusion:** The acute and chronic exercise downregulated expression of GMTs in rats and patients with type 2 diabetes. Expressions of CNNM2, TRPM6, and TRPM7 correlated well with HbA1c and WC in diabetes. GMTs may serve as a potential tool in the evaluation of efficacy in diabetic care.

## Vascular effects of VEGF and EGF are mediated through TRPM7

Zhi-Guo Zou<sup>1</sup>, Neves KB<sup>1</sup>, Alves-Lopes R<sup>1</sup>, Graham D<sup>1</sup>, Montezano AC<sup>1</sup>, Rios FJ<sup>1</sup>, Touyz RM<sup>1</sup>

<sup>1</sup> Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, U.K.

**Purpose:** Transient Receptor Potential Melastatin (TRPM) 6 and 7 are novel cation channels important in Mg<sup>2+</sup> and Ca<sup>2+</sup> homeostasis. We identified TRPM7 as a major regulator of vascular function and showed that TRPM7-induced Mg<sup>2+</sup> transport is influenced by vasoactive agents, including angiotensin II, bradykinin and aldosterone. Previous studies showed that growth factors, such as EGF (epidermal growth factor) influence TRPM6 activity and that cancer patients treated with EGF receptor inhibitors exhibit hypomagnesemia and hypertension by unclear mechanisms. Whether growth factors influence vascular TRPM7 is unknown. Here we questioned if VEGF (vascular endothelial growth factor) and EGF mediate vascular effects through TRPM7.

**Materials and methods:** Studies were performed in primary human VSMCs, wild type (WT) and TRPM7-kinase deficient (TRPM7<sup>+/-</sup>) mice. VSMCs were stimulated with VEGF or EGF (10 or 50ng/ml) in the absence and presence of vatalanib, gefitinib (0.1-1μM), 2-APB (30μM) and NS8593 (40μM), inhibitors of VEGF receptor, EGF receptor, and TRPM7 respectively. Ca<sup>2+</sup> and Mg<sup>2+</sup> concentration was assessed using Cal-520 and Mg-green probes and VEGF/EGF signaling was assessed by immunoblotting. Vascular function was assessed by myography in isolated small mesenteric arteries from WT and TRPM7<sup>+/-</sup> mice and treated with EGF or VEGF (50ng/ml). TRPM7 expression in aortas and kidneys from WT mice treated with vatalanib or gefitinib (100mg/Kg/day, 2 weeks) was assessed by immunoblotting.

**Results:** VEGF and EGF increased expression of TRPM7 (50% and 67% respectively) and MagT1 (125% and 100% respectively), and promoted influx of Ca<sup>2+</sup> (8% and 10%) and Mg<sup>2+</sup> (8%), effects that were reduced by vatalanib, gefitinib, NS8593, and 2-APB. EGF but not VEGF increased phosphorylation of PKC (43%), p38MAPK (47%), and ERK1/2 (120%). These responses were reduced by gefitinib, however only ERK1/2 phosphorylation was inhibited by NS8593, and 2-APB. Mice treated with vatalanib or gefitinib showed reduced expression of TRPM7 in aortas (50% and 74% respectively) and kidneys (36% and 66% respectively). Vessels exposed to EGF were less responsive to acetylcholine (ACh)-induced relaxation, [maximum response: WT (veh 97%±3% vs EGF 63%±10%, p<0.05), TRPM7<sup>+/-</sup> (veh 89%±5% vs EGF 69%±5%, p<0.05)]. VEGF increased sensitivity to sodium nitroprusside (SNP)-induced relaxation [LogEC50: WT (veh 7±0.12 vs VEGF 7.4±0.12, p<0.05), TRPM7<sup>+/-</sup> (veh 6.7±0.15 vs VEGF 6.9±0.09)].

**Conclusion:** EGF and VEGF regulate VSMCs through TRPM7-dependent pathways. These processes involve MAP kinases and influence vascular function. Our findings identify novel mechanisms whereby growth factors influence vascular contraction/relaxation and suggest that TRPM7-regulated Mg<sup>2+</sup> and Ca<sup>2+</sup> are important.

## Comparative effects of sodium–glucose cotransporter 2 inhibitors on serum magnesium levels in patients with type 2 diabetes: a network meta-analysis of randomized controlled trials

Huilin Tang<sup>1</sup>, Qi Dai<sup>2</sup>, Jingjing Zhang<sup>3</sup>, Yiqing Song<sup>1</sup>

<sup>1</sup> Department of Epidemiology, Richard M. Fairbanks School of Public Health, Indiana University, Indianapolis, Indiana, USA

<sup>2</sup> Department of Medicine, School of Medicine, Vanderbilt Ingram Cancer Center, Vanderbilt University Medical Center, Vanderbilt University, Nashville, Tennessee, USA

<sup>3</sup> Division of Nephrology, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, Pennsylvania, USA

**Purpose:** Previous studies have reported that sodium-glucose co-transporter 2 (SGLT2) inhibitors, a new class of antidiabetic drugs, affect the serum electrolytes levels, especially magnesium. The aims of this study are to integrate direct and indirect trial evidence to evaluate the relative effects of all SGLT2 inhibitors against each other on magnesium levels in patients with type 2 diabetes (T2D).

**Materials and methods:** We systematically searched PubMed, Embase, and Cochrane Central Register of Controlled Trials (CENTRAL) up to December 2018 to identify eligible randomized controlled trials (RCTs) that reported the mean (standard deviation) changes from baseline to end of follow-up in serum magnesium. Random-effects pairwise and network meta-analyses were performed to calculate the weighted mean difference (WMD) and 95% confidence interval (CI).

**Results:** Of the 4,751 citations retrieved from electronic databases, 24 RCTs involving 17,396 patients with five SGLT2 inhibitors (canagliflozin, dapagliflozin, empagliflozin, ertugliflozin, and ipragliflozin) were included in this meta-analysis. All SGLT2 inhibitors were significantly associated with increase in serum magnesium as compared with placebo (WMD = 0.08 mmol/L; 95% CI = 0.06 – 0.09). Subgroup analyses by types of SGLT2 inhibitors showed that each SGLT2 inhibitor compared to placebo could significantly increase serum magnesium (range: 0.05-0.20 mmol/L). Significant increases in serum magnesium were observed in the network meta-analysis among the patients taking canagliflozin (WMD = 0.08 mmol/L), dapagliflozin (WMD = 0.15 mmol/L), and empagliflozin (WMD = 0.06 mmol/L) compared to those taking placebo. No statistically detectable differences of serum magnesium levels were evident between any two of SGLT2 inhibitors but between dapagliflozin and empagliflozin (dapagliflozin versus empagliflozin: WMD = 0.09 mmol/L). However, significant heterogeneity was observed in these analyses.

**Conclusion:** This study confirmed that SGLT2 inhibitors could significantly increase serum magnesium, indicating a potentially similar class-effect. However, more data for long-term efficacy and safety of raising serum magnesium in T2D patients with different clinical phenotypes are needed for further investigation.

## Magnesium supplementation improves diabetic mitochondrial and cardiac diastolic function

Man Liu<sup>1</sup>, Euy-Myoung Jeong<sup>2,3</sup>, Hong Liu<sup>1</sup>, An Xie<sup>1</sup>, Eui Young So<sup>2,3</sup>, Guangbin Shi<sup>2</sup>, Go Eun Jeong<sup>4</sup>, Anyu Zhou<sup>2,3</sup>, Samuel C. Dudley Jr<sup>1</sup>

<sup>1</sup> Division of Cardiology, Department of Medicine, the Lillehei Heart Institute, University of Minnesota at Twin Cities, Minneapolis, MN

<sup>2</sup> Cardiovascular Research Center, Lifespan Rhode Island Hospital, Providence, RI

<sup>3</sup> The Warren Alpert Medical School, Brown University, Providence, RI

<sup>4</sup> Brown University, Providence, RI

**Purpose:** In heart failure and type II diabetes mellitus (DM), the majority of patients have hypomagnesemia, and magnesium (Mg) supplementation has improved cardiac function and insulin resistance. Recently, we have shown that DM can cause cardiac diastolic dysfunction (DD). Therefore, we hypothesized that Mg supplementation would improve diastolic function in DM.

**Materials and methods:** High fat diet-induced diabetic mouse hearts showed increased cardiac DD and hypertrophy.

**Results:** Mice with DM showed a significantly increased E/e' ratio in the echocardiogram, left ventricular end diastolic volume (LVEDV), incidence of DD, left ventricular posterior wall thickness in diastole (PWTd), and ratio of heart weight to tibia length (HW/TL) when compared to controls. DM mice also had hypomagnesemia. Ventricular cardiomyocytes isolated from DM mice exhibited decreased mitochondrial ATP production, a  $1.7 \pm 0.2$ -fold increase of mitochondrial ROS, depolarization of the mitochondrial membrane potential, and mitochondrial  $\text{Ca}^{2+}$  overload. Dietary Mg administration (50 mg/mL in the drinking water) for 6 weeks increased plasma Mg concentration and improved cardiac function. At cellular level, Mg improved mitochondrial function with increased ATP, decreased mitochondrial ROS and  $\text{Ca}^{2+}$  overload, and repolarized mitochondrial membrane potential.

**Conclusion:** In conclusion, Mg supplementation improved mitochondrial function, reduced oxidative stress, and prevented diastolic dysfunction in DM.

## Effect of magnesium bicarbonate-rich water on high normal and hypertension grade 1 blood pressure: a double-blind, placebo-controlled, two-center trial

Mašenjka Katić<sup>1</sup>, Sandy Lovković<sup>2</sup>

<sup>1</sup> NutriProject Consultancy, Zagreb, Croatia, HR

<sup>2</sup> Jamnica d.d., Zagreb, Croatia, HR

**Purpose:** To evaluate the potential of magnesium bicarbonate-rich natural mineral water MgMivela on cardiovascular parameters, in particular to blood pressure, in subjects with high normal or hypertension grade 1 blood pressure levels.

**Materials and methods:** This randomized, double-blind, placebo-controlled, two-center pilot study enrolled 50 subjects, 18-65 years old, with high normal or hypertension grade 1 blood pressure (130-159/85-99 mmHg). After a two week run-in phase, 33 subjects were assigned to 1l a day (500 ml in the morning and 500 ml in the late afternoon/early evening) of MgMivela (339 mg/l magnesium, 2074 mg/l bicarbonate) and 17 subjects to placebo water (15 mg/l magnesium, 543 mg/l bicarbonate), for the following 8 weeks. There were four visits at the study centers (Charité University Medicine Berlin and Analyze & Realize GmbH, Germany) during the trial (screening, baseline, control and the final visit). Blood pressure, heart rate measurements, physical examination, blood and urine laboratory parameters, body weight, dietary diary and physical activity questionnaire, assessment of adverse effects and global evaluation of benefit and tolerability of the water consumed were assessed. Statistical analysis was performed by applying General Estimating Equations, Wald Square Chi-square testing, exact Fisher test, Mann-Whitney U test and Wilcoxon test.

**Results:** There was a significant time-dependant difference in the reduction of blood pressure, showing that consumption of MgMivela over eight weeks yielded in a greater reduction of blood pressure compared to the placebo, with mean difference in systolic blood pressure of -6,1 mmHg vs -1.2 mmHg ( $p < 0.001$ ) and diastolic blood pressure of -4.9 mmHg vs -2.8 mmHg ( $p < 0.001$ ) respectively.

**Conclusion:** The consumption of 1l MgMivela water daily over the period of eight weeks was shown to be excellently tolerated and associated with beneficial effects on blood pressure in subjects with high normal or hypertension grade 1 blood pressure.

Clinical trial registration *DRKS-ID: DRKS00009383*



# OC10

## Global human Mg nutrition: need and supply

Andrea Rosanoff

*Center for Magnesium Education & Research LLC*

**Purpose:** Does the global Mg Supply meet the nutritional Mg need of the growing and aging global human population?

**Materials & Methods:** Using FAO food supply data and food Mg values from the USDA-NAL database, Kumssa et al., 2015 derived the 1991-2011 per capita human dietary Mg global supply and compared it with the UN/FAO human nutritional Mg requirements, weighted for global age distribution. This analysis showed that the unprocessed global Mg Supply easily meets human global Mg need with a small risk of dietary Mg deficiency. However, the UN/FAO Mg RDA is a low estimate of human Mg need, not taking into account the global trend of rising body weights, possibility of CLMD with the physiological transfer of Mg body stores (mostly bone) during suboptimal Mg intakes, nor impacts of stress on Mg requirement. Additionally, food processing losses of Mg can be substantial.

**Results:** Global Mg supply needs to be corrected for Mg processing losses (estimated -8 to -60%), and human Mg requirements need to be corrected for rising body weights (+6 – 11% in 20 years) plus physiological requirements to reduce potential depletion of Mg stores (7 mg Mg/kg vs 4 mg Mg/kg) and estimated impact of high stress levels (10 mg Mg/kg).

**Conclusion:** Global Mg Supply production outpaced the human Mg need due to the rise in human global population, 1991-2011. Even though the global human population rose +25% 1991-2011 (UN data), the per capita human Mg Global Supply rose +9%. Mg supply with processing losses < 38% may meet global human Mg requirement while minimizing or preventing depletion of Mg stores in healthy non-stressed people, but lifelong consumption of diets with larger Mg processing losses, common in several developed countries, can be expected to show higher levels of Mg related chronic diseases mainly seen in the elderly.

## Associations of dietary and urinary magnesium, calcium and their ratio with blood pressure: intermap

Linda Van Horn<sup>1</sup>, Rachel Gibson<sup>2</sup>, Alan Dyer<sup>1</sup>, Katsuyuki Miura<sup>3</sup>, Hirotsugu Ueshima<sup>3</sup>, Liancheng Zhao<sup>4</sup>, Martha Daviglus<sup>5</sup>, Paul Elliott<sup>2</sup>, Elaine Holmes<sup>6</sup>, Jeremiah Stamler<sup>1</sup>, Queenie Chan<sup>2</sup>

<sup>1</sup> Department of Preventive Medicine, Northwestern University, Chicago IL, US

<sup>2</sup> Department of Epidemiology and Biostatistics, Imperial College London, London, UK

<sup>3</sup> Department of Public Health, Shiga University of Medical Science, Shiga, Otsu, Japan

<sup>4</sup> Department of Epidemiology, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing, PRC

<sup>5</sup> Institute for Minority Health Research, University of Illinois at Chicago, Chicago IL, US

<sup>6</sup> Division of Integrative Systems and Digestive Medicine, Imperial College London, London, UK

**Purpose:** To investigate associations of magnesium (Mg) and calcium (Ca) intake and Ca/Mg ratio with blood pressure (BP).

**Materials and methods:** Cross sectional data from the International Study of Macro-Micronutrients and Blood Pressure (INTERMAP) on 4,680 men and women ages 40-59 years from Japan, China, United Kingdom and the United States (US) were included. Multiple linear regression was used with control for possible confounders.

**Results:** Mean total Mg and Ca intakes were 324 and 747 mg/24h, and excretion was 228 and 684 mg/24h, respectively. Ca/Mg (food and supplements) was 2.4 and urinary ratio was 3.2. Multiple regression showed 2 SD higher Mg intake associated with -1.2mmHg (P=0.04) systolic BP (SBP), and 2SD higher Ca intake with -1.7mmHg (P=0.002) SBP and -0.9mmHg (P=0.01) diastolic BP (**Table 1**). In US only, the highest quartile of urinary Ca/Mg was associated with the lowest DASH dietary score (least healthy diet), lowest intakes of total Mg, betaine and folate, highest Ca and vitamin D intakes and highest BP levels, versus other quartiles.

**Conclusion:** BP was inversely associated with dietary Mg, Ca, Ca/Mg ratio and urinary Mg excretion. Urinary Ca excretion and Ca/Mg ratio were directly related to BP. Whether ratios of other combined nutrients are related to BP deserves investigation.

Table 1. Estimated differences and standard errors (SE) in systolic blood pressure (SBP) and diastolic blood pressure (DBP) (mm Hg) for predication higher by two standard deviation (SD) of dietary and urinary Mg, Ca and their ratio, INTERMAP (n=4680)

	$\Delta$ SBP	SE	P-value	$\Delta$ DBP	SE	P-value
Total Mg intake (2SD=262 mg/24h)	-1.24	0.61	0.04	-0.68	0.41	0.10
Total Ca intake (2SD=767 mg/24h)	-1.67	0.54	0.002	-0.94	0.37	0.01
Dietary Ca/Mg ratio (2SD=1.8)	-0.95	0.45	0.03	-0.71	0.31	0.02
Urinary Mg excretion (2SD=160 mg/24h)	-0.21	0.54	0.69	-0.45	0.36	0.21
Urinary Ca excretion (2SD=654 mg/24h)	1.41	0.48	0.003	0.70	0.32	0.03
Urinary Ca/Mg ratio (2SD=2.8)	1.38	0.44	0.002	0.90	0.30	0.003

Controlled for sex, age, center, energy, special diet, supplement intake, cardiovascular disease/diabetes diagnosis, physical activity, smoking, family history of high blood pressure, education, urinary sodium, urinary potassium, 7-day alcohol intake and body mass index.

# OC12

## Magnesium – impact of food processing on the bioavailability of this shortfall nutrient

Roger Clemens<sup>1</sup>, Peter Pressman<sup>2</sup>

<sup>1</sup> USC School of Pharmacy, Los Angeles, CA USA

<sup>2</sup> Daedalus Foundation, Mt. Vernon, VA 22121 USA

**Purpose:** Magnesium is one of the many shortfall nutrients. This nutrient is central in cellular energetics and an array of metabolic systems, including those involved in hepatic detoxification of xenobiotics. This issue is particularly important among an older adult (≥51 years) population which is often associated with the stress of polypharmacy. Little is known about impact of various forms of food processing (e.g., thermal and non-thermal) and Mg bioavailability.

**Materials and methods:** The bioavailability of Mg in a variety of food matrices and following several food processing conditions were evaluated. The apparent uptake and activity of Mg among human subjects and in animal models were compared with analytical data from the identified commercial foods.

**Results:** Preliminary data from rat hepatocytes and microsomal membranes suggest inadequate Mg intake may reduce glucuronidation via decreased UDP-glucuronyl transferase activity, while overt presentation of Mg deficiency was not observed. Additional data suggest numerous nutrient interactions with dietary Mg, such as Ca, protein and dietary fiber, may influence the uptake and subsequent impact of Mg-related biological activities. This intake may also be affected by its influence on a sensory quality, such that Mg salts are typically bitter. On the other hand, these divalent salts may provide several technical effects in processed foods, including reducing syneresis and minimizing biofilm formation.

**Conclusion:** Magnesium is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body. Current data indicate dietary intake of Mg is insufficient. These data which rely on analytical analysis are insufficient to adequately assess Mg status. Magnesium assessment by serum Mg levels does not reflect its metabolic status. Our incomplete understanding of Mg in food suggest some components in plant-based diets known to reduce mineral uptake may actually be decreased following food processing.



## OC13

## Observational data from a database of 23 thousand russian women, pregnant or with sex hormone-related conditions: epidemiology, diagnosis and clinical significance of hypomagnesemia

Svetlana V. Orlova<sup>1</sup>, Galina B. Dikke<sup>2</sup>, Eliso M. Dzhobava<sup>3</sup>, Nataliya V. Ialtceva<sup>4</sup>, Kirill M. Starostin<sup>5</sup>, Sofya P. Konchits<sup>5</sup>

<sup>1</sup> *The Peoples' Friendship University of Russia (RUDN), Moscow, Russia*

<sup>2</sup> *The Academy of Medical Education named F.I. Inozemtsev, St. Petersburg, Russia*

<sup>3</sup> *The Credo Experto Group of Medical Centers, Moscow, Russia*

<sup>4</sup> *The Yaroslavl State Medical University Institute of Postgraduate Education, Yaroslavl, Russia*

<sup>5</sup> *Sanofi-Aventis group, Moscow, Russia*

**Background:** Although magnesium deficiency (MD) is correlated with several health-related issues, including gynecological disorders, detection of MD remains a challenge in routine clinical practice. Available methods of assessment of magnesium status are rather expensive, optional or rarely clinically substantiated, and are often not reimbursed in Russia. This justifies the need of a simple, reliable and affordable tool to detect MD.

**Materials and methods:** The current work aimed at conducting secondary analysis of pooled data collected during 4 large observational studies: "MAGIC1", "MAGIC2", "MAGYN" and "MAGYN2". "MAGIC1" and "MAGIC2" covered cohorts of 1130 and 2117 pregnant women, respectively, recruited during routine visits to maternity welfare centres. "MAGYN" and "MAGYN2" covered cohorts of 9168 and 11424 women with hormone-related conditions respectively, in 21 cities of the Russian Federation. Cross-sectional data included socio-demographic factors, comorbidities, obstetrics and gynaecology anamnesis, and quality of life (QoL). Magnesium status was evaluated, using serum magnesium level and magnesium assessment questionnaire. Besides cross-sectional observational data, a sub-cohort including 3247 pregnant women and 4243 women with hormone-related condition who received magnesium supplementation was included into longitudinal study analysis with 4 weeks follow-up.

**Results:** This communication will shed the light on the following outcomes (1) relevance of an optimized short questionnaire for MD assessment, (2) description of hypomagnesemia prevalence and related risk factors and comorbidities among women in the Russian Federation, based on large real world data, (3) identification of links between MD and various socio-demographic, clinical parameters and its correlation with QoL.

**Conclusions:** Given the high incidence of MD in the Russian women, it would be very valuable for general clinical practice to develop a relevant, non-invasive routine and available method to assess MD. This would also allow to better understand the clinical impact of MD on QoL of women in various sex hormone-related conditions including pregnancy.

**Funding source:** with the sponsorship of Sanofi



## Osteogenic signaling pathways stimulated by magnesium

Charles Sfeir<sup>1,2,3,4</sup>, Chu-Chih Hung<sup>1,2,4</sup>, Dandan Hong<sup>1,2,4</sup>, Amy Chaya<sup>1</sup>, Kai Liu<sup>1,3,4</sup>, Andrew Brown<sup>1,3,4</sup>

<sup>1</sup> The Center for Craniofacial Regeneration, University of Pittsburgh, Pittsburgh, PA, USA

<sup>2</sup> Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

<sup>3</sup> Department of Periodontics and Preventive Dentistry, University of Pittsburgh, Pittsburgh, PA, USA

<sup>4</sup> The McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA

**Purpose:** resorbable magnesium based alloys are biocompatible and they were shown *in vivo* and *in vitro* by our group to enhance bone regeneration. Successful examples of bone regeneration using magnesium alloys in both animal models and clinical studies have been reported. However, the biological mechanisms have not been investigated in depth. To elucidate the osteogenic effects of magnesium, we stimulated human bone marrow stromal cells (hBMSCs) with culture medium containing adjusted concentrations of magnesium ion, and analyzed expression of genes and proteins related to osteogenesis.

**Materials and methods:** hBMSCs were cultured for three weeks in  $\alpha$ -MEM containing 5% FBS, with or without osteogenic factors (100 nM dexamethasone, 50  $\mu$ M ascorbic acid, and 10 mM  $\beta$ -glycerophosphate). Magnesium ion concentration was adjusted from 0.8 (physiological concentration; control) to 10 mM by adding MgSO<sub>4</sub> to culture medium. Extent of ECM mineralization was assessed using Alizarin red stain. mRNA expression of osteogenic markers was analyzed by qPCR array and confirmed by qPCR. Signaling pathway was analyzed by Ingenuity® IPA software. The production of proteins involved in the Wnt signaling pathway, collagen type X (COL10), vascular endothelial growth factor (VEGF), and upstream signaling molecules of these proteins were analyzed by Western blot.

**Results:** Mineralization of ECM was significantly enhanced at 10 mM of MgSO<sub>4</sub>. The Wnt signaling pathway as well as the HIF pathways were activated. mRNA expression and protein expression by Western blots revealed that components of these pathways were activated.

**Conclusion:** 10 mM of magnesium ion upregulated expression of several osteogenic genes and proteins of hBMSCs. Magnesium ion released from resorbing bone fixation devices may promote bone regeneration by activating the Wnt signaling pathways and enhancing the production of COL10 and VEGF via multiple intracellular signaling pathways.

# OC15

## Magnesium and its transporters: impact on osteogenic differentiation of human bone mesenchymal stem cells

Sara Castiglioni, Valentina Romeo, Jeanette Maier

*Department of Biomedical and Clinical Sciences Luigi Sacco, University of Milano, Milano, IT*

**Purpose:** To assess the effect of low and high magnesium (Mg) as well as Mg transporters on osteogenic differentiation of human bone mesenchymal stem cells (hMSCs).

**Materials and methods:** hMSCs isolated from adult human bone marrow are cultured in physiological (1.0 mM), low (0.1 mM) or high (3, 6, 10 mM) Mg concentrations. TRPM7 and MagT1 are transiently silenced using specific siRNAs. Osteogenic differentiation is induced by exposing the cells to an osteogenic cocktail containing  $2 \times 10^{-8}$  M  $1\alpha,25$ -Dihydroxyvitamin D<sub>3</sub>, 10 mM  $\beta$ -Glycerolphosphate and 0.05 mM Ascorbic Acid. Extracellular calcium (Ca) deposition is evaluated staining the cells with 2% Alizarin Red S.

**Results:** Magnesium deficiency reversibly accelerates the expression of osteogenic markers as well as extracellular calcium deposition in hMSCs. This event is associated with an increased production of reactive oxygen species (ROS). Accordingly, the antioxidant NAC prevents the enhancement of hMSCs differentiation by low extracellular Mg. Since ROS are recognized as fundamental in promoting cell differentiation partly through the enhancement of autophagy, it is noteworthy that low Mg activates this process. Intriguingly, similar results are obtained when the kinase and cation channel TRPM7 or the magnesium transporter MagT1 are silenced. On the contrary, high Mg inhibits hMSCs osteogenic differentiation probably through inhibition of autophagy. We detected a marked reduction of calcium deposition by hMSCs in medium containing 3 mM Mg, while the cells cultured in 6 and 10 mM Mg presented a slight but significant increase of the calcified matrix.

**Conclusion:** We argue that both low and high Mg concentrations as well as Mg transporters modulate osteoblastogenesis partly through the modulation of autophagy.



## Regulation of and by the murine TRPM7 channel-kinase

Pavani Beesetty<sup>1</sup>, Tetyana Zhelay<sup>1</sup>, Taku Kaitzuka<sup>2</sup>, Masayuki Matsushita<sup>3</sup> and J. Ashot Kozak<sup>1</sup>

<sup>1</sup> *Department of Neuroscience, Cell Biology and Physiology, Boonshoft School of Medicine, Wright State University, Dayton, OH 45409, USA*

<sup>2</sup> *Department of Molecular Physiology, Kumamoto University, Kumamoto 860-8556, Japan*

<sup>3</sup> *Department of Molecular and Cellular Physiology, University of the Ryukyus, Okinawa 903-0215, Japan*

TRPM7 is a ubiquitously expressed dual function cation channel-protein kinase. Mg<sup>2+</sup> and other divalent metal cations are both permeant ions and regulators of TRPM7 channels. TRPM7 kinase, belonging to eEF-2 kinase family, is regulated by these cations as well but with a diverging profile. The relationship between the kinase and channel activities of this protein has been the focus of numerous studies yet its details remain unclear. We showed that TRPM7 can autophosphorylate at serine/threonine residues without noticeably altering channel activity. Moreover, point mutations abolishing kinase activity leave the channel function largely intact, demonstrating that kinase activity and autophosphorylation are not necessary for ion conduction. We investigated the consequences of kinase inactivation in a mouse model where K1646R kinase-dead (KD) variant replaced wildtype TRPM7. The spleens of the mutant animals were larger and evidence of extramedullary hematopoiesis was found. Proliferation and enlargement were significantly reduced in KD mouse T cells in response to anti CD3/CD28 stimulation. Store-operated calcium entry, required for efficient proliferation, was diminished. TRPM7 protein expression was upregulated in activated T cells of both WT and KD mice. Effects of kinase inactivation in lymphocytes did not depend on extracellular Mg<sup>2+</sup> and Ca<sup>2+</sup> concentrations, however, suggesting that Mg<sup>2+</sup>/Ca<sup>2+</sup> influx was not responsible.

The mechanism of channel inhibition by cytosolic Mg<sup>2+</sup> has remained a mystery since its discovery. Using voltage-sensitive phospholipid phosphatase expression and site-directed mutagenesis of TRPM7, we investigated the dependence of channel sensitivity to these cations on phosphoinositides by patch clamp electrophysiology. We found that in PIP<sub>2</sub>-depleted cells, Mg<sup>2+</sup> and proton inhibition of the channels was more effective. Point mutations in the channel drastically reducing channel sensitivity to Mg<sup>2+</sup>, also decreased its sensitivity to polyamines and protons and PI(4,5)P<sub>2</sub> depletion. We conclude that inhibition by Mg<sup>2+</sup> reflects sequestration of plasma membrane PI(4,5)P<sub>2</sub>, the necessary cofactor for channel activation.

## Cadmium exposure enhances TRPM7 expression and intracellular magnesium leading to EMT in mammary epithelial cells

Alison Vanlaeys<sup>1</sup>, Gregory Fouquet<sup>1</sup>, Sylvie Pasco-Brassart<sup>2</sup>, Thibaut Lefevre<sup>1</sup>, Pierre Rybarczyk<sup>1,3</sup>, Frédéric Hague<sup>1</sup>, Philippe Kischel<sup>1</sup>, Isabelle Duthille-Dhennin<sup>1</sup>, Bertrand Brassart<sup>2</sup>, Halima Ouadid-Ahidouch<sup>1</sup> and Mathieu Gautier<sup>1</sup>

<sup>1</sup> EA4667, University of Picardie Jules Verne, Amiens, Fr

<sup>2</sup> UMR CNRS/URCA 7369, University of Reims Champagne-Ardennes, Reims, Fr

<sup>3</sup> Pathological Anatomy and Cytology Department, Amiens's Hospital, Amiens, Fr

**Purpose:** Cadmium is a pollutant that can be absorbed by epithelial cells through divalent cation transporters leading to neoplastic transformation. Among these transporters, the transient receptor potential melastatin-related 7 (TRPM7) is known to allow metal constitutive entries and is involved in cancer cell fates. The aim of this study was to show how TRPM7 is involved in neoplastic transformation induced by cadmium exposure in mammary epithelial cells.

**Materials and methods:** MCF10A non-cancer epithelial mammary cells were exposed to non-toxic concentration of cadmium for 40 weeks. TRPM7 expression was assessed by western-blot and its activity was studied by both patch-clamp and Manganese quench techniques. The effects of cadmium and TRPM7 silencing on intracellular calcium and magnesium were evaluated by using fluorescent probes as Fura-2 and MagFura-2. Finally, the migratory and invasive properties of cadmium-exposed cells were studied by using non-modified or modified Boyden chambers.

**Results:** Cadmium exposure induces TRPM7 overexpression followed by an increase of membrane currents and constitutive cation entry. Cells exposed to cadmium have a slight increase of intracellular calcium but the increase is much higher for magnesium levels. TRPM7 silencing restores calcium level but strongly decreases intracellular magnesium under control basal level. Moreover, cadmium exposure enhances both cell migration and invasion and TRPM7 silencing strongly decreases these cell fates. Furthermore, we observe that MCF10A exposed to cadmium became rounded and have less cell-to-cell junctions. Cadmium exposure decreases epithelial markers and increases the mesenchymal ones. Importantly, TRPM7 silencing is able to reverse these phenotypic modifications.

**Conclusion:** Our data show that cadmium exposure enhances TRPM7 expression and activity in mammary epithelial cells. TRPM7 overexpression induces intracellular magnesium increase and stimulates MCF10A cell migration and invasion. These neoplastic properties could be linked to a TRPM7-dependant epithelial-to-mesenchymal transition reprogramming in cell exposed to cadmium. These findings provide new insights in the regulation of cancer cell fates by TRPM7 and magnesium.



## OC18

## The anticancer EGFR-TKI, erlotinib, causes cutaneous, cardiac and hypomagnesemic side effects that were inhibited by aprepitant, a substance P receptor-blocker

W. B. Weglicki, J H Kramer, J J Chmielinska, CF Spurney and I T Mak

*Department of Biochemistry and Molecular Medicine, The George Washington University School of Medicine and Health Sciences, and the Department of Pediatrics, The Childrens National Medical Center, Washington, DC, USA*

**Purpose:** To determine the effect of SP-receptor blockade on the side effects of the EGFR-TKI, erlotinib (Tarceva), which has been used successfully to combat small cell lung and other various cancers, which may impact the patient's quality of life and even cause treatment interruption.

**Materials and Results:** In early studies of erlotinib-mediated side effects in a rodent model, we observed neurogenic inflammation associated with elevated circulating substance P (SP, 25% increase), ROS/RNS stress (8-isoprostane increased 210%, neutrophil superoxide increased 3-fold), progressive hypomagnesemia (-9% to -26%), skin lesions/hair loss, increased cardiac nitrotyrosine staining, and mildly impaired cardiac contractile function. During 9 weeks of erlotinib treatment (10 mg/kg/day), co-treatment with the SP receptor blocker, aprepitant (Emend: 2 mg/kg/day): significantly ( $p < 0.05$ ) restored by ~50% the 26% decrease in circulating magnesium levels, inhibited ( $p < 0.01$ ) the increased level of SP, and restored cardiac LV ejection fraction and fractional shortening to near normal levels ( $p < 0.02$ ). Most surprisingly, the skin lesions/hair loss due to erlotinib treatment were largely prevented by SP receptor blockade. In a more recent study, when the rodents were placed on a 40% Mg-deficient diet for 12 weeks, a 21% lowering ( $p < 0.01$ ) of circulating Mg occurred, and co-treatment with erlotinib lowered the Mg level further to 30% below control ( $p < 0.01$ ). This combination of erlotinib treatment with dietary hypomagnesemia enhanced the severity of skin lesions and the impairment of cardiac contractile function.

**Conclusion:** These results indicate that co-existing hypomagnesemia may worsen the above side effects during erlotinib therapy and neurogenic inflammation. Our intervention studies with the FDA-approved anti-nausea drug, aprepitant, suggest a novel potential therapy to prevent these known clinical side effects experienced by EGFR-TKI-treated cancer patients.

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## Magnesium pidolate vs magnesium sulphate: which salt performs better to protect the brain?

Romeo V.<sup>1</sup>, Locatelli L.<sup>1</sup>, Scrimieri R.<sup>1</sup>, Castiglioni S.<sup>1</sup>, Maier J.A.M.<sup>1</sup>, Cazzaniga A.<sup>1</sup>

<sup>1</sup> "L. Sacco" Department of Biomedical and Clinical Sciences, University of Milan, Via G.B. Grassi 74, 20157 Milan, IT

**Purpose:** The blood-brain barrier (BBB) consists of an interdependent network of cells designed to isolate the central nervous system from the systemic circulation. The loss of BBB integrity or changes of its permeability are associated with several neuropathological disorders and approaches to augment or stabilise the BBB are lacking. The aim of this study was to evaluate the effect of magnesium sulfate ( $\text{MgSO}_4$ ) and Mg pidolate in *in vitro* BBB-models.

**Materials and methods:** Co-cultures using the Transwell system were performed. Astrocytes were cultured in the lower part of the transwell and endothelial cells on the upper side. The change of permeability was studied by measuring bovine serum albumin-fluorescein isothiocyanate conjugate passage through the BBB and by transendothelial electrical resistance across the apical and basal compartments. Mg levels in the lower chamber was evaluated using the DCHQ5 fluorescent probe. The modulation of ZO-1 was investigated by immunofluorescence and the levels of some Mg transporters by western blot.

**Results:** High concentrations of Mg salts reduce BBB permeability and allow the accumulation of Mg in the lower chamber. No modulation of the levels of TRPM7 and MagT1 was detected. ZO-1 seems to be differently distributed upon exposure to high concentration of Mg salts. We also demonstrate that 5 mM of Mg salts protect against the increase in permeability induced by LPS. Mg pidolate seems to perform better than  $\text{MgSO}_4$ .

**Conclusion:** Our results can be summarized as follows: i) Mg is involved in the modulation of BBB's permeability, thereby indicating that high concentration of Mg salts could be used to prevent the accumulation of toxic molecules in the brain; ii) Mg pidolate is more efficient than  $\text{MgSO}_4$  in preventing BBB permeability alteration by inflammatory stress; iii) Mg pidolate tends to cross the BBB more than  $\text{MgSO}_4$ .

Sanofi has sponsored this study.

## Magnesium acetyltaurate: a prospective drug candidate for retinal neuroprotection in glaucoma

Igor Iezhitsa<sup>1,2\*</sup>, Renu Agarwal<sup>1</sup>, Lidawani Lambuk<sup>1</sup>, Natasha Najwa Nor Arfuzir<sup>1</sup>, Azliana Jusnida Ahmad Jafri<sup>1</sup>, Puneet Agarwal<sup>3</sup>, Alexander Spasov<sup>2</sup>, Alexander Ozerov<sup>2</sup>

<sup>1</sup> Center for Neuroscience Research, Faculty of Medicine, Universiti Teknologi MARA Sungai Buloh Campus, Selangor, Malaysia

<sup>2</sup> Research Institute of Pharmacology, Volgograd, State Medical University, Volgograd, Russia

<sup>3</sup> School of Medicine, International Medical University, Negeri Sembilan, Malaysia

\*Corresponding Author [iezhitsa@salam.uitm.edu.my](mailto:iezhitsa@salam.uitm.edu.my)

**Background:** Glutamate excitotoxicity is well-recognized pathophysiological factor for the development of glaucomatous neuropathy. Activation of NMDA receptors was proposed as a mechanism of neuronal degeneration in retina. Endothelin-1 (ET-1) enhances glutamate-induced retinal cell death, possibly through ET receptors and ET-1 may act synergistically with glutamate to damage retinal neurons. Glutamate-induced neurotoxicity is triggered by Ca<sup>2+</sup> influx into neurons by NMDA receptors and ET-1 is known to increase the intracellular Ca<sup>2+</sup> concentration in glial and neuronal cells. Additionally ET-1 specifically stimulates the efflux of glutamate via ET receptors from astrocytes, suggesting that ET-1 exacerbates neurodegeneration. Owing to that, several studies have been done to investigate therapies for RGC protection by targeting ET-1 and NMDA receptors. Accordingly, NMDA and/or ET receptor antagonists have been suggested to inhibit excitotoxicity in RGCs and to delay progression of visual loss in glaucoma.

**Purpose:** The purpose of the present study was to examine the potential of neuroprotective effect of Mg acetyltaurate (MgAT) on RGC death induced by NMDA and ET-1. The choice of MgAT was due to structural combination of magnesium (Mg) and taurine which together were expected to provide neuroprotection through multiple mechanisms such as inhibition of ET1 effect, antagonism of NMDA receptors, and antioxidant properties.

**Materials and methods:** In two series of experiments (ET1 and NMDA series) rats were divided into 5 groups of 6 rats each and were given intravitreal injections. Group 1 (PBS group) was injected with vehicle; group 2 (NMDA/ET1 group) was injected with either NMDA or ET1 while groups 3 (pre-treatment -), 4 (co-treatment) and 5 (post-treatment) were injected with MgAT, 24 hours before, in combination or 24 hours after either NMDA or ET1 injection. NMDA, ET1 and MgAT were injected in PBS at doses 160 nmol and 320 nmol, respectively. Seven days after intravitreal injection, the histological changes in the retina were evaluated using haematoxylin & eosin (H&E) staining. The extent of retinal cell apoptosis was assessed by TUNEL assay and Caspase-3 immunohistochemical staining.

**Results:** The estimation of neutrophilic factor, oxidative and nitrosative stress, pro/anti-apoptotic factors and caspase-3 activity in retina was done using enzyme-linked immunosorbent assay (ELISA) technique. The morphometric measurements on H&E staining showed reduction in number of ganglion cells in NMDA/ET-1 group compared to the MgAT pre- and co-treated groups. Similar results were observed in TUNEL and Caspase-3 staining. The results were further corroborated by the estimation of neutrophilic factor, oxidative stress, pro/anti-apoptotic factors and caspase-3 activity in retina. In conclusion, our current study revealed that pre-treatment with intravitreal MgAT prevents RGC apoptosis induced by NMDA/ET1.

**Conclusion:** Overall, our data demonstrated that the pretreatment with MgAT was more effective than co- and post-treatment. Protective effect of MgAT was associated with activation of BDNF-related neuroprotective mechanisms and reduction of retinal oxidative and nitrosative stress.



## Dietary magnesium alleviates murine colitis through upregulation of TRPM6 and modulation of intestinal microbiota

Valentina Trapani<sup>1</sup>, Valentina Petito<sup>2</sup>, Andrea Quagliariello<sup>3</sup>, Giuseppe Pietropaolo<sup>1</sup>, Sofia Reddel<sup>3</sup>, Federica Del Chierico<sup>3</sup>, Lorenza Putignani<sup>3,4</sup>, Antonio Gasbarrini<sup>2,5</sup>, Franco Scaldaferrì<sup>2,5</sup> and Federica I. Wolf<sup>1</sup>

<sup>1</sup> *Istituto di Patologia Generale, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "A. Gemelli" IRCSS, Rome, Italy*

<sup>2</sup> *Istituto di Patologia Speciale Medica, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "A. Gemelli" IRCSS, Rome, Italy*

<sup>3</sup> *Human Microbiome Unit, Ospedale Pediatrico Bambino Gesù IRCCS, Rome, Italy*

<sup>4</sup> *Parasitology Unit, Ospedale Pediatrico Bambino Gesù IRCCS, Rome, Italy*

<sup>5</sup> *UOC di Medicina Interna e Gastroenterologia, Area di Gastroenterologia e Oncologia Medica, Dipartimento di Scienze Gastroenterologiche, Endocrino-Metaboliche e Nefro-Urologiche, Fondazione Policlinico Universitario "A. Gemelli" IRCSS, Rome, Italy*

**Purpose:** Magnesium is mainly absorbed through the intestine. Therefore, chronic inflammation in the intestine is likely to result in a magnesium deficit, which could in turn exacerbate inflammation. The aim of the present study was to assess whether dietary magnesium availability could affect the course of inflammatory bowel disease (IBD).

**Materials and Methods:** The correlation between magnesemia and IBD activity was examined in 30 patients. The effect of dietary magnesium modulation was evaluated in a murine model of dextran sodium sulphate (DSS)-induced colitis. The role of the TRPM6 channel was investigated in a panel of colon cancer cell lines.

**Results:** In IBD patients, serum magnesium levels inversely correlated with disease activity and inflammatory markers. In mice, magnesium deficiency aggravated DSS-induced colitis, and viceversa colitis severely compromised intestinal magnesium absorption due to mucosal damage and reduction in TRPM6 expression. On the other hand, Mg supplementation resulted in better restoration of mucosal integrity and channel expression upon DSS challenge. Notably, distinctive gut microbiota profiles were associated with inflammation and with a magnesium-rich diet. *In vitro*, TRPM6 expression and function was strictly associated with epithelial cell functions.

**Conclusion:** Our results highlight the importance of evaluating and correcting magnesemia in IBD patients. The murine model suggests that Mg supplementation may represent a safe and cost-effective strategy to reduce inflammation and restore mucosal function, as well as to modulate gut microbiome. We confirm that TRPM6 expression and function in the colon is essential for systemic magnesium homeostasis and health.

## Combination antiretroviral therapy (cART) altered redox/nitrosative genes, enhanced systemic, renal and cardiac dysfunction in HIV-1 transgenic rats: protection by Mg-supplementation

I.T. Mak, J. H. Kramer, L. ElZohary J. J. Chmielinska, C. Spurney and W. B. Weglicki

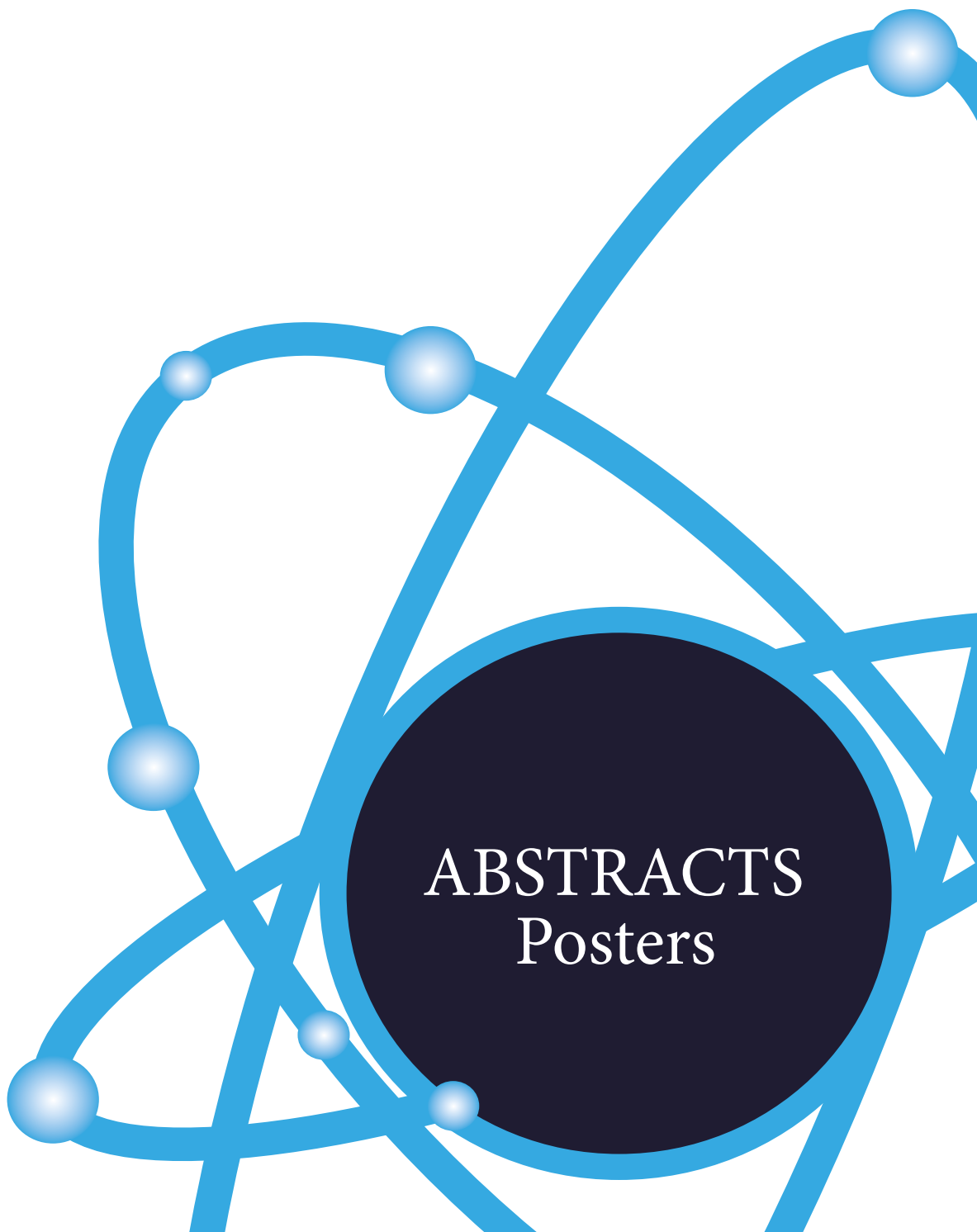
*Department of Biochemistry and Molecular Medicine, The George Washington University School of Medicine and Health Sciences, and the Department of Pediatrics, The Childrens National Medical Center, Washington, DC*

**Purpose:** We determined whether a clinically used cART drug regimen promoted redox/nitrosative stress, renal and cardiac toxicity in control and HIV-Tg rats.

**Materials and Results:** Treatment with cART (Truvada+atazanavir/ritonavir) up to 18 weeks, induced significant hypomagnesemia (-25%,  $p<0.01$ ) in the HIV-Tg rats along with significant ( $p<0.05$ ) renal dysfunction (increased plasma creatinine & urea), elevated plasma 8-isoprostane (265% increase,  $p<0.01$ ), RBC GSSG 2-3 folds along with a 3-fold ( $p<0.01$ ) increased basal neutrophil superoxide production; plasma 3-nitrotyrosine (NT) also increased 3.5-fold in the HIV-Tg rats. In association, hepatic master transcription factor for antioxidant defense, Nrf2 (nuclear erythroid-related factor-2) expression (by real-time RT-PCR) was down-regulated 50-75% ( $p<0.01$ ) along with altered down-stream related expression of heme oxygenase-1 (HmOX-1) and glutathione-S-transferase (GST) in the HIV-Tg rats; however, iNOS and TNF $\alpha$  mRNAs were increased 2- & 4-fold ( $p<0.01$ ). Prominent renal and ventricular NT staining was observed in HIV-Tg+cART rats; echocardiography detected moderate but significant decreases in cardiac systolic (Fractional Shortening, -17%,  $p<0.05$ , and diastolic (E/A, -26%,  $p<0.01$ ) function. Mg-supplementation (6-fold in the diet), substantially prevented ( $p<0.05$ ) the increased levels of 8-isoprostane, RBC-GSSG, plasma NT and suppressed neutrophil activation. Striking, Mg-supplementation also restored ( $p<0.01$ ) the expression of Nrf2, HmOX-1 and GST in HIV-Tg rats; both iNOS and TNF $\alpha$  up-regulations were blunted. In association, renal and cardiac NT staining was substantially ( $p<0.05$ ) reduced; both renal dysfunction and decreased cardiac systolic, diastolic function were attenuated 60-70% ( $p<0.05$ ).

**Conclusions:** cART treatment synergized with HIV-1 expression in: down-regulating the Nrf2 pathway, up-regulating iNOS and TNF $\alpha$  expression, enhancing systemic redox/nitrosative stress contributing to renal & cardiac dysfunction. The renal and cardio-protective effects of Mg-supplementation might be mediated by its antioxidant actions at the genomic and systemic levels through the Nrf2 pathway.

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ABSTRACTS  
Posters

## P01

## Monitoring the serum Ca/Mg ratio in response to magnesium supplementation

Rebecca Costello<sup>1</sup>, Forrest Nielsen<sup>2</sup>, Andrea Rosanoff<sup>3</sup>, Michael Plesset<sup>3</sup> and Ronald Elin<sup>4</sup>

<sup>1</sup> NIH Office of Dietary Supplements, Bethesda, MD

<sup>2</sup> USDA Grand Forks, ND

<sup>3</sup> Center for Magnesium Education and Research, Pahoia, HI

<sup>4</sup> University of Louisville, KY

**Purpose of Study:** Chronic latent magnesium (Mg) deficiency (CLMD) along with decreased dietary intake of Mg is common and may incur increased risk for several chronic diseases. To evaluate the risk profile of women participating in the USDA Mg depletion studies by their serum calcium (Ca)/serum Mg ratios (mg/dL) and EKG parameters.

**Methods Used:** Three USDA depletion-repletion studies in 39 healthy postmenopausal women were analyzed for changes in the Ca/Mg ratio with or without QT interval changes on the EKG during the study period (mean of 5 complexes/reading corrected for heart rate; QTc), consuming a depletion diet between 104 to 146 mg Mg/day lasting from 72 to 93 days.

**Results:** Serum measures throughout the study period were paired with EKG QTc measures. Mean serum Mg was 1.99 + 0.18 mg/dL, mean serum Ca was 9.33 +0.37 mg/dL. Mean QTc was 0.419 ± 0.02 msec.

Ca/Mg Ratio by Group	N	Baseline	Depletion	Repletion	p-value (baseline vs. repletion)
127	13	4.63 ± .218	4.98 ± .282	5.22 ± .270	.00002
132	13	4.45 ± .036	4.58 ± .389	4.74 ± .269	.0004
135	13	4.53 ± .256	4.34 ± .310	4.39 ± .312	.074
Normal QTc	28	4.55 ± .314	4.59 ± .457	4.71 ± .471	.030
Increased QTc > .450 msec	11	4.52 ± .220	4.75 ± .295	4.95 ± .301	.0004

Repletion mean differences were statistically significant between groups 127 vs 132 (p=0.001), 127 vs 135 (p=0.001) and 132 vs 135 (p=.005) due to an elevation of serum Ca. A low serum Mg (<2.1) and increased QTc were present in 7/11 women in group 127.

**Conclusion:** A Mg repletion diet lead to elevations in the Ca/Mg ratio primarily due to increases in serum Ca, not serum Mg, which was also reflected by EKG changes. The EKG, in combination with serum Mg, may help detect CLMD.

## P02

### Magnesium and depression in patients at risk of suicide

Emily Young<sup>1</sup>, Andrea Rosanoff<sup>2</sup>, Ph.D., Rebecca Costello<sup>2</sup>, Ph. D., Michael R. Plesset<sup>2</sup>, Bernadette P. Marriott, Ph. D.<sup>1</sup>

<sup>1</sup> Medical University of South Carolina, Charleston, SC, USA

<sup>2</sup> Center for Magnesium Education and Research, Pahoa, HI, USA

**Purpose:** To examine magnesium (Mg) status and depression in individuals at risk of suicide via the interplay of dietary Mg intake, serum Mg, dietary and serum Ca:Mg ratios, and the use of medications known to affect the absorption, distribution, metabolism, and/or excretion (ADME) of these electrolytes.

**Materials and methods:** Using baseline data for 103 subjects determined to be at risk of suicide by the Better Resiliency Among Veterans and Non-Veterans with Omega-3's protocol, we analyzed the relationship between Mg markers and five mental health screening tools. Due to the variable effects on Mg's ADME by the large number of medications primary analyses considered subjects reporting medications with a major effect on Mg, such as proton pump inhibitors (PPI).

**Results:** 73% of the population (n=75) met the Beck Depression Inventory (BDI-2) criteria for depression. Of the depressed individuals, 61% reported taking at least one prescription anti-depressant (and as many as 10 or more prescription drugs). Similarly, a subgroup of 18 individuals taking a PPI at baseline met criteria for depression and 11 individuals also reported taking at least one prescription anti-depressant. Mean serum Mg for the entire population was  $2.07 \pm 0.24$  mg/dL (normal 1.7 – 2.2), mean dietary Mg intake was  $298 \pm 131$  mg/day, mean serum Ca:Mg ratio  $4.8 \pm 0.88$ , and mean dietary Ca:Mg ratio  $3.4 \pm 1.6$ . Regarding the correlation between Mg markers and depressive symptoms, outlier points within the data dominated Pearson's r, and removing these points showed the remainder of the data to be basically trendless. Spearman's rho and logarithmic analyses also failed to elucidate significant trends or findings.

**Conclusion:** No significant relationship between magnesium status and depression was found in a high-suicide-risk cohort, the majority of whom reported depression despite >50% reporting pharmaceutical depression treatment at baseline.



# P03

## Overview of the NIH research portfolio on magnesium

*Rebecca B. Costello<sup>1</sup> and Karen Regan<sup>1</sup>*

<sup>1</sup> *Office of Dietary Supplements, National Institutes of Health, Bethesda, MD*

**Purpose:** The National Institutes of Health (NIH) accomplishes its mission to improve the health of the Nation by funding research in the causes, diagnosis, prevention, and cure of human diseases. Toward that end, NIH funds research on a multitude of diseases, conditions, and related topics, including nutrition. Nutrition-related research at NIH exceeds \$1.5 billion a year. We sought to evaluate the level of NIH funding for magnesium-related grants since our last reporting in 2010 (FY 2011- FY 2018).

**Materials and Methods:** A search of the NIH RePORTER database for dietary magnesium-related research funded between 2011-2018 was completed. Projects included as magnesium-related were not necessarily 100% magnesium-related but had at least one aim that included magnesium intake or metabolism and related outcomes. The search revealed the following portfolio of grants funded during this time period.

**Results:** Eighty-eight (88) unique projects were funded for a total of almost \$109 million by 16 Institutes and Centers (ICs) during the period reviewed. There was a noted decrease in funded projects between 2011 and 2018. In 2011, a total of 43 projects (22 basic, 12 clinical, 3 RCTs, 3 epidemiological, 2 bioengineering, 1 conference) were funded by 14 NIH ICs. In 2018, a total of 25 projects, (17 basic, 2 clinical, 4 RCTs, 2 epidemiological) were funded by 8 ICs. The two ICs funding the largest number of magnesium-related projects were the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) and the National Institute of General Medical Sciences (NIGMS). In 2018, NIH invested \$7.5 million dollars to support magnesium-related research considerably down from \$17.6 million dollars provided in 2011.

**Conclusion:** NIH funding for magnesium-related grants has decreased by 43% compared to 2011 suggesting that it is not a priority area of research, possibly due to the lack of funding announcements focused on mineral research.

## Necessity of determining ionized magnesium in relation to total magnesium in blood

Branimir Radosavljević<sup>1</sup>, Svetlana Ignjatović<sup>2</sup>

<sup>1</sup> Institute of Chemistry in Medicine, Faculty of Medicine, University of Belgrade, Belgrade, RS

<sup>2</sup> Center for Medical Biochemistry, Clinical Center of Serbia and Faculty of Pharmacy, University of Belgrade, Belgrade, RS

**Purpose:** Ionized magnesium (iMg) is the only physiologically active form of Mg in circulation. However, in most of the world's biochemical laboratories total Mg (tMg) level is exclusively determined. Without evidence of significant variations in tMg levels in pathological and physiological states, the role of Mg is neglected, but the established method for determining iMg level throws a completely new light on the significance of Mg.

**Materials and methods:** Venous blood was anaerobically collected from 91 patients with acute myocardial infarction (AMI) (at admission, during the following four days and on the tenth day), from 38 soldiers of infantry and 34 soldiers of medical corps, and from 62 smokers. The reference interval of the domestic population was also established. Determinations of serum iMg levels were performed promptly by electrolyte analyzer (AVL 988/4), tMg levels were determined colorimetrically by xylydyl blue and creatine kinase (CK) activities were determined spectrophotometrically. Lipid status, certain AMI indicators and routinely determined biochemical parameters were also examined.

**Results:** The analytic characteristics of the ionoanalyzer were excellent. Within first 24 hours after AMI, the mean values of iMg (0.695 mmol/L) in patients and in the reference group (0.744 mmol/L) differed significantly, unlike tMg concentrations. Patients' iMg values were significantly lower all days except for the tenth day. However, tMg concentrations were not reduced a single day, and were even significantly higher from the third day. At maximum CK levels of above 1800U/L the patients showed significantly decreased iMg concentrations (0.64mmol/L vs. 0.71mmol/L), unlike tMg concentrations. The soldiers had increased tMg levels, but only the soldiers of infantry had decreased iMg levels. The smokers had the same iMg levels as nonsmokers, but increased tMg levels. The concentrations of tMg were decreased with advancing age, unlike iMg concentrations.

**Conclusion:** These results convincingly suggest the necessity of routine iMg determination.



## Magnesium and insulin stimulate glucose uptake in differentiated bovine adipocytes

S. Jurek<sup>1</sup>, M.A. Sandhu<sup>1,2</sup>, S. Trappe<sup>1</sup>, M. Kolisek<sup>1,3</sup>, G. Sponder<sup>1</sup> and J.R. Aschenbach<sup>1</sup>

<sup>1</sup> Freie Universität Berlin

<sup>2</sup> PMAS-Arid Agriculture University, Rawalpindi, Pakistan

<sup>3</sup> Comenius University Bratislava, Slovakia

**Purpose:** After parturition, dairy cows show a dramatic increase in the demand for energy. Especially, the requirement of glucose increases because of the onset of lactation. The feed uptake lags behind energy demand. This negative energy balance results in an increased mobilization of body fat and the synthesis of ketone bodies. Both are linked to insulin deficiency and/or insulin resistance, analogical to diabetes mellitus in humans. Magnesium plays a crucial role in insulin signaling and many enzymatic pathways. Therefore, the aim of the study was to investigate the influence of different magnesium and insulin concentrations on glucose uptake as a measure of insulin sensitivity in bovine adipocytes.

**Materials and methods:** Subcutaneous adipose tissue was collected from young calves and preadipocytes were cultivated as an explant culture. After 2 days of induction, adipocytes were incubated with various magnesium concentrations (0.1mM, 0.3mM, 1mM and 3mM) and various insulin concentrations (25pM, 250pM and 25nM) for 14 and 21 days. Glucose uptake was investigated using the fluorescent glucose analogue 6-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose (6-NBDG) and normalized to DAPI fluorescence of cell nuclei. Statistics were conducted by two-way ANOVA.

**Results:** After an incubation over 14 days, the normalized glucose uptake (glucose/nuclei ratio) showed a trend to rise with higher insulin concentrations ( $P = 0.063$ ) with no effect of the magnesium concentration. After 21 days of culture, the glucose/nuclei ratio at 25pM and 250pM insulin was lower than at 25nM insulin concentrations ( $P < 0.05$ ). Furthermore, a low magnesium concentration of 0.1mM caused a decrease in glucose uptake compared to all other tested magnesium concentrations ( $P < 0.05$ ).

**Conclusion:** Increasing concentrations of insulin stimulate glucose uptake of cultivated bovine adipocytes. Magnesium has an additional positive impact on glucose uptake of adipocytes, which becomes especially evident at later stages of adipocyte differentiation.

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# P06

## Magnesium in hypertensive heart diseases

*Klaus Kisters, Uwe Gröber*

*Med. Clinic I, St. Anna-Hospital, Herne, University of Bochum, Germany & ESH Excellence Centre*

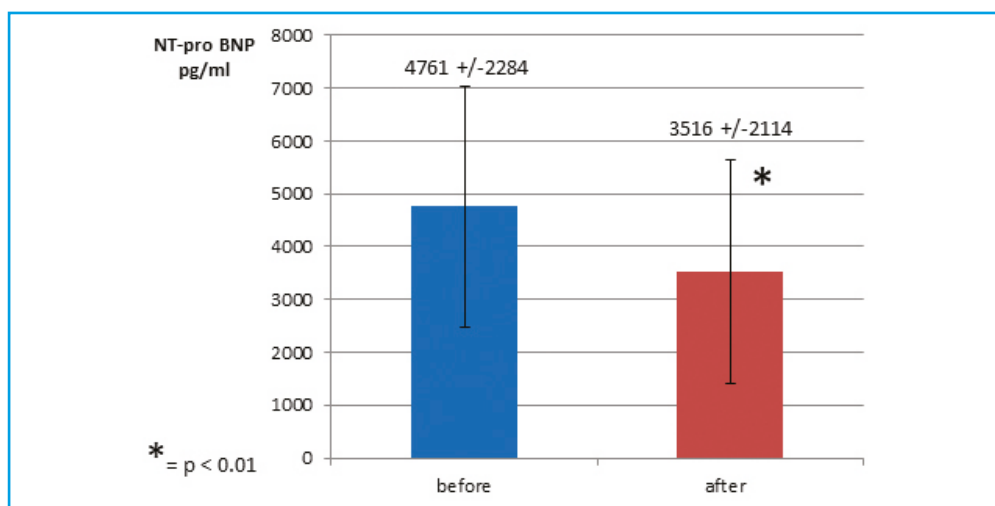
**Purpose:** Magnesium has an important role in hypertension (1, 2).

According to the results of the MACH study additional magnesium orotate therapy has shown a positive effect on life expectancy and quality of life in patients with severe forms of heart insufficiency (3). Both magnesium orotate can be cardioprotective.

**Material and methods:** In the presented data here additional magnesium orotate therapy was tested in 11 patients with hypertensive heart disease NYHA III-IV as compared to 10 patients with hypertensive heart disease NYHA II-IV as controls. Additional magnesium orotate therapy was 4500 mg magnesium orotate daily for one week.

**Results:** NTproBNP levels decreased significantly in the magnesium orotate group versus controls (figure) ( $p < 0.01$ ). Under therapy quality of life improved significantly as well. Kidney function remained stable in the normal range (4).

**Conclusion:** An additional therapy with magnesium orotate is of benefit in hypertensive heart disease.



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## TRPM7 is a potential pharmacological target in progressive kidney fibrosis

Sayuri Suzuki<sup>1</sup>, Reinhold Penner<sup>1,2</sup>, Andrea Fleig<sup>1,2</sup>

<sup>1</sup> Queen's Center for Biomedical Research, The Queen's Medical Center, Honolulu, HI

<sup>2</sup> Cancer Biology Program, University of Hawaii Cancer Center, Honolulu, HI

**Purpose:** Unilateral Ureteral Obstruction (UUO) is a representative model of chronic nephritis with progressive tubulointerstitial injury and renal fibrosis occurring by inflammation, cell proliferation and apoptosis. Blocking tubular epithelial cell proliferation is crucial to preventing the progression of kidney damage.

TRPM7 belongs to the Transient Receptor Potential Melastatin family of ion channels, is a Mg<sup>2+</sup>-conducting ion channel fused with a functional kinase. TRPM7 plays a key role in a variety of diseases, including malaria invasion, neuronal death in ischemia, cancer and cardiac fibrillation. TRPM7 is aberrantly over-expressed in lung, liver and heart fibrosis. It is also overexpressed after renal ischemia-reperfusion, an event that induces kidney injury and fibrosis. However, the role of TRPM7 is unclear in kidney fibrosis. In this study, we establish TRPM7 is an anti-fibrotic target *in vivo* and provide a novel pharmacological tool for drug development against kidney fibrosis.

**Materials and methods:** We created UUO mouse model on male C57BL/6 male, 5 weeks old, compared the expression level of TRPM7 in UUO kidneys and control kidneys at Day7 using qRT-PCR, western blotting assay and immunohistochemistry. Next, we injected NS8593 as TRPM7 inhibitor to UUO mice whether NS8593 suppresses TRPM7 in UUO kidney and attenuates renal fibrosis.

**Results:** The expression levels of TRPM7 mRNA and protein in UUO kidneys were higher than control kidneys, TRPM7 particularly increased on tubular epithelial cells. The TRPM7 positive cell number increased twice on both of renal tubular epithelial cells and tubulointerstitial cells in UUO kidneys compared to control kidneys. NS8593 completely blocked TRPM7 mRNA in UUO kidneys compared to control group. And it decreased the produce of extracellular matrix, attenuated renal fibrosis.

**Conclusion:** TRPM7 up-regulated in progressing inflammatory renal damage, NS8593 protected from the progressive kidney fibrosis. NS8593 could be capable of inhibiting kidney cell proliferation and fibrosis in renal disease.

## Incidence of hypomagnesemia and hypermagnesemia in a university hospital

A. Catalano, D. Chilà, F. Bellone, N. Morabito, S. Loddo, S. Benvenga, F. Corica

*Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy*

**Purpose:** Magnesium is the fourth most abundant cation circulating in the body involved in a number of fundamental processes such as glycemic control, muscle contraction, energy production, bone metabolism. Magnesium disorders are often underestimated in the clinical practice. The aim of our study was to evaluate the incidence of hypomagnesemia and hypermagnesemia in hospitalized patients.

**Materials and methods:** This retrospective study was based on data from laboratory tests of all inpatients whose serum levels of magnesium were measured between January 1, 2015 and December 31, 2017. Measurements of magnesium were carried out by a single centralized laboratory. Hypomagnesemia was defined by serum magnesium levels  $<1.5$  mg/dl and hypermagnesemia by serum calcium levels  $> 3.8$  mg/dl.

**Results:** A total of 11,469 subjects were considered. Hypomagnesemia accounted for 8.58% (n=985) and hypermagnesemia for 1.58% (n=182) of recruited subjects over the entire observation period. The incidence of hypermagnesemia increased from 1.38% in 2015 to 1.93% in 2017. The highest incidence of hypomagnesemia (5.27%, n=604) was found in subjects over 65yr, while a lower incidence was observed in patients aged 19-65yr and  $<19$ yr (2.67%, n=307 and 0.64%, n=74, respectively). Incidence of hypermagnesemia showed no differences over time and according to age.

**Conclusion:** Magnesium disorders and particularly hypomagnesemia are frequently observed in hospitalized elderly patients. Physicians have to check for magnesium levels because its disturbances may be of potentially reversible consequences for human health if adequately treated.



## Stressed cerebral organoids: can magnesium help?

Alessandra Cazzaniga, Valentina Romeo, Laura Locatelli, Roberta Scrimieri, Sara Castiglioni, Jeanette A.M. Maier

*Dipartimento di Scienze Biomediche e Cliniche L. Sacco, Università di Milano, Milano I-20157, Italy*

**Purpose:** Several studies have documented an association between magnesium and depression, anxiety and stress, even though the mechanistic pathways are largely unknown. Magnesium (Mg) increases the availability of GABA, the primary inhibitory transmitter in the CNS, and inhibits the activity of the NMDA receptor, which is the receptor for the excitatory neurotransmitter glutamate.

**Materials and methods:** A novel approach to study the effects of Mg in stress, anxiety or depression, is represented by the use of human mini-organoids. These are generated from induced human pluripotent stem cells (iPS) cells. Mini-brains will be exposed to different concentrations of magnesium salts with or without stress mediators (i.e. adrenalin, cortisol). The levels of glutamate, GABA and their receptors will be measured.

**Results:** Human Mini-brain organoids are embedded in droplets of a gelatinous protein mixture within a spinning bioreactor, providing the structural support and the nutrient/oxygen exchange that allows growth of organoids up to 4 mm in diameter. Retinoic acid, which is critical for cortical neurogenesis, is added to accelerate development. Neurons appear after 10 days, and in about 30 days defined brain regions form. Mini-brain organoids will be generated using media with various concentration of Mg in the presence or not of stress mediators

**Conclusion:** The human mini-brain organoids is an innovative experimental model to study neural networks in human brain and, therefore, might highlight the molecular mechanisms underlying Mg involvement in stress, anxiety and depression.

# P10

## Magnesium deficiency syndrome

*Corneliu D. Zeana, MD, PhD*

*UMF Carol Davila Bucharest and AIS Clinic and Hospital Bucharest, ROMANIA*

Magnesium is one of the most important mineral in biology. More than 300 enzymes are activated by magnesium in humans. During the last hundred years and especially within the last decades, the Magnesium input dropped dramatically, to more than half of the quantities ingested before. Responsible factors for this reduction of magnesium concentration in the food are: industrialization of the agriculture, especially excessively use of chemicals, irrigations, acid rains, followed by the semiindustrial food preparation. We entered the era of generalized magnesium deficit.(2) As a consequence of the magnesium carence, beyond the limits of the biological compensatory mechanisms, some manifestations expressing the magnesium deficit become manifest: in the vegetal world (some trees have pale leaves, even yellow, due to less than normal chlorophyll content), animal (cow tetany) and humans. Jean Durlach was the first that established a link between magnesium deficit, latent tetany and mitral valve prolapse (Durlach syndrome)(1). We describe a new syndrome manifest in the children of the magnesium deficient mothers suffering from spasmodophilia and mitral valve prolapse (Durlach syndrome). These women are magnesium looser, meaning that they have a diminished capability of conserving the magnesium in the body. This trait is probably inherited because cluster of such cases are encountered in these families, especially spasmodophilia, mitral valve prolapse and stress sensitivity. Their children have been followed by us from the birth to the adulthood. They developed generalized convulsions during the first year of age, rarely after this age, very few up to three years, and no longer manifest thereafter so that epilepsy was ruled out. Convulsions were triggered by high fever, brisk noise, but also without identifiable cause. These children presented somatic stigma of magnesium deficit, especially chest deformities: plat thorax, flat dorsal curvature, rarely funnel chest. In the late childhood and adolescence they develop spasmodophilic episodes. Palpitations are a common complaint. Mitral valve prolapse clearly visualized by echocardiography is evident but rarely of severity. Later, in adulthood, urolithiasis is frequent, with or without urinary stones elimination. Three cases with the above exposed manifestations have been described: Two women, aged 42 and 44 respectively and one male, 38 years old. Satisfactory results are obtained with magnesium supplementation of long duration. In the majority of cases, under magnesium supplementation the mitral valve prolapse is ameliorated to the resumption of the normal aspect of the valve.

### Summary

A new syndrome expressing the magnesium deficiency is described. The association of spasmodophilia, mitral valve prolapse, generalized convulsions in infancy, skeleton deformities, heart arrhythmias and urolithiasis defines this syndrome. Familial predisposition is notable. Good results are obtained by magnesium supplementation of long duration.

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## Involvement of CaSR, claudin-14 and 16 in the development of hypermagnesiuria associated with tubulo-interstitial nephropathy

*Taisuke Shimizu*<sup>1</sup>, *Kaori Takayanagi*<sup>1,2</sup>, *Akira Kurosawa*<sup>1</sup>, *Masaaki Terao*<sup>1</sup>, *Hiroaki Hara*<sup>1</sup>, *Takatsugu Iwashita*<sup>1</sup>, *Maiko Yamasaki*<sup>1</sup>, *Yumiko Nakamura*<sup>1</sup>, *Mariko Sato*<sup>1</sup>, *Yu Shimozato*<sup>1</sup>, *Akira Ikari*<sup>3</sup>, *Hajime Hasegawa*<sup>1</sup>

<sup>1</sup> *Department of Nephrology and Hypertension, Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan*

<sup>2</sup> *Ishikawa Kinenkai, Kawagoe Ekimae Clinic, Saitama, Japan*

<sup>3</sup> *Laboratory of Biochemistry, Gifu Pharmaceutical University, Gifu*

**Purpose:** Hypermagnesiuria is well known clinical feature of tubulo-interstitial nephropathy (TIN), and would be a diagnostic tool for TIN. We studied the changes in the expression of renal magnesium transporting molecules with the development of TIN in rats with unilateral ureter obstruction (UUO).

**Materials and methods:** Ureter-ligated kidney was sampled at day-0 (control), day-1 (early phase) and day-7 (late phase). The development of TIN was assessed by immunohistochemistry and the real-time PCR of fibrosis-related genes (MCP-1, TGF- $\beta$ ). The respective expressions of claudin-10, 14, 16, 19, and transient receptor potential (TRP) M6 as magnesium-transporting molecules were also studied. The expression of calcium sensing receptor (CaSR) as an inhibitory regulator of claudin-14 was additionally studied.

**Results:** The gene expression of claudin-16 was decreased in the late phase of UUO ( $100.2 \pm 2.9\%$  at day-0,  $90.3 \pm 6.3\%$  at day-1,  $36.4 \pm 1.6\%$  at day-7) which was consistent with the increased urine magnesium excretion (FEMg:  $3.13 \pm 0.81$  at day-0,  $4.72 \pm 0.54$  at day-1,  $10.7 \pm 0.81\%$  at day-7). Immunohistochemistry showed an apparent reduction of the immunoreactivity of claudin-16 in the late phase. The expression of TRPM6 was reduced even in the early phase. The immunohistochemistry and gene expression of MCP-1 and TGF- $\beta$  showed that TIN was not apparent in the early phase but was significant in the late phase. The density of peritubular capillaries was diminished in the late phase but not in the early phase. Expression of claudin-14 and CaSR was up- and down-regulated, respectively.

**Conclusion:** Our findings may indicate that the hypermagnesiuria in TIN is principally caused by the dysfunction of magnesium reabsorption in the thick ascending limb of Henle resulting from a significant decrease in the claudin-16 expression. The down-regulation may be closely related to the development of TIN through CaSR-claudin-14-claudin-16 regulatory network.

## A focus on magnesium in epilepsy research and treatment – a review

*Marija Stanojević<sup>1,2</sup>, Srdjan Lopovic<sup>1</sup>, Jelena Nesovic Ostojic<sup>1</sup>, Svetolik Spasic<sup>1</sup>, Sanjin Kovacevic<sup>1</sup>, Vladimir Nedeljkov<sup>1</sup>, Milica Prostran<sup>3</sup>*

<sup>1</sup> *Institute for Pathological Physiology, Faculty of Medicine, University of Belgrade, Belgrade, RS*

<sup>2</sup> *Department of Medical Neurobiology, Institute for Medical Research, Israel-Canada, Hebrew University - Hadassah School of Medicine, Jerusalem, IL*

<sup>3</sup> *Institute for Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, RS*

**Purpose:** This review aims to provide an overview on some of the gathered findings on the role of magnesium in epilepsy research and epilepsy treatment.

**Materials and methods:** Reexamination of the literature on magnesium in epilepsy was made. PubMed search included both preclinical and clinical studies.

**Results:** Magnesium has multiple neuroactive actions (analgetic, antimigrainous, antidepressant, anxiolytic, neuroprotective, anticonvulsive).  $Mg^{2+}$  exerts an overall stabilizing effect on nerve cell function. Neuronal  $Mg^{2+}$  requirements are high and its level higher in the cerebro-spinal fluid (CSF) than in the serum. Magnesium status is usually assessed by measuring the total serum  $Mg^{2+}$  level ( $tMg^{2+}$ ); however, serum level of ionized  $Mg^{2+}$  ( $iMg^{2+}$ ) is considered to be a better indicator. Magnesium deficiency has epileptogenic effect. Magnesium deprived diet induces seizures in experimental animals. In humans hypomagnesiemia increases seizure frequency. In pharmacoresistant epilepsy (PhRE) hypomagnesiemia increases the risk of sudden unexpected death from epilepsy. In epilepsy patients  $iMg^{2+}$  levels are found to be low in serum and CSF. Low serum  $iMg^{2+}/tMg^{2+}$  ratio was found interictally in patients with PhRE. In animal models of epilepsy  $Mg^{2+}$  shows antiepileptic effect. In humans, anticonvulsant  $Mg^{2+}$  effect is clinically used to control seizures in eclampsia, uremia, porphyria and hypomagnesiemia. Magnesium infusion can also help control refractory status epilepticus. The intact blood-brain barrier normally prevents  $Mg^{2+}$  penetration into the brain. Its increased permeability in seizures permits  $Mg^{2+}$  loss by leakage from the interstitial brain fluid, but also allows for  $Mg^{2+}$  level in the brain to be restored by *i.v.* treatment. Oral magnesium supplementation has been reported to help reduce seizure frequency.

**Conclusion:** More research studies focusing specifically on  $Mg^{2+}$  in basic and clinical epileptology are needed to further enlighten its role in the underlying pathophysiological mechanisms and adequately evaluate the promising benefit of magnesium supplementation in epilepsy, especially for the PhRE cases.



## Magnesium (Mg) deficiency enhances oxidative stress and migratory potential of colon cancer cells

*Gopal Kumar*<sup>1,2</sup>, *Swati Madankumar*<sup>2</sup>, *Xiangying Xue*<sup>2</sup>, *Prodyot Chatterjee*<sup>2</sup> and *Christine N Metz*<sup>1,2</sup>

<sup>1</sup> *Elmezzi Graduate School of Molecular Medicine, Manhasset, NY*

<sup>2</sup> *The Center for Biomedical Sciences, Feinstein Institute for Medical Research, Manhasset, NY*

**Purpose:** The purpose of this study was to assess the role of Mg deficiency (which increases Ca:Mg ratio), oxidative stress and TRPM7 signaling in promoting colon cancer cell motility in an in vitro model system.

**Materials and methods:** CT26 colon cancer cells were grown in media containing either 100%Mg, 50%Mg or 10%Mg (with constant concentrations of Ca) or in media containing either 100%Mg/100%Ca, 50%Mg/50%Ca or 10%Mg/10%Ca for 48hrs. qPCR and western blotting of cell lysates were performed to assess various markers associated with FAK, NFκB, E-cadherin and p53 signaling, as well as oxidative stress. Cells were grown under similar conditions and the DCFDA assay was performed for oxidative stress and Fluo-4-AM was used to measure intracellular Ca. Cell motility was measured by scratch assay.

**Results:** We observed that MgD cells showed higher intracellular Ca with increased expression of TRPM7 (that regulates intracellular Mg & Ca homeostasis). MgD also increased oxidative stress as measured by DCFDA assay and *Gpx1* mRNA expression. Furthermore, MgD enhanced the motility of the colon cancer cells. This was consistent with the observed degradation of full length E-cadherin as well as that of N-terminal FAK, a cytoplasmic tyrosine kinase, which negatively regulates FAK activity by increasing the cycling of the focal adhesions. MgD also degraded IκBα and the TAD containing C-terminal of p65. We also observed degradation of the p53 protein, as well as its transcriptional repression in MgD cells; loss of p53, promoting genomic instability, would be expected to increase metastatic potential in the long run. When the Ca:Mg ratio was kept as 1 these effects were not observed.

**Conclusion:** These data support that Mg plays a crucial role in the intracellular dynamics of Ca, with an increased Ca:Mg ratio, leading to increased oxidative stress and increased migration of colon cancer cells in vitro (and possibly their metastatic potential).



## Cetuximab Impacts on magnesium uptake in the colon

Giuseppe Pietropaolo<sup>1</sup>, Daniela Pugliese<sup>2</sup>, Francesca Luongo<sup>1</sup>, Federica I. Wolf<sup>1</sup> and Valentina Trapani<sup>1</sup>

<sup>1</sup> *Istituto di Patologia Generale, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "Agostino Gemelli" IRCCS, Rome, IT*

<sup>2</sup> *IBD Unit, Presidio Columbus Fondazione Policlinico Universitario "Agostino Gemelli" IRCCS, Università Cattolica del Sacro Cuore, Rome, IT*

**Purpose:** Magnesium (Mg) is an important micronutrient involved in various cell functions. Systemic Mg homeostasis mainly depends on the concomitant action of intestine and kidney, for its uptake and reabsorption, respectively. A significant proportion of patients treated with Cetuximab (CTX), a monoclonal antibody targeting the epidermal growth factor receptor (EGFR), develops a mild to severe hypomagnesemia. This effect has been ascribed to a specific down-regulation of the TRPM6 Mg channel that occurs in kidney epithelial cells. Given the importance of the intestine in maintenance of Mg homeostasis, we investigated: 1) whether CTX could also affect Mg homeostasis in the gut, 2) whether magnesium supplementation could impact on CTX efficacy.

**Materials and methods:** We used a colon epithelial cell line (CaCo2) to assess the effects of CTX, with or without EGF stimulation or Mg supplementation on Mg influx, viability and migration. Mg influx was evaluated by live cell imaging of Mag-Fluo-loaded cells. Cell viability and migration were evaluated by MTT tests and scratch assays, respectively.

**Results:** CTX significantly reduced magnesium influx, while EGF stimulation doubled it. Cetuximab inhibition of magnesium influx was rescued by EGF. EGF stimulation also enhanced wound healing capacity, while cetuximab reduced it. In CTX-treated cells, both Mg or EGF supplementation overrode the inhibitory effect of CTX on wound healing capacity. Mg supplementation did not affect CTX EC50.

**Conclusion:** CTX reduces magnesium uptake and cell migration of colon epithelial cells. Mg supplementation or EGF treatment rescues these effects. Our data suggest that Mg supplementation may represent a safe and cost-effective strategy to promote mucosal healing without impairing cetuximab efficacy.



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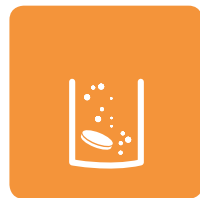
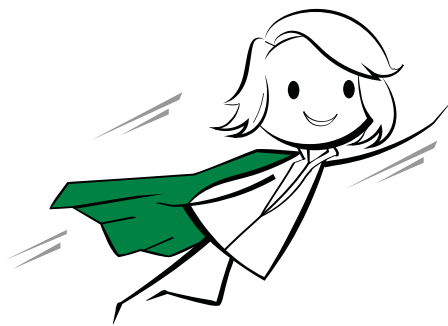


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## NOTES