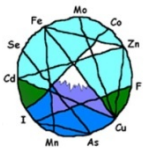
Pucón-Chile
Tema 13
2008

13 th International Meeting on Trace Elements in Man and Animales Pucón-Chile November 9th-13th, 2008.

Trace elements from the Andes to the world



Pucón-Chile
Tema 13
2008

Book of abstracts

13th International Meeting on Trace Elements in Man and Animals
Pucón-Chile
November 9th-13th, 2008.

Welcome message

Dear colleagues:

On behalf of the TEMA Parent Committee and the local Organizing Committee, we are pleased to welcome you to the 13th International Meeting on Trace Elements in Man and Animals.

We have chosen as a theme "From the Andes to the World" highlighting the fact that the Andes mountains are a major site in planet Earth of accumulated iron, copper, zinc, manganese, tin, selenium, molybdenum among others. In fact, the very word Andes is thought to derive from the quechua (original language of the indigenous people of Peru) word "ante", used by the Inca people to name copper. They "cultivated" rather than "mined" copper in the "anta-chakras" (copper fields) and left offerings to the gods as they collected the mineral for processing. The Andes span virtually all of the Americas from North to South and have served as back bone to unite the various people that inhabit this continent.

We look forward to an exiting scientific program that includes 6 conferences, 13 Symposia, 30 oral presentations and 118 Posters. Outstanding researchers will discuss the different aspects of trace elements from cellular metabolism and molecular biology in human, animal and plant sciences to the applications of this knowledge.

We invite you to enjoy the amazing volcanoes-and-lakes area and hope that during your stay you will have a chance to appreciate not only the science and the scenery but also the culture and the hospitality of our people.

Sincerely,

The Organizing Committee

Committees

Parent Committee

Mary L'Abbé, Chair, Canada
Harry McArdle, Secretary/Treasurer, UK.
Dennis J. Thiele, USA.
Bo Lönnerdal, USA
J.(Sean) J. Strain, Northern Ireland, UK
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International Atomic Energy Agency (IAEA)
Latin American Zinc Association (LATIZA)

Venue

The 13th International Meeting on Trace Elements
in Man and Animals
will be held at the Gran Hotel Pucón
November 9-13, 2008.

Map of Pucón

PROGRAM

PROGRAM OF EVENTS

Sunday, November 9, 2008

	Arrival & registration
20:00	Welcome dinner (Lonquimay Room)

Monday, November 10, 2008

7:00-8:30	Breakfast
8:30-9:30	Plenary session. State of the art on copper (Araucanía Room) Magdalena Araya. Copper effects in human nutrition Leo Klomp. Copper homeostasis disorders; a tale of dogs, mice and men.
9:30-10:30	Plenary session. State of the art on iron (Araucanía Room) Elizabeth Theil. Frontiers in iron nutrition and antioxidant metabolism. Caroline C. Philpott. A cytosolic iron chaperone that delivers iron to ferritin.
10:30-11:00	Coffee break
11:00-12:00	Plenary session. State of the art on zinc (Araucanía Room) Michael Hambidge. Human zinc deficiency: new insights into zinc homeostasis provide a quantitative basis for prevention and treatment of a new public health challenge. Juan Pablo Liuzzi. Zinc research: current status and future perspectives.
12:00-13:00	Plenary session. State of the art on Selenium (Araucanía Room) Roger Sunde. Molecular selenium nutrition – from discovery to molecular biology biomarkers for selenium status and requirements. Vadim N Gladyshev. Selenoproteins and their roles in redox biology.
13:00- 14:00	Lunch (Restaurant)
15:00 - 17:00	*Symposium 1.* Advances in Proteomics. Analytical, structural and functional aspects (Araucanía Room) D Behne (chair). Trace elements as binding partners of proteins. Dirk Schaumloeffel. Detection of trace element-containing proteins. Brian Gibney. Modern methods of analysis of Zn proteins *Symposium 2.* Transition metals and oxidative stress (Lonquimay Room) Hernán Speisky (chair). Cu(I)-glutathione complex: a new biological source of superoxide radicals? Marco Tulio Nuñez. An iron-calcium connection in nmda receptor signaling and hippocampal synaptic plasticity. Xingen Lei. Hidden roles of Se-GPX1 and Cu,Zn-SOD *Symposium 3.* Advances in the use of stable isotopes for applications in humans (Coñaripe Room) Nancy Krebs (chair). Zinc homeostasis: taking current knowledge to the field Leland V. Miller. Zinc absorption: modeling the impact of dietary factors Lena Davidsson. Iron absorption in children: implications for global challenges.

Sue Fairweather-Tait. Copper homeostasis: how stable isotope applications inform understanding

17:00 -17:30 Coffee break

17:30 - 19:00 Posters session with cheese & beer (Llaima Room)

P001. The development of a food iron bioavailability index (FIBI). Marcia J. Cooper, Kevin A. Cockell and Mary R. L'Abbé

P002. Dietary intake modelling supports a low level of iron supplementation for pregnant Canadian women. Kevin A Cockell, Hélène Lowell, Doris C Miller and George H Beaton

P003. Iron and zinc dialyzability in an infant diet including bread with different iron sources or absorption promoters. Maria Julieta Binaghi, Nestor Pellegrino, Patricia Ronayne and Mirta Valencia.

P004. Mineral dialyzability from expanded products fortified with bovine hemoglobin. Silvina R. Drago, M. Julia Birocco, Rolando J. González and Mirta E. Valencia

P005. Effect of *lupinus angustifolius* chelating peptides on mineral dialyzability from fortified foods. Silvina R Drago, Manuel Alaiz and Javier Vioque

P006. Effect of globin, erythrocyte stroma, cow, chicken and fish meat on heme-iron bioavailability. Valerie Weinborn, Manuel Olivares, Miguel Arredondo, Eva Hertrampf and Fernando Pizarro.

P007. Effect of purified animal and vegetal proteins on heme-iron bioavailability. Valerie Weinborn, Manuel Olivares, Miguel Arredondo, Eva Hertrampf and Fernando Pizarro.

P008. Iron supplementation in previously anemic bolivian children normalized hematologic parameters, but not immunologic parameters. Edgar A. Sejas, Patrick Kolsteren, Tom Hoeree, Daniel B Roberfroi.

P009. Nutritional status of zinc in children with down sindrome. Adriana de Souza Lima, Bárbara Rita Cardoso and Silvia Maria Franciscato Cozzolino.

P010. Assessment of glutathione peroxidase activity in Alzheimer's disease patients. Bárbara Rita Cardoso, Wilson Jacob Filho, Omar Jaluul, Thomas Prates Ong and Silvia Maria Franciscato Cozzolino

P011. Errors in estimating zinc requirements in populations. Farzad Amirabdollahian and R. Ash

P012. Phytate intake and molar ratio of phytate: zinc in the diet of the UK population. Farzad Amirabdollahian and R. Ash

P013. Zinc supplementation has divergent effects on plasma high-density lipoprotein cholesterol concentrations in humans: a meta-analysis. Meika J Foster, Peter Petocz, Samir Samman

P014. Ratios of IGF-1, IGF binding protein-3 and zinc in cancer and benign human prostate hiperplasia. Adam Daragó, Andrzej Sapota, Jan Taczalski and Anna Kilanowicz

P015. Effect of red wine extract supplementation on copper, polyphenols and total antioxidant status in elderly people. Grzegorz Mielcarz, Aleksander Barinow-Wojewodzki

P016. Potencial indicators to detect early effects of copper exposure in humans. Miguel Arredondo, Silvia Flores, Héctor Núñez, Valeria Candia, Fernando Pizarro, Magdalena Araya.

P017. Copper status and their relation to thyroid hormone profile in male and female goitrous patients. Ghulam Abbas Kandhro, Tasneem Gul Kazi, Sirajuddin, Hassan Imran Afridi, Naveed Kazi, Mohammad Balal Arain, Raja Adil Sarfraz, Abdul Qadir Shah, Nasreen Syed and Jameel Ahmed Baig

P018. Chronic exposure to high copper doses in non-human primates: a model of copper loading. Héctor Núñez, Miguel Arredondo, Marco Méndez, Fernando Pizarro, Manuel Olivares, Ricardo Uauy and Magdalena Araya.

P019- effects of Brazilian nuts intake on the selenium blood concentrations and on erythrocyte glutathione peroxidase activity in morbid obese women. Cristiane Cominetti, Maritsa Carla de Bortoli, Thomas Prates Ong, Fernando Salvador Moreno, Arthur Belarmino Garrido Jr. and Silvia Maria Franciscato Cozzolino

P020. Mercury level and selenium status in women living in Cubatão, São Paulo, Brazil. Maritsa C. de Bortoli, Cristiane Cominetti, Luciana A. Farias, Déborah I.T. Fávaro and Silvia M.F. Cozzolino

P021. Nutritional status of selenium of patients with thyroid disorders of Fortaleza/CE–Brazil. Carla Soraya Costa Maia; Liliane Viana Pires; Luciana Sigueta Nishimura, Alexandre Coelho Pimentel; Rafael Barofaldi Bueno; Renan Magalhães Montenegro Junior; Virgínia de Oliveira Fernandes and Sïlvia Maria Franciscato Cozzolino.

P022. Assessment of nutritional status on the selenium of patients with Turner syndrome in different stages of development. Liliane Viana Pires,

José Alexandre Coelho Pimentel, Rafael Barofaldi Bueno, Luciana Sigueta Nishimura, Carla Soraya Costa Maia, Adriana Siviero-Miachon, Tatiana Fabbri T, Angela Maria Spinola-Castro and Silvia Maria Franciscato Cozzolino.

P023. Relationships between respiratory diseases, nutrition items and trace elements in ingested food. Maria do Carmo Freitas and Adriano M.G. Pacheco.

P024. Total diet study: Fe, Cr, Zn and Se content estimation of São Paulo State (Brazil) diet by instrumental neutron activation. Roseane P. Avegliano , Vera A. Maihara[2]and Fábio F. Silva

P025. Determination of iron, seleniun and zinc in milk formulas commercialized in São Paulo city- Brazil. Paola Santos, Vera A. Maihara, Mitiko Saiki, Maria Esther Ceccon, Jane Oba, Cecília Yu, Roseane P. Avegliano

P026. Micro distribution of biologically important metals in primary invasive ductal carcinoma of breast. Michael Farquharson, Alia Al-Ebraheem, Russell Leek and Adrian Harris.

P027. Trace elements in dietary supplements. Tore Syversen, Marte Aurstad Aspnes, Kristin Gellein and Lars Evje.

P028. Lead and arsenic levels in reproductive age women with different nutritional status living in Santiago, Chile. Yareni Gutierrez, L. Muñoz , Gabriela Salazar, Miguel N Llanos and Ana M Ronco

P029. Trace elements in hemochromatosis. Bjørn J. Bolann and Rune J. Ulvik

P030. Iron, zinc, and calcium content of commercially-produced cereal-based complementary foods from Africa and Mongolia fail to meet the estimated needs for 9-11 month breast-fed infants. Karl B Bailey, Rebecca Lander, Tserennadmid Enkhargal and Rosalind S Gibson

P031. The correlation of arsenic levels in drinking water with the biological samples of skin disorders. Tasneem Gul Kaz, Muhammad Balal Arain, Ghulam Abbas Kandhro, Muhammad Khan Jamali, Hassam Imran Afridi, Nusrat Jalbani, Raja Adil Sarfraz, Jameel Ahmed Baig, Abdul Qadir Shah.

P032. Study on dietary iron intake in Chinese adult man and interaction between iron and lead in rats. Junquan Gao

P033. Dietary exposure assessments of heavy metals and trace elements in China. Junquan Gao and Xiaowei Li

P034. Factorial design and CCD used as optimization procedures for direct determination of iron in canine serum samples by GF AAS with in-situ matrix removal. Carolina Carvalho de Souza, Henrique José Ferraz Fabrino, Waldomiro Borges Neto, Aldair junior Woyamos Pinto, José Bento Borba da Silva and Wagner Luiz Tafuri

P035. Copper deficiency in calves may increase susceptibility to infectious keratoconjunctivitis. Leonardo Minatel, Gabriela Cintia Postma, Susana Cristina Underwood, María Elena Dallorso and Julio César Carfagnini.

P036. Copper and iron bioavailability in anemic rats fed fructans-containing yacon (*Smallanthus Sonchifolius*) flour-supplemented diets. Alexandre Rodrigues Lobo, Maria Lúcia Cocato, Primavera Borelli, Amanda Crisma, Karina Nakajima and Célia Colli.

P037. Variations in metallothionien, copper and zinc concentrations in the livers and kidneys of dogs, cats and horses. Carmen I Fuentealba, M George Cherian, JC Lau

P038. Liver biopsy in cattle. A safe procedure to evaluate copper accumulation in the liver. Marco García-Vaquero, Isabel Blanco-Penedo, José Luis Benedito, Marta López-Alonso, Betiana Gutiérrez and Marta Miranda

P039. Dietary fluoride effect on liver weight and body weight of New Zealand white rabbits. Artur Canella Avelar and Walter Motta Ferreira.

P040. Effect of plant species, harvest time and soil type on mineral content of organic forage crops. Jakob Sehested, Søren Krogh Jensen and Karen Søegaard

P041. Cadmium levels in edibles fish species of gulf and Pacific coasts of Mexico. Marisela Méndez-Armenta, M Isabel Castro-González, Sergio Montes, Camilo Ríos and Sara Montaño

P042. Phosphorus, potassium, calcium, protein and N-3 PUFA content in marine fishes as an option in patients with renal diseases´ diet. María Isabel Castro-González, Daniela Miranda Becerra and Sara Montaño Benavides.

P043. HO1, BAX and BCL-2 gene expression in cells incubated at different iron concentrations. Marcela Fuentes and Miguel Arredondo

P044. An iron-calcium connection in NMDA receptor signaling and hippocampal synaptic plasticity. Pablo Muñoz-Carvajal, Alexis Humeres, Cecilia Hidalgo and Marco T. Núñez

P045. Participation of aldosterone in hypotension secondary to iron deficiency anemia. Aki Konomi and Katsuhiko Yokoi.

P046. Iron and zinc dialyzability in quinoa crops: preliminary study. Binaghi Maria Julieta, Dyner Luis, Zuleta Angela, Bertero Hector and Pallaro Anabel.

P047. Calcium increases iron uptake by Caco 2 cell line. Diego Gaitán, Manuel Olivares, Miguel Arredondo and Fernando Pizarro.

P048. Impact of iron-deficiency on ^{210}Pb-absorption along the murine intestine. Klaus Schümann, Noel Solomons, Monica Orozco, Bernd Elsenhans.

P049. Effects of zinc supplementation on gene expression of zinc transporters and metallothionein in hemodialysis populations. Amanda Amorim, Rubens Santana, Maraysa Carvalho, Rafael Brandão, Hélida Andrade, José Adail Castro, Adalberto Silva, José Tiburcio Neto, Carmem Barros, Erasmo Oliveira, Waldemar Bartchewsky, Marcelo Ribeiro, Semiramis Monte, Nadir Nogueira

P050. In-silico identification and characterization of efflux heavy metals resistance proteins in *Acidithiobacillus Ferrooxidans*. Mauricio Latorre, Pablo Moreno, Andrés Aravena, Verónica Cambiazo, Alejandro Maass and Mauricio González

P051. CIND, a copper-induced nitroreductase involved in protein denitrosylation in *Lactococcus Lactis*. Frédéric Mourlane and Marc Solioz.

P052. Role of selenoproteins in brain function and development. Ulrich Schweizer.

P053. Cancer chemoprotection through selenium: a nutriproteomics approach to identify selenium biomarkers. Andrea Mahn, Héctor Toledo, Manuel Ruz and Ricardo Vega

P054. Effect of inorganic and organic selenium supplementation on growth rate, blood metabolites, antioxidant status and immune response in lambs. Anil K. Garg, Neeraj Kumar, Vinod K. Chaturvedi and Vijai P. Varshney

P055. Effect of selenium supplementation on milk yield in grazing dairy cows. Javier Neumann, Alejandro Ceballos, Helga Böhmwald and Fernando Wittwer

P056. The health promoting quality of food obtained from crops foliary enriched with selenium. Ivana S. Djujic

P057. Selenium determination by neutron activation analysis (NAA) applied to poultry production and health. María Dallorso, Sara Resnizky, Rodrigo Invernizzi and Ernesto Benavídez

P058. Congruency and divergency of calcium in the biological matrice of the human hair with the other osteotrophic and non-osteotrophic members of the multielement profile. Juraj Prejac, Asja Čelebić, Jasmina Stipetić-Ovčarićek, Renata Poljak-Guberina, Petra Nola-Fuchs, Anatoly Viktorovich Skalny, Margarita Germadievna Skalnaya, BerislavMomčilović.

20:00 Dinner & social activity (Restaurant)

Tuesday, November 11, 2008

7:00-8:30 Breakfast
8:30-9:00 Plenary session. Leone Memorial Lectureship (Araucanía Room)
Carmen Donangelo. Zinc utilization in women during reproduction: evidence of physiologic adaptation.
9:00-10:30 *Symposium 4.* Selenoprotein P metabolism and functions in animals and humans (Araucanía Room)
John Arthur (chair). Selenoprotein P: variations in response to selenium supplementation
Ray Burk. Selenoprotein P and "selenium status"
Lutz Schomburg. Selenoprotein P and disease in humans and animals.

9:00–10:30 *Oral abstracts presentation session 1.* Copper, zinc, and iron basic aspects (Lonquimay Room).
Mauricio González (chair)
9:00–9:15 *O01*. The zinc transporter 3 (ZnT3) sorting and transport activity are regulated by covalent ZnT3-oligomers. Gloria Salazar and Victor Faundez.
9:15-9:30 *O02.* Dietary iron affects proteins involved in iron metabolism in weanling pigs. Stephanie L Hansen and Jerry W Spears.
9:30-9:45 *O03.* Effect of zinc on hepatic copper and iron concentrations in experimentally induced copper toxicity in rats. Carmen Fuentealba, Susan Haywood, Kent G. Hecker and Jim Trafford
9:45-10:00 *O04.* Live imaging of metal-ion transport in oocytes expressing the human divalent metal-ion transporter DMT1: substrate profile and selectivity of DMT1. Anthony C Illing, Ali Shawki, Christopher L Cunningham, and Bryan Mackenzie
10:00-10:15 *O05.* Dual-localization of ZnT2 to mitochondria and exocytotic vesicles redistributes zinc pools in mammary cells. Young Ah Seo, Veronica Lopez and Shannon L Kelleher
10:15-10:30 *O06.* Is copper chaperone for cu/zn superoxide dismutase a potential biomarker of mild copper supplementation? Miriam Suazo, Talía del Pozo, Marco Méndez, Mauricio González and Magdalena Araya.
9:00–10:30 *Oral abstracts presentation session 2.* Health hazards (Coñaripe Room)
Mary L'Abbé (chair)

9:00–9:15 *O07*. Long term effects of cadmium on forearm bone density in a chinese population. Xiao Chen, Guoying Zhu, Taiyi Jin, Agneta Åkesson , Ingvar A. Bergdahl , Lijian Lei, Shifang Weng and Yihuai Liang

9:15-9:30 *O08.* Glutathione modulation influences methyl mercury induced toxicity in albino rats. Varsha Singh, Deepmala Joshi, Sadhana Shrivastava, Sangeeta Shukla and Mohammed Abdullah

9:30-9:45 *O09.* Effects of complex I inhibition on mitochondrial iron homeostasis, in an experimental model of parkinson's disease. Natalia Mena, Julio Salazar, Enrique Armijo, H. Stephen, Etienne Hirsch and Marco Tulio Núñez.

9:45-10:00 *O10.* Competition between oligomeric silicic acid and transferrin for aluminium binding and implications for aluminium toxicity. Sylvaine FA Bruggraber, Ravin Jugdaohsingh, William Cook and Jonathan J. Powell

10:00-10:15 *O11.* Comparison of prenatal biomarkers of low-level methyl mercury exposure. Janja Tratnik, Irena Rupnik and Milena Horvat

10:15-10:30 *O12.* Rapid screening of toxic elements via X-ray fluorescence spectrometry. Richard Jacobs, Janet McDonald and Peter Palmer.

10:30-11:00 Coffee break

11:00-13:00 *Symposium 5.* Trace elements and the central nervous system (Araucanía Room)

Joe Prohaska (chair). Impact of copper deficiency on brain energy metabolism

Raymond Burk. Selenoprotein P and brain function

John Beard. Iron deficiency and neural functioning

Symposium 6. New approaches to define TE biomarkers (Lonquimay Room)

Magdalena Araya (co-chair)

Harry McArdle (co-chair). Identification of biomarkers for micronutrient status – a comparison of genomics and proteomics approaches.

Ioav Z Cabantchik. Misdistribution of iron: causes, pathological implications and attempts of correction.

Ruan Elliott. What is the true potential of transcriptomic methods for biomarker discovery in trace element research?

John Beattie. Zinc biomarker discovery: light at the end of the tunnel?

Symposium 7. Interventions with trace elements in susceptible populations (Coñaripe Room)

Manuel Ruz (chair)

Rosalind Gibson. Interventions for combating micronutrient deficiencies in Africa: problems, progress, and future solutions.

Emorn Wasantwisut. Trace elements interventions in Asia: knowledge vs challenges

Daniel Lopez de Romaña. Recent trace elements interventions in Latin America

Noel Solomons. Interventions with trace elements (TES) in susceptible populations: when does safety trump efficacy?

13:00 - 14:00 Lunch (Restaurant)

15:00	Social activity. Trip to “Termas del Huife”
20:00	Dinner (Restaurant)

Wednesday, November 12, 2008

7:00-8:30	Breakfast
8:30-9:00	Plenary session. Underwood Lecture (Araucanía Room) Janet King. Why are indicators of zinc status so elusive?
9:00 – 10:30	*Oral abstracts presentation session 3.* Trace elements in animal metabolism (Araucanía Room) Fernando Wittver (chair)
9:00–9:15	*O13.* Effect of selenium supplementation on somatic cell counts in grazing dairy cattle. Alejandro Ceballos, Juan Kruze, Daniel Uribe, Javier Sanchez, Ian Dohoo, Herman Barkema, Jeff Wichtel, Javier Neumann and Fernando Wittwer.
9:15-9:30	*O14.* Persistence of blood changes associated with alteration of the dietary electrolyte balance following feed withdrawal, transportation and lairage in market weight swine. Lily N. Edwards, Terry E. Engle, Temple Grandina and David B. Anderson.
9:30-9:45	*O15.* Effects of copper on ruminal fermentation and biohydrogenation of unsaturated fatty acids *in vitro*. Jennifer S. Schutz and Terry E. Engle.
9:45-10:00	*O16.* Dietary silicon: a beneficial mediator of immunoregulating and stress proteins in mammals? Sarah Ratcliffe, Ravin Jugdaohsingh and Jonathan J Powell
10:00-10:15	*O17.* Effects of long-term copper deficiency on gene expression profiles of copper transporters and chaperones in the liver of cattle. Robert S. Fry, Melissa S. Ashwell, Stephanie L. Hansen, Terry E. Engle, Hyungchul Han and Jerry W. Spears
10:15-10:30	*O18.* Effect of dietary antagonists on copper metabolism of sheep. Alexander M. Mackenzie, Carolyn M. Atkin, Nia Griffith, Claire L. Williams, Simon G. Edwards and Robert G. Wilkinson
9:00 – 10:30	*Oral abstracts presentation session 4.* Iodine and selenium (Lonquimay Room) Sean Stain (chair)
9:00–9:15	*O19.* The effect of high iodine intake on thyroid hormones and selenium status in older people. Christine Thomson, Jenny Campbell, Jody Miller and Sheila Skeaff
9:15-9:30	*O20.* Probiotics and selenium metabolism: does it matter whether the bacteria are dead or alive? Woravimol Krittaphol, Philip Wescombe, Arlene McDowell, John R. Tagg, Christine D. Thomson and J. Paul Fawcett
9:30-9:45	*O21.* Iodine status and cognitive function of women of childbearing age and their five year-old children in Sidama, Southern Ethiopia. Alemtsehay Bogale, Cherinet Abuye, Kassu Gurmu, Yewelsew Abebe, K Michael Hambidge and Barbara J Stoecker
9:45-10:00	*O22.* Selenium in milk – selenium speciation and health effects. Tien Hoac, Peter Olsson, Vasileios Pagmantidis, Gitte Ravn-Haren, Jan Stagsted, Susanne Bügel, Gunilla Önning, Jacob H. Nielsen, Lars O. Dragsted and Björn Åkesson

10:00-10:15 *O23.* Selenium and human cancer. From epidemiological data to molecular biology study. Wojciech Wasowicz, Ewa Jablonska, Edyta Reszka and Jolanta Gromadzinska

10:15-10:30 *O24.* Selenoprotein W MRNa expression analysis in human colonic mucosa using affymetrix HGU133 plus 2.0 microarrays and real-time PCR. Jeannette Molnár, Orsolya Galamb, Ferenc Sipos, Sándor Spisák, Kinga Tóth, Norbert Solymosi, Annamária Németh, Zsolt Tulassay, Béla Molnár

9:00 – 10:30 *Oral abstracts presentation session 5.* Iron and zinc metabolism and interventions (Coñaripe Room)
Shannon Kelleher (chair)

9:00–9:15 *O25.* One-day zinc kinetics, a versatile tool for analysis of human zinc metabolism and its application. Katsuhiko Yokoi, Harold H. Sandstead, Norman G. Egger, Nancy W. Alcock, V.M. Sadagopa Ramanujam, Hari H. Dayal and James G. Penland

9:15-9:30 *O26.* Hepcidin and its role in regulating intestinal iron transport. Paul Sharp, Bomee Chung, Timothy Chaston, Joanne Marks, Edward Debnam and Surjit Srai

9:30-9:45 *O27.* Intakes and impact of dietary factors affecting iron availability in the Medical Research Council National Survey of Health and Development (MRC NSHD) (1946 British birth cohort) between 1982 and 1999. Anna P. Rickard, Mark D. Chatfield, Jonathan J. Powell and Alison M. Stephen

9:45-10:00 *O28.* Short-term inhibitory effect of calcium on iron absorption persists after an extended course of calcium supplementation in young women with limited iron stores. Karima Benkhedda, Mary R. L'Abbé and Kevin A. Cockell

10:00-10:15 *O29.* Maternal dietary zinc supplementation prevents cognitive impairment in adult offspring of mice exposed to infection (LPS) in early pregnancy. Allan M Rofe, Nancy Tran, Jenny N.T. Fung, Brooke L Summers, Joanne S Chua and Peter Coyle

10:15-10:30 *O30.* Zinc and iron absorption and nutritional status is reduced after gastric bypass in morbidly obese patients. Manuel Ruz, Fernando Carrasco, Pamela Rojas, Attila Csendes, Karin Papapietro, Jorge Inostroza, Annabella Rebolledo, Karen Basfifer, Fernando Pizarro, Manuel Olivares, Nancy Krebs, Jamie Westcott, Michael Hambidge.

10:30-11:00 Coffee break

11:00-13:00 *Simposium 8.* Trace elements in neurodegeneration (Araucanía Room)
Tulio Nuñez (chair)
George Perry. Evidence of increased copper and iron but not zinc in Alzheimer's disease: energy dispersive X-ray spectroscopy (EDS) elemental microanalysis.
Carlos Opazo. Untuning of protein-metal interactions in Alzheimer disease.
Etienne Hirsch. Iron in Parkinson's disease.
Moussa Youdim. Multifunctional iron chelators neuroprotective and neurorestorative drugs for alzheimer's disease.

Symposium 9. Trace elements and vascular disease (Lonquimay Room)
Y. James Kang (chair). Copper metabolic disorder in heart failure
John Beattie. Zinc in vascular health and disease
Markus Brielmeier. The morphological and functional integrity of

cardiomyocytes critically depends on the selenoprotein mitochondrial thioredoxin reductase.
Huiqi Xie. Copper and zinc metabolic changes and manipulation in hypertension.

Symposium 10. Animal nutrition current problems (Coñaripe Room)
Xin Gen Lei (chair)
Dennis Miller. Prebiotics and iron bioavailability: Using the piglet as a model to study effects and mechanisms.
Jerry Spears. Trace mineral transporters: implications for animal nutrition.
Zongyong Jiang. Potential of supplemental selenium in improving meat quality of food animals.

13:00-14:00 Lunch (Restaurant)

15:00-17:00 *Symposium 11.* Trace elements interactions in health (Araucanía Room).
Bo Lönnerdal (chair). Iron-Zinc Interactions: effect of maternal Fe and Zn supplementation on offspring Fe and Zn homeostasis.
James Collins. The Menkes copper ATPase (ATP7A) is novel iron responsive gene in the mammalian intestine.
Richard Hurrell. Contradictory findings on the influence of vitamin A on iron bioavailability in human subjects.
Ralf Biebinger. Interactions of vitamin a and iodine deficiencies: effects on the pituitary TSHB-gene and the thyroid axis.

Symposium 12. Increasing awareness on selected trace elements (Lonquimay Room)
Michael Garrick (chair). Increasing awareness of selected trace elements.
Jerry Roth. Role of NF-KB and parkin in regulating DMT1 expression and manganese toxicity.
Laura Garrick. Accumulation of trace metals as an assay for transport (by DMT1)
Brian Mackenzie. Substrate profile and selectivity of the human divalent metal-ion transporter DMT1.

Symposium 13. Trace element transport and its regulation (Coñaripe Room)
Jim Camakaris (chair). The pivotal role of the Menkes copper ATPase in copper transport and regulation of its function
José Arguello. Ferrying Cu across the membrane: molecular mechanism of Cu-ATPases.
Miguel Arredondo. New insights in heme and none-heme iron transport
Robert Cousins. Zinc transport and signaling

17:00-7:30 Coffee break

17:30-19:00 Posters session with cheese & beer (Llaima Room)

P059. Gender dependent homology of the human hair and whole blood multielement profile. Berislav Momčilović, Jadran Morović, Juraj Prejac, Sandra Morović, Vesna Sitar-Srebočan, Anatoly Viktorovich Skalny, Margarita Germadievna Skalnaya.

P060. Depression dependent homology of the human hair and whole blood multielement profile. Berislav Momčilović, Jadran Morović, Juraj Prejac, Sandra Morović, Ninoslav Mimica, Irena Bezić, Tanja Radionov, Ankica Svirč, Anatoly Viktorovich Skalny, Margarita Germadievna Skalnaya.

P061. Mucin does not improve heme iron bioavailability. Gustavo Cediel, Manuel Olivares, Fernando Pizarro.

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P070. Effect of copper on iron bioavailability. Manuel Olivares, Fernando Pizarro, Alejandra Martin and Manuel Ruz.

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P072. Assessment of selenium nutritional status of Riverine children from Rondônia State, Western Amazon. Ariana Vieira Rocha, Rafael Barofaldi Bueno, Cristiane Cominetti, Maritsa Carla Bortoli, Liliane Viana Pires Milena Barcza Pinto, Luís Marcelo Aranha Camargo and Silvia Maria Franciscato Cozzolino.

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P093. Managing mineral balance within beef-cattle systems. Isabel Blanco-Penedo, Richard F. Shore, Marta Miranda, José Luis Benedito and Marta López-Alonso

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P107. Mechanism of selenium cytoprotection against cholesterol oxide-induced vascular damage in rats. Kaixun Huang, Hongmei Liu, Qingzhi Wu, Rong Tang and Huibi Xu.

P108. Thimerosal reduces the expression of MT-1 mRNA in the cerebellum microglia cell line, C8-B4. Takeshi Minami and Eriko Miyata.

P109. The role of mitochondrial ferritin in cellular iron metabolism. Enrique A. Armijo, Natalia Mena and Marco T. Núñez

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P113. A comparative study of hair elements and pancreas function. Tatjana D. Lalic and Ivana S Djujic

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P115. A simpler analytical method for phytate. Donald Oberleas, Alemzewed Challa, Barbara Stoecker and Barbara F Harland.

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P117. The elementome matrix map for a complex contextual interactions in the human whole blood. Rastko Momčilović, Juraj Prejac, Dalibor Veber, Nikola Ivičić, Jadranka Pongračić, Anica Benutić, Glenn Irvin Lykken, Berislav Momčilović.

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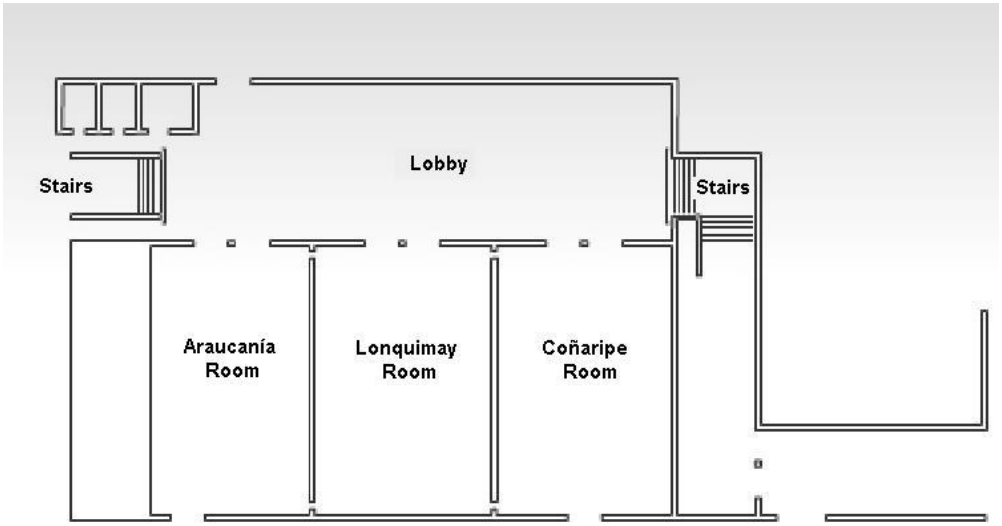
20:00 Dinner & social activity (Araucania Room)

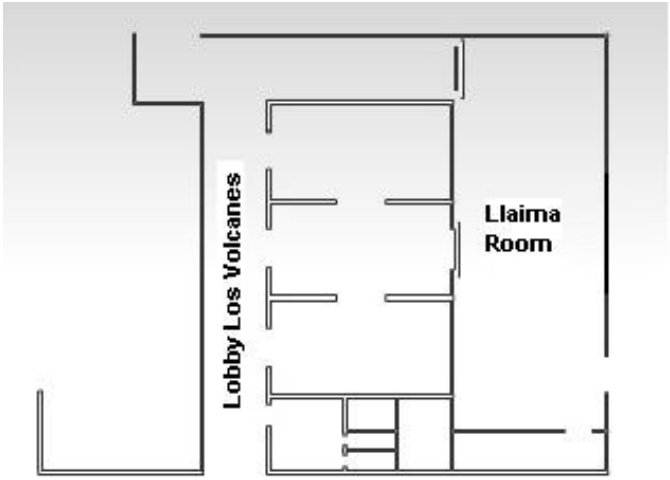
Thursday, November 13, 2008

7:00-9:00 Breakfast
Check-out

MEETING ROOMS



POSTER ROOM



ABSTRACTS

S01. COPPER EFFECTS IN HUMAN NUTRITION. Magdalena Araya, Manuel Olivares, Fernando Pizarro
Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, Chile.
maraya@inta.cl

Nutrigenomics raises the challenge to identify subgroups of population at risk of marginal copper deficiency or excess and protect them from adverse effects. For doing this, solid knowledge about copper metabolism and regulation is needed. Copper essentiality is clearly established; its presence is necessary for the adequate function of proteins indispensable for life, such as cytochrome c, superoxide dismutase, ceruloplasmin and many others. A biological ranking seems to ensure the most vital functions. Effects of severe deficiency and severe copper excess are associated with well known genetic conditions, Menkes disease and Wilson disease, respectively. However, how frequent is acquired copper deficiency and what are its early functionally relevant effects are not clear; in the same way and despite considerable efforts, it is still not clear what are the dosages and length of excess copper exposure necessary to induce increased deposit of the metal in liver and other tissues leading to damage. A relevant question is whether copper exposure as encountered in daily life poses health risks to the human population. Extensive studies in normal adults showed that nausea and vomiting are the earliest adverse effects derived from acute copper exposure, with a no-observed-effect level (NOEL) of 2 mg Cu/L for water. Exposure for months to copper dosing at the high range of the upper limit intake given by the Institute of Medicine of the National Academy of Science at different ages, in women and in men, has failed to demonstrate changes in general health markers, in liver function, oxidative stress and blood biochemical indicators. More recently, Cebus monkeys that received 7.5 mg Cu/kg/day orally, mixed with food, for 3 years remained healthy, with no changes in blood biochemistry, liver function and histology. We interpret these studies as suggesting that copper would be less toxic that initially thought a decade ago; however, knowing of the considerable differences among species, these results can not be inferred to humans. There is little evidence published about copper deficiency. In the last few years a series of studies have started to explore this area in humans, identifying specific deficient groups among young children and individuals over 65 years. Further studies are needed to better characterize these groups and define the best strategies for copper fortification/ supplementation. To improve understanding of the copper effects in humans there is a clear need for more knowledge about molecular mechanisms and paths responsible for copper cell metabolism and the effects on the whole body.
Supported by Fondecyt grant 1070595

S02. NEW INSIGHTS IN HEME AND NON-HEME IRON TRANSPORT. Solange Le Blanc, María José Mendiburo, Sebastián Flores, Miguel Arredondo,
Laboratorio de Micronutrientes, INTA, Universidad de Chile
marredon@inta.cl

Iron (Fe) is an essential and potentially toxic metal to human. Because its redox properties, it is involved in multiple essential metabolic processes. In diet, Fe can be found as heme or non-heme Fe and its absorption occurs mainly in small intestine and is carried out by enterocytes, specialized absorptive intestinal cells. The uptake of heme Fe and non-heme Fe occurs through different pathways, and both must be tightly regulated to maintain body Fe homeostasis. Non-heme iron uptake is mediated by the presence in the apical membrane of an oxide-reductase (DCytb) and DMT1 transporter. Inside the cell, Fe is complexed with small peptide forming the Labile Iron Pool compartment. Intracellular iron can be storage as ferritin or could be delivered to the basolateral side through the IREG1 (Ferroportin) transporter. In this membrane, hephaestin participated oxidating Fe+2 to Fe+3. In this form, Fe can be transported by transferrin molecule. The mechanism by which the heme Fe is taken up by the duodenal enterocytes has been much more difficult to elucidate. Recently, two proteins involved in heme transport have been identified: 1) HCP1, an intestinal heme transporter, found in mouse duodenum, which is highly expressed in Caco-2 cell line, -a model of human intestinal epithelium-, 2) FLVCR, a heme exporter found in human erythroid cells, which is expressed in high levels in Caco-2 cells, and may also be involved in heme absorption from the diet.

S03. SELENOPROTEIN P: VARIATIONS IN RESPONSE TO SELENIUM SUPPLEMENTATION. John R. Arthur
Vascular Health Division, University of Aberdeen, Rowett Institute of Nutrition and Health, Bucksburn, Aberdeen, AB21 9SB, U.K.
j.arthur@abdn.ac.uk

Selenium is essential for the maintenance of health in humans and animals. The involvement of selenium in this function in humans revolves around the products of the 25 selenoprotein genes that have been identified in the genome sequence, by the use of bioinformatic techniques. Most of these selenium-containing proteins have previously been characterised by biochemical techniques and all contain selenocysteine (Sec) at their active site or as an essential component of their biological function. Selenoproteins are involved in almost every aspect of cell metabolism and include proteins essential for selenium transport and selenoprotein synthesis (selenoprotein P and selenophosphate synthetase 2). In human populations in Europe, dietary selenium intake is often below recommended levels and thus expression of these selenoproteins is lower than maximum levels that are achievable with higher intakes. Many studies have also associated low selenium status with diseases or potentially detrimental physiological/biochemical changes in humans, leading some to suggest increasing selenium intake as part of a strategy to improve general health in the population. However, such a strategy requires properly controlled intervention trials to assess effectiveness and safety of the micronutrient. Thus in volunteer trials we have tested the responses of healthy UK residents to selenium supplements (<100□g Se /day) chosen to reflect dietary selenium intakes that could be achieved by dietary means. After supplementation increased levels of selenoproteins were detected in both plasma and blood cells. The magnitudes of these responses were associated with two common polymorphisms in selenoprotein P that also interacted with the gender and the BMI of the subjects. Thus we have demonstrated individual variation in the response to increased selenium intakes and this should to be taken into account when considering the potential effect of increased selenium intakes on public health.

Meplan, C., Crosley, L.K., Nicol, F., Horgan, G.W., Mathers, J.C., Arthur, J.R., Hesketh, J.E. (2008). Functional effects of a common single nucleotide polymorphism (GPX4c718t) in the glutathione peroxidase 4 gene: interaction with gender. American Journal of Clinical Nutrition 87: 1019-1027.

Meplan, C., Crosley, L.K., Nicol, F., Beckett, G.J., Howie, A.F., Hill, K.E., Horgan, G., Mathers, J.C., Arthur, J.R., Hesketh, J.E. (2007). Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner. (The SELGEN Study). FASEB Journal 21: 3063-3074

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S04. IRON DEFICIENCY AND NEURAL FUNCTIONING. John L Beard
Department of Nutritional Sciences, Penn State University, University Park, PA, USA
jbeard@psu.edu

Infants who experience iron deficiency during the first 6-12 months of life are likely to experience persistent effects of the deficiency that alter functioning in adulthood. There is accumulating data regarding the underlying biological processes that may explain these observations in humans. A lack of sufficient iron intake may significantly delay the development of the central nervous system due to alterations in morphology, neurochemistry, and bioenergetics. Morphological changes are reported in apical dendrites in hippocampus of rodents with early ID; these changes persist into adulthood. Metabolomic and genomic alterations are also apparent and again persist into adulthood in rodent models despite the subsequent correction of brain iron content. These data point clearly toward alterations in myelinogenesis. The neurochemistry of various eurotransmitters is altered by early ID with a decreased expressed of DA transporters and receptors on the cell surface, increased extracellular DA. These alterations may be related to the dynamics of iron movement or flux in and out of brain regions and across the blood brain barrier. GABA metabolism is also changed with early ID and this may be independent, or co-dependent, with the changes in monoamine metabolism. Depending on the stage of development at the time of iron deficiency, there may be an opportunity to reverse adverse effects, but the success of repletion efforts appear to time-dependent. Chilean children have persistent alterations in Auditory Evoked Potentials, serum prolactin levels, and cognitive functioning despite correction of their early ID before 2 years of age. Publications in the past several years describe the emerging picture as to the consequences of iron deficiency in both human and animal studies. The mechanisms for iron accumulation in the brain and perhaps redistribution are being understood. The data in human infants are consistent with altered myelination of white matter, changes in monoamine metabolism in striatum, and functioning of the hippocampus. These studies indicate that gestation and early lactation are likely critical periods when iron deficiency will result in long lasting damage.

S05. ZINC BIOMARKER DISCOVERY: LIGHT AT THE END OF THE TUNNEL? John H. Beattie[1], Graham W. Horgan[1], Sylvia Hay[1], Nancy F. Krebs[2], John Draper[3], Shaobo Zhou[3] and Manfred Beckmann[3]
[1]Division of Vascular Health, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen AB21 9SB, UK
[2]Section of Nutrition, Department of Pediatrics, University of Colorado School of Medicine, Denver, Colorado, USA
[3]Institute of Biological Science, Edward Llwyd Building, University of Wales Aberystwyth, Aberystwyth, Ceredigion SY23 3DA, Wales, UK.
J.Beattie@rowett.ac.uk

Conventional approaches to biomarker discovery have yielded an array of methods for the assessment of zinc status over 50 years, but all of them suffer from the disadvantage of lacking sensitivity and/or specificity. In the absence of anything better, analysis of plasma or serum zinc remains the only biochemical indicator recommended by WHO/UNICEF to evaluate population zinc status. For this purpose (population analysis), assay of plasma zinc may suffice albeit with poor sensitivity, but as a diagnostic indicator of marginal deficiency in individuals, it is hopelessly inadequate. Other proposed assays including urinary zinc, blood cell zinc, plasma and blood cell metallothionein, thymulin, retinol and retinol binding protein and various zinc-dependent enzymes, have proved less than ideal in methodology, specificity and/or sensitivity. It is entirely possible that there is no single biomarker that can reliably be used to assess zinc status and so a piecemeal approach to biomarker discovery is perhaps destined to fail. We are examining holistic approaches to biomarker discovery using proteome and metabolome profiling. In addition to discussing the utility of biomarkers such as metallothionein for the assessment of zinc status, this new systems approach, including plans for a proposed human study, will be presented and discussed. We believe that this approach will result in the generation of biomarker combinations that will specifically, and with sensitivity, identify zinc deficiency from other confounding factors.
JHB, GWH and SH are funded by the Rural and Environment Research and Analysis Directorate of the Scottish Executive.

S06. ZINC IN VASCULAR HEALTH AND DISEASE. John H. Beattie, Susan J. Duthie, Chris J. McNeil, In-Sook Kwun and Margaret-Jane Gordon
Division of Vascular Health, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen AB21 9SB, UK
J.Beattie@rowett.ac.uk

Inadequate zinc nutrition has long been associated with direct and measurable biological consequences, such as reduced growth and impaired immune response. In contrast, the impact of zinc nutrition on the development of chronic diseases has hardly been considered because of a lack of reliable zinc status biomarkers and therefore an absence of relevant epidemiological studies. Limited evidence indicating an association between zinc deficiency and vascular health has suggested that key arterial targets for zinc include PKCα, and that vascular smooth muscle cell differentiation may be adversely affected, as evidenced by zinc deficiency influences on transgelin isoform expression [1]. We have utilized a mouse model of atherosclerosis to investigate the impact of zinc nutrition on the development of arterial plaques and on a range of different parameters indicating chronic vascular disease, including plasma lipids and cholesterol, soluble adhesion factors, adipokines and cytokines. At weaning, apoE-null mice and wild-type mice on the same C57BL/6 genetic background were given diets that were marginally deficient in zinc, ranging from 3 to 8 mg Zn/kg diet for 15 and 25 weeks, and compared with animals given a zinc adequate diet of 35 mg Zn/kg diet. Since acute zinc deficiency reduces food intake, necessitating inclusion of pair-fed groups, the lowest zinc intake level was selected to ensure that food intake and animal growth were not markedly affected by zinc intake. Aortic arch plaque development was analysed quantitatively using an oil red O staining technique. Blood plasma analytes were measured using Luminex xMAP technology and a Konelab autoanalyser with Labmedics kits. Pilot study data indicated a significant ($p<0.05$) increase in aortic arch plaque development with decreasing zinc intake, but this finding was not reproduced in the full 25-week study. Nevertheless, by 15 and 25 weeks, total cholesterol and sVCAM 1 were among several parameters found to be significantly ($p<0.05$) raised in apoE-null mice at the lowest zinc intake compared to the appropriate control group. Biochemically therefore, zinc deficiency increased parameters associated with a greater risk of developing atherosclerosis and heart disease. Further studies are in progress to confirm these results and to investigate the metabolic and signaling pathways affected by altered zinc status. JHB, SJD, CJM and MJG were funded by the Rural and Environment Research and Analysis Directorate of the Scottish Executive. ISK was funded by the Korea Science and Engineering Foundation (KOSEF) (grant No. F01-2006-000-10172-0) and the Korea Research Foundation (KRF) (grant No. KRF-2007-313-C00810)

1. Beattie J.H., Gordon M-J., Rucklidge G.J., Reid M.D., Duncan G.J., Horgan G.W., Cho Y.E. and Kwun I.S. (2008). Aorta protein networks in marginal and acute zinc deficiency. Proteomics, 8: 2126-2135.

S07. TRACE ELEMENTS AS BINDING PARTNERS OF PROTEINS. Dietrich Behne
Hahn-Meitner-Institut, D-14109 Berlin, Germany
dietrich.behne@freenet.de

There are several indications that in addition to the metalloproteins already known there is a large number of proteins which likewise contain certain trace elements, either as part of the active centers of enzymes or as structural components. It has been shown that the biological significance of most essential metals and metalloids is due to such functions, and the investigation of relationships between these elements and proteins is therefore a useful approach in order to obtain initial information on the existence of further metalloproteins not yet identified. Some other trace elements assumed to have biological effects might also act as constituents of metalloproteins and should therefore be included in these studies. In this introductory overview the metals and metalloids are discussed which should be taken into consideration as potential binding partners of proteins and which are therefore of interest in metalloprotein research.

S08. THE MORPHOLOGICAL AND FUNCTIONAL INTEGRITY OF CARDIOMYOCYTES CRITICALLY DEPENDS ON THE SELENOPROTEIN MITOCHONDRIAL THIOREDOXIN REDUCTASE. Claudia Kiermayer and Markus Brielmeier.Department of Comparative Medicine, Helmholtz Zentrum München - German Research Center for Environmental Health (GmbH), Neuherberg, Germany
brielmeier@helmholtz-muenchen.de

Introduction: Cardiomyopathies are associated with Selenium deficiency in man and several animal species. So far, only very limited information is available about the role of individual selenoproteins in this context. Knockout mice for single selenoprotein genes provide suitable models to study loss-of-function phenotypes and search for candidate genes. Thioredoxin reductase 2 (Txnrd2) together with thioredoxin 2 (Txn2) constitutes the mitochondrial thioredoxin system playing a crucial role in the maintenance of cellular redox homeostasis. Ubiquitous deletion of Txnrd2 in mice is embryonic lethal and associated with abnormal heart development, while constitutive, heart-specific Txnrd2 inactivation leads to dilated cardiomyopathy and perinatal death1. Methods: To study the specific role of this redox-active enzyme system in the adult myocardium, the inducible cardiomyocyte specific αMHC-MerCreMer mouse2, in which the activity of Cre recombinase is Tamoxifen (TM)-dependent, was used to inactivate loxP site flanked (fl) Txnrd2 (Txnrd2fl/fl) alleles in adult mice3. Results: As confirmed by Real-Time PCR and Western Blot experiments, Txnrd2 mRNA and protein expression was efficiently reduced after induction in knockout animals. Morphological analysis revealed left ventricular hypertrophy (LVH) in aged Txnrd2 knockout mice. Ultrastructural analysis showed that mitochondria in knockout cardiomyocytes degenerated and that in these myocytes the accumulation of the age pigment lipofuscin was increased. Echocardiography revealed a significant increase in left ventricular end-systolic diameters (LVESD) in knockouts, while fractional shortening (FS) and ejection fraction (EF) were significantly decreased. Six and 10 months after knockout induction systolic and diastolic blood pressures were significantly lower in knockout mice. In summary, heart-specific Txnrd2 ablation in adult mice caused degeneration of mitochondria associated with left ventricular hypertrophy as an adaptive response to contractile dysfunction. Increased accumulation of lipofuscin was suggestive of accelerated aging of the myocardium. Outlook: Txnrd2 heart-specific knockout mice might represent a useful model to examine the effect of exercise, nutrition or therapeutic agents on chronic ROS-related myocardial damage.
This work was supported by the German Research Foundation (DFG).
References: (1) Conrad et al. Mol Cell Biol. 2004 Nov;24(21):9414-23. (2) Sohal et al. Circ Res. 2001 Jul 6;89(1):20-5. (3) Kiermayer et al. Genesis. 2007 Jan;45(1):11-6.

S09. SELENOPROTEIN P AND SELENIUM STATUS IN HUMAN BEINGS. Raymond F. Burk.
Vanderbilt University, Nashville, Tennessee, USA
raymond.burk@vanderbilt.edu

Selenoprotein P (Sepp1) and glutathione peroxidase-3 (Gpx3) are the only known selenoproteins in plasma. Plasma selenium is made up of selenium in Sepp1, Gpx3, a variable amount of ingested selenomethionine, and a small amount (<3% of the total) of small-molecule forms. Animal studies have shown that Sepp1 and Gpx3 are useful indices of selenium nutritional status. However, they do not rise when amounts of selenium greater than the nutritional requirement are given and therefore are not useful indices of supra-nutritional intake. We have carried out studies of Sepp1 in groups of people to assess its value as an index of selenium status. Selenium-replete subjects have 64 µg selenium/L plasma in Sepp1 and 17 µg/L in Gpx3. Supplementing these subjects with selenium did not increase the plasma concentration of Sepp1 or its glutathione peroxidase activity. Selenium-deficient subjects in China had Sepp1 concentrations that were 23% of those in selenium-replete subjects and glutathione peroxidase activities that were ~40%. Supplementation of the subjects with selenium resulted in an increase of glutathione peroxidase activity to the 'replete' value with a lower dose of selenium than was needed for a similar increase of Sepp1. Thus, when Gpx3 is optimized Sepp1 may not be optimized, but when Sepp1 is optimized Gpx3 will also be optimized. Because the marker for adequacy of selenium should represent as many of the 25 human selenoproteins as possible, Sepp1 fills the role of that marker better than Gpx3.
Supported by NIH grant DK 58763.

S10. SELENOPROTEIN P AND BRAIN FUNCTION. Raymond F Burk.
Vanderbilt University. Nashville, Tennessee 37221, USA.
raymond.burk@vanderbilt.edu

Studies with selenoprotein P (Sepp1) null mice have indicated that Sepp1 is involved in maintaining selenium in brain and testis. Our group examined the testis using immunocytochemistry (ICC) and noted vesicles containing Sepp1 at the periphery of the seminiferous tubules. We postulated that Sepp1 was being taken up via a receptor. We solubilized testis and passed it over a column that had Sepp1 bound to it. Eluate was analyzed by mass spectrometry and the lipoprotein receptor ApoER2 was detected. ApoER2-/- mice could not take up Sepp1 into the testis and had abnormal spermatozoa that had the appearance of selenium-deficient ones. ApoER2 is also present in the brain and ApoER2-/- mice had low brain selenium. However, we did not detect Sepp1-containing vesicles within the brain of wild type mice. Thus, ApoER2 is responsible for uptake of Sepp1 into testis and maintenance of selenium in brain. An experiment was performed to assess the effect of apoER2 on the function of selenium in the brain. Feeding selenium-deficient diet to apoER2-/- mice led to neurological dysfunction and death with characteristics exhibited by Sepp1-/- mice fed the same diet. Thus, apoER2 is necessary for maintenance of brain selenium and for prevention of neurological dysfunction and death under conditions of selenium deficiency. This implies an interaction of apoER2 with Sepp1 in the brain.
Supported by NIH grant ES 02497.

S11. MISDISTRIBUTION OF IRON: CAUSES, PATHOLOGICAL IMPLICATIONS AND ATTEMPTS OF CORRECTION. Z. Ioav Cabantchik, Yang-Sung Sohn, William Breuer and Or Kakhlon.
Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Safra Campus at Givat Ram, Jerusalem 91904, Israel
ioav@cc.huji.ac.il

Iron accumulation has been identified in brain damaged areas of patients with neurodegenerative disorders. Hitherto, the causes of iron accumulation in specific brain regions, the chemical nature of the accumulated metal and, most importantly, the causal relationship of neuronal damage to iron, have remained elusive. Although damage caused by iron as been viewed as the sine qua non of iron accumulation, it is not the bulk, but rather a relatively small fraction of iron appearing in redox active (labile) forms that in aeration catalyzes the formation of noxious-damaging radicals. Therapeutically targeting a minor fraction of body iron that accumulated in a restricted brain area depends on the ability to selectively act on that chemical species while sparing the bulk of complexed bioiron or other biometals (Cu or Zn) that are essential for the organism and especially for brain functions. We deal here with Friedreich's ataxia (FRDA) as a paradigm of an inherited disorder that is etiologically associated with regional cell damage evoked by mis-distribution of iron in a distinct area of the brain. We will describe our recent attempts: a. to define biochemically the nature of iron misdistribution and its functional consequences in cell models of FRDA; b. to explore the feasibility of overcoming frataxin-deficiency by functionally correcting the cellular properties affected by iron misdistribution using cell models of FRDA and siderophores as cell iron relocators or translocators and c. to translate to the clinical setting the basic findings obtained in model cells with an iron chelator in clinical use for systemic iron overload, first on patients with FRDA and subsequently on patients with neurodegeneration with brain iron accumulation (NBIA). Finally we will discuss the prospects of drug-induced brain iron chelation for treating the more widespread neurodegenerative disorders Parkinson's disease (PD), Alzheimer’s disease (AD) and Huntington's disease (HD), in which accumulated (toxic) iron has been implicated in regional oxidative damage. This work was supported by the Association Francaise contre les Myopathies (AFM), AFIRN (French-Israeli Organization for Research in Neuroscience), the Israel Science Foundation (ISF) and by the EEC Framework 6 (LSHM-CT-2006-037296 Euroiron1).

S12. THE PIVOTAL ROLE OF THE MENKES COPPER ATPase IN COPPER TRANSPORT AND REGULATION OF ITS FUNCTION. James Camakaris[1], Nicholas Veldhuis[1], Ann Gaeth[1], Nickless Palstra[1], Valentina Valova[2], Phillip Robinson[2], Adam Southon[1], Richard Burke[3]
[1]University of Melbourne, Parkville, Victoria, Australia
[2]Children's Medical Research Institute, Westmead , NSW, Australia
[3]School of Biological Sciences, Monash University , Clayton ,Victoria, Australia
j.camakaris@unimelb.edu.au

Copper is an essential trace element, required by several critical cuproenzymes, but whose concentrations must be carefully regulated in cells as it is potentially highly toxic.
The Menkes copper-transporting P-type ATPase (MNK,ATP7A) functions as a transmembrane copper pump and plays a pivotal role in copper homeostasis. It is involved in copper transport across epithelial and endothelial cell barriers, copper detoxification via copper efflux, and supply of copper to cuproenzymes in the secretory pathway. The function of MNK is controlled via its sub-cellular localisation. It normally resides in the trans-Golgi network but at elevated copper it traffics via vesicles to the plasma membrane. In polarised gut and kidney epithelial cells it is targeted to the basolateral membrane where it functions in copper absorption and reabsorption. Our studies on copper-responsive MNK trafficking are directed towards understanding the molecular mechanisms involved. We have identified motifs in cytosolic domains of MNK which are important in these processes and also sites which are phosphorylated in response to copper which suggests that the function of MNK is regulated by signal transduction pathways which respond to copper and some hormones. We are also defining the tethering and cytoskeletal elements involved in post-Golgi trafficking of MNK and are investigating interactions at particular membrane domains using "fluorescence recovery after photobleaching"(FRAP).
We have developed a Drosophila model to further investigate copper homeostasis. We have identified DmATP7 as the sole Drosophila copper ATPase orthologue. Investigation of DmATP7 expression has revealed strong expression in the gut and neuronal tissues and, importantly, expression in particular regions of the gut was induced by dietary copper. This induction was mediated by the metal transcription factor, MTF1. We have found that DmATP7 can correct the copper accumulation phenotype of cultured fibroblasts from patients with Menkes disease, can donate copper to the cuproenzyme, tyrosinase, in the human cells, and exhibit copper-responsive vesicular trafficking. These exciting findings suggest that copper-dependent trafficking of copper ATPases evolved prior to the duplication event that resulted in the Menkes (ATP7A) and Wilson (ATP7B) copper ATPases in vertebrates. We are using genetic approaches in the Drosophila system to identify genes and proteins that interact with copper ATPases.

S13. THE MENKES COPPER ATPase (ATP7A) IS NOVEL IRON RESPONSIVE GENE IN THE MAMMALIAN INTESTINE. James F. Collins, Ping Hua, Yan Lu, P. N. Ranganathan
Food Science & Human Nutrition department, University of Florida
jfcollins@ufl.edu

The Menkes Copper ATPase (Atp7a) gene is strongly induced during iron-deficiency in the rat intestine, an observation that was initially made by Gene Chip analysis of the intestinal mucosa in iron-deficient rats across several stages of postnatal development. Interestingly, the Atp7a gene is induced throughout the length of the rat small and large intestines during iron-deficiency, in a similar fashion to Divalent Metal Transporter 1 (Dmt1). Significantly more protein was also detected in the proximal small bowel during iron-deficiency, utilizing Atp7a-specific antibodies raised against 2 peptides of the rat Atp7a protein. We thus hypothesize that Atp7a may be involved in copper loading during iron-deficiency, which may be part of a compensatory mechanism to increase iron and nutrient absorption during iron-deficiency. The Atp7a gene is also induced and the protein more strongly expressed in iron-deprived IEC-6 cells; actinomycin D treatment abolished this induction indicating transcriptional regulation. We thus sought to characterize the rat Atp7a gene promoter to determine the precise mechanism of induction during iron-deficiency. As a first step, we next mapped the 5' end of the Atp7a transcript by RNA ligase-mediated 5' RACE. Results revealed 3 splice variants present at the 5' end of the gene. All clones began with a sequence that mapped ~60 kb upstream of the rat Atp7a gene in the genomic databases, which is homologous to exon 1 in mouse and human. We found that exon 1 is spliced to exon 1A (a novel exon), to exon 2 or to exon 3. The first 2 splice variants would encode an Atp7a protein containing the full length N-terminus, but the ex1/ex3 splice variant would encode a protein that was truncated by 69 amino acids (missing copper binding domain 1). Preliminary studies also suggest that these splice variants exist in mouse and human, and that the gene structure is conserved. A qRT-PCR strategy was devised that allowed quantification of all 3 variants; results revealed ~7-fold induction of all splice variants in the intestine of iron-deficient rats. In order to determine if the splice variants resulted in the production of different versions of Atp7a protein, we developed polyclonal antibodies against the extreme N-terminus of the rat Atp7a protein and showed strong induction of protein expression in iron-deficient rats and unique immunolocalization in IEC-6 and Caco-2 cells, and in rat intestine. Surprisingly, we detected 2 specific proteins of 97 kDa and 64 kDa in the nucleus; these versions of Atp7a protein bound to a copper affinity resin and to DNA. The 97 kDa protein was found in the cytosol and nucleus, while the 64 kDa protein was only detected in the nucleus. Another version of the protein, which was approx. 190 kDa was found only in the cytosol and co-localized with a golgi specific marker; this version of the protein was not detected by the N-terminal antiserum, suggesting that it does not contain copper binding domain 1. Our studies have thus revealed novel variants of Atp7a protein that have different intracellular locations and that could be functionally important during iron-deficiency.

S14. ZINC TRANSPORT AND SIGNALING. Robert J. Cousins.
Center for Nutritional Sciences and Food Science and Human Nutrition Department, University of Florida, PO Box 110370, Gainesville FL 32611-0370 USA.
cousins@ufl.edu

There is increasing evidence that many of the twenty four zinc transporters contribute to signaling pathways and therefore specific cell functions. Some transporters are regulated by the dietary zinc supply while others respond to immune stimuli such as LPS, IL1, IL6, and nitric oxide, or hormones such as glucocorticoids and prolactin. Cell type-specificity also characterizes zinc transporter expression/function. Three separate examples of transporter-mediated cell signaling are presented here. In the mouse, control of ZIP4 which is necessary for enteric zinc absorption by enterocytes is regulated by the transcription factor KLF4. Decreased dietary zinc intake increases KLF4 expression which leads to increased KLF4 binding to the ZIP4 promoter and increased ZIP4 synthesis, translocation to the plasma membrane of enterocytes, and increased zinc uptake to maintain zinc homeostasis. In mouse RBC's, ZnT1, ZIP8, and ZIP10 are highly expressed. Erythroid development produces increased ZIP8 and ZIP10 and subsequent increased ZnT1. These regulatory events may limit availability of zinc ions to prevent binding to protoporphyrin instead of iron. Dietary zinc depletion results in increased ZIP10 and decreased ZnT1 in mature RBC membranes and appear to influence intracellular Zn content through altered transport kinetics. In human T-cells, ZIP8 expression is involved in cell activation leading to IFNY expression via differential cell signaling. ZIP8 overexpression decreases labile zinc in secretory lysosomes and increases IFNY secretion, while ZIP8 knockdown with siRNA has the reverse effect. ZIP8 via control of the IFNY promoter through regulation of calcineurin phosphatase activity and phosphorylation of the CREB transcription factor.
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S15. IRON ABSORPTION IN CHILDREN; IMPLICATIONS FOR GLOBAL CHALLENGES.
Lena Davidsson
Nutritional and Health Related Environmental Studies Section, Division of Human Health, IAEA, Vienna, Austria.
davidsson@iaea.org

The prevalence of iron deficiency is unacceptably high globally, in particular among infants, children and women of child-bearing age in developing countries. Effective, food based strategies to combat iron deficiency are therefore urgently needed. As only a fraction of dietary iron is absorbed and utilized, access to data on iron bioavailability from foods, diets and iron fortificants is crucial in the development of food fortification strategies and interventions based on dietary diversification. From a methodological point of view, the rapid incorporation of newly absorbed iron into erythrocytes is a great advantage. Stable isotope technique to evaluate iron bioavailability has been developed based on the incorporation of stable iron isotopes into erythrocytes 14 days after administration of labelled test meals. In most studies, the incorporation rate is assumed to be constant, 80-90 % in adults and infants respectively. However, when the incorporation rate cannot be assumed to remain stable, for example during pregnancy, incorporation of a stable isotope administered intravenously can be used to correct for changes in incorporation rate. Large interindividual variation in iron bioavailability has been demonstrated, primarily due to differences in iron status between subjects, and paired comparisons are therefore essential when evaluating iron bioavailability from different foods or food fortificants. By using a double isotope technique, i.e., administration of two stable isotopes of iron (57Fe and 58Fe) - on consecutive days - information about iron bioavailability from two different test meals can be obtained simultaneously. Over the last few years, this technique has been used to generate new data on, in particular, iron bioavailability from iron compounds used in food fortification programs and information about dietary enhancers and inhibitors of iron absorption in infants and children.

S16. ZINC UTILIZATION IN WOMEN DURING REPRODUCTION: EVIDENCE OF PHYSIOLOGIC ADAPTATION. Carmen Marino Donangelo
Laboratório de Bioquímica Nutricional e de Alimentos, Instituto de Química, CT Bloco A Lab 528 A, Universidade Federal do Rio de Janeiro, 21949-900 Rio de Janeiro.
donangel@iq.ufrj.br

Zinc plays critical roles in cell proliferation and function thus being essential for embryogenesis, fetal growth and milk secretion. It is estimated that the total zinc demand in a full-term human pregnancy is about 100 mg, and that the total breast milk zinc output during 6 months of lactation is about 160 mg. These increased zinc demands during pregnancy and lactation, equivalent to ≈ 6% and 9.6 % respectively, of the whole-body zinc of a non-pregnant woman, may be met by increased zinc intake or by adjustments in zinc homeostasis. Because women do not typically increase their zinc intake during pregnancy and lactation, homeostatic adjustments in zinc utilization appear to be the primary mechanisms to meet the increased zinc demands of women during reproduction, particularly in those with low zinc intakes. Homeostatic adjustments may increase zinc absorption, reduce endogenous fecal zinc excretion, and modify the distribution of tissue zinc and the kinetics of exchangeable zinc pools. In women with adequate dietary zinc intakes, a longitudinal study from preconception to lactation, and a cross-sectional study, found that fractional zinc absorption increased up to ~80% during lactation. In women consuming lower zinc diets, cross-sectional studies, and a longitudinal study from early pregnancy to 8 wks post-partum, indicated that fractional zinc absorption increased up to ~70% both during pregnancy and lactation. A study measuring endogenous fecal zinc losses during lactation found that intestinal conservation of endogenous zinc contributed ~50% to the adaptation of zinc homeostasis in lactating women on low dietary zinc. Studies of zinc kinetics during pregnancy and lactation are scarce and results still incipient. In general, comparisons between zinc physiological studies during pregnancy and lactation are not possible due to differences in study design, composition of habitual diet, and methodological approach. Although several randomized, well-controlled zinc supplementation trials have been done during pregnancy in different populations, the potential benefit of supplemental zinc on homeostatic adjustments during pregnancy and lactation is still unclear. In one study, zinc supplementation of pregnant women with low zinc diets had no effect on gestational age and infant birth weights but improved maternal and neonatal zinc status, and also improved fetal bone growth and neurobehavioral development. Because about half of the world population is at risk of inadequate zinc intakes, longitudinal comprehensive studies of zinc homeostatic adjustments during pregnancy and lactation in these populations are needed in order to provide the physiological basis for adequate zinc intervention studies.

S17. WHAT IS THE TRUE POTENTIAL OF TRANSCRIPTOMIC METHODS FOR BIOMARKER DISCOVERY IN TRACE ELEMENT RESEARCH?

Ruan Elliott
Institute of Food Research, Colney, Norwich, NR4 7UA, UK.
ruan.elliott@bbsrc.ac.uk

Functional genomic methods have been advocated for some considerable time as presenting enormous potential for advancement of nutritional research by providing new avenues for defining diet-gene interactions and for biomarker development. But to what extent have “omic” technologies so far delivered on this promise? Transcriptomic technology will be used as an example here to evaluate this question.

Many early attempts to exploit transcriptomics produced equivocal results. This was due to compromised data quality resulting from limitations of the platform and protocols and to a lack of appropriate statistical and analytical tools. However, transcriptomic technology platforms have now achieved a level of technical maturity that means the production of high quality genome-wide expression profiling data is achievable by all. Universal standards for transcriptomic data handling have been adopted and infrastructures developed that ensure secure long-term data storage and facilitate data exchange. Innovative statistical approaches and data analysis tools have been developed specifically for transcriptomic data, enabling much more detailed, accurate and informative conclusions to be drawn from well designed studies. On top of this, the costs of transcriptome analysis have reduced substantially and continue to do so, driven by a competitive commercial market. This makes it increasingly feasible to undertake larger studies and incorporate more appropriate numbers of biological replicates than have often been used in the past.

In the context of trace element research, transcriptomic methods have already been widely applied. Studies performed to date have identified a host of genes regulated at the mRNA levels by varying exposures to trace elements and, albeit to a more limited extent, have helped provide new insights into biological processes affected. However, most of the work published to date has been performed in cell lines and animal models with a notable paucity of human nutritional intervention studies. It is also notable that the wealth of new information gained through transcriptomic studies has, to date, not been successfully translated into the development of new biomarkers of mineral status. These observations reflect (1) an apparent reticence to test transcriptomic methods in nutritional intervention studies where the magnitude of the effect on gene transcription is likely to be low and may be masked by normal biological variation in volunteers and (2) a fundamental problem of specificity in using single genes as biomarkers since expression of most, perhaps all, genes can be regulated by more than one environmental factor.

Nevertheless, evidence obtained from the limited number of human studies performed suggests that with appropriate study design it is possible to apply transcriptomic methods successfully in human dietary intervention studies. Indeed, analytical methods now available to investigators enhance the sensitivity of transcriptomic methods to such an extent that they are arguably capable of detecting more subtle changes in gene expression patterns than techniques such as real-time RT-PCR. The development of gene expression-based biomarkers still poses a major challenge for which the exploitation of advanced mathematical modelling approaches to identify diagnostic gene expression profile signatures, rather than single genes, may provide a solution.

S18. COPPER HOMEOSTASIS: HOW STABLE ISOTOPE APPLICATIONS INFORM UNDERSTANDING. Susan J Fairweather-Tait, Linda J Harvey, Jack R Dainty.
University of East Anglia, Norwich, Institute of Food Research, Norwich UK.
s.fairweather-tait@uea.ac.uk

Currently, there are no established sensitive and specific biomarkers for measuring copper status, and therefore dietary requirements have to be estimated using data from studies on copper metabolism. Copper homeostasis is maintained through changes in both absorption and excretion. In order to measure true copper absorption it is essential to differentiate between unabsorbed dietary copper (apparent absorption) and endogenous copper secreted into the GI tract. This can be achieved by employing an enriched source of the stable isotope 65Cu (30.8% natural abundance) to label dietary copper. Using the stable isotope technique, with holmium as a fecal marker, female vegetarians were shown to have a significantly lower true copper absorption than women consuming diets containing red meat ($p=0.0013$) or poultry/fish ($p=0.0008$). In a small pilot study 65Cu was used to test the hypothesis that iron supplements (100mg/d) would have an adverse effect on copper and zinc [1] status in pregnant women. There was no effect on copper absorption, although all women had lower true absorption ($p=0.0054$) and endogenous losses ($p=0.014$) of copper at 24 weeks compared with 16 weeks gestation. The iron supplemented women also had lower serum copper ($p<0.0001$) and ceruloplasmin ($p=0.0005$) concentrations. Copper bioavailability was shown to differ between foods (29% in soya beans, 35% in mushrooms, 49% in red wine, 52% in sunflower seeds) and, like non-heme iron, the native copper in some foods (e.g. soya beans) can be labelled extrinsically, but in other foods (e.g. sunflower seeds) it is necessary to label the copper intrinsically whilst the plant is growing [2]. Compartmental modelling was utilised to study copper metabolism in healthy men given an oral dose of 65Cu. The appearance in plasma and feces was monitored and the exchangeable body pool was estimated to be 43 mg, which is lower than published autopsy data (110 mg) but consistent with the fact that most copper is contained in a very slowly exchanging bone pool. In the exchangeable tissues, 84% was located outside the plasma, liver and gut compartments, most likely in muscle and other soft tissues. It was estimated that 74% of newly absorbed copper was removed by the liver on first pass before undergoing a delayed entry into the plasma bound to ceruloplasmin (80%) or excreted back into the GI tract (20%). The estimated copper flux from salivary, gastric, duodenal and pancreatic routes was 1.4 mg/d and from biliary excretion 1.0 mg/d. The model predicted that that 99% of plasma copper was bound to ceruloplasmin, with a half-life of 27d [3].
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S19. ACCUMULATION OF TRACE METALS AS AN ASSAY FOR TRANSPORT (BY DMT1). Laura Garrick1, Lin Zhao1, Saied Ghadersohil1, Jacqueline Stonehuerner2, Andrew Ghio2, Michael Garrick1
1Department of Biochemistry, SUNY at Buffalo, Buffalo, NY, USA/
2Environmental Protection Agency, Chapel Hill, NC, USA
lgarrick@buffalo.edu

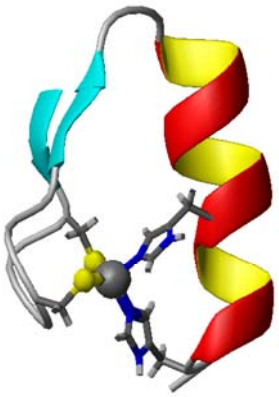
We tested the hypothesis that over expression of Divalent Metal Transporter 1 (DMT1) in HEK293 cells would lead to a net accumulation of its metal ion substrates. DMT1 has four isoforms distinguished at the N- and C-termini: 1A/+IRE; 1A/-IRE; 2/+IRE; and 2/-IRE. Rat 1A/+IRE and mouse 2/-IRE DMT1 were cloned into HEK293 cells such that their expression was regulated by doxycycline. The two cell lines successfully took up 59Fe or 54Mn in a DMT1-dependent fashion (Garrick et al 2006 Biochem. J. 398:539). To test for metal accumulation, it was necessary for survival of the cells during incubation periods longer than one hour to incubate the cells at neutral pH rather than at pH 5.5-6.0 (optimal for DMT1). After incubation (typically 4 hr in Hanks Buffered Salt Solution), cells were separated from the medium and washed then metals were assayed in the supernatant and cell digest by inductively coupled plasma - atomic emission spectroscopy (ICP-AES). Mn accumulated in a time and DMT1 dependent fashion. Fe accumulation was also DMT1 dependent, but uptake from media during doxycycline induction interfered with following the time course. Other metals that accumulated in a DMT1 dependent fashion included Cd, Ni, Co and Cu1+. Vanadyl, Tl, Ga, Cu2+, Pt (as chloroplatinate) and Ca did not accumulate; while Pb, Zn, Mg and Ti yielded ambivalent data. This assay clearly allows one to assess which metals DMT1 transports. Constraints imposed by the conditions of pre-incubation and incubation limit interpretation of data for some metals.

S20. INCREASING AWARENESS OF SELECTED TRACE ELEMENTS. Michael Garrick
Department of Biochemistry, SUNY at Buffalo, Buffalo, NY, USA
mgarrick@buffalo.edu

This abstract introduces the symposium on increasing awareness of selected trace elements, a title for which an initial draft was Mn and other metals. As themes, we considered Mn and manganism, how mammals and pathogens compete for trace elements and promiscuity of nominally specific transporters. Because coverage is otherwise missing for one topic, the introduction will mention how it is well established that the innate immune response to pathogens includes iron withholding and some pathogens in turn have learned to take advantage of this response. Recently a report (Corbin et al 2008 Science 319:962) shows that a protein named Calprotectin binds Mn to inhibit the growth of S. aureus, showing that “we” combat “them” by withholding other trace elements than Fe. Dr. Jerome Roth will discuss research on manganism and Parkinsonism where a probable common factor is the metal iron transport function of Divalent Metal Transporter 1 (DMT1). The regulation of DMT1 expression at multiple levels is proving relevant to the two disorders. Dr. Laura Garrick will present a new assay for DMT1 that depends on net accumulation of its metal ion substrates. The assay shows that Mn^{2+}, Fe^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+} and Cu^{1+} accumulate. Pb, Zn, Mg and Ti may also do so, but there are interesting challenges in applying the assay. Dr. Bryan Mackenzie also enumerates potential substrates for DMT1, primarily by using a fluorescent assay. While his results frequently agree with hers, it will be of particular interest to consider discrepancies for trace elements like V and Cu^{1+}. We expect to stimulate a vigorous discussion on the contrasts and invite mention for apparent promiscuity of other transporters and proteins that function in metal ion homeostasis like Zip14 or Zip8 (Zrt- and Irt-like Proteins, initially identified as a Zn transporter) and Steap 2,3,4 (Six-Transmembrane Epithelial Antigen of Prostate, now identified as ferri- and cupric-reductase).

S21. MODERN METHODS OF ANALYSIS OF ZN PROTEINS. Brian R. Gibney
Brooklyn College – The City University of New York, Department of Chemistry, Brooklyn, NY 11210
bgibney@brooklyn.cuny.edu

Nature utilizes a variety of cofactors and prosthetic groups to augment protein structure and function. Zn(II) is one of the most pervasive metal cofactors in biology, serving fully 10% of the human proteome in catalytic and structural capacities. The zinc finger transcription factors, representative structure shown at right, are unstructured without Zn(II) and fold into their biologically active structure upon addition of Zn(II). Thus, they represent the prototypical example of a metal-induced protein folding event.



The $\beta\beta\alpha$ fold of a Cys_2His_2 zinc finger transcription factor

Our approach to the study of metalloproteins is to engineer and fabricate peptide structures that incorporate metal cofactors toward the goal of generating molecular *maquettes*, protein-based synthetic analogues. We have developed a 16 amino acid peptide containing the canonical ligand sets found in zinc finger transcription factors, Cys_4, Cys_3His_1 and Cys_2His_2, to determine the thermodynamic contribution of Zn(II) to metal induced protein folding reactions (1-3). These model peptide studies provide the basis for this tutorial lecture which will examine modern chemical, biophysical and spectroscopic methods of analysis for Zn(II) proteins. The chemical methods focus on the central issue of determining the affinity of the protein for Zn(II) and include potentiometry and calorimetry. Biophysical methods will include methods to determine the stability of the Zn(II) protein via chemical and thermal denaturation. The spectroscopic methods surveyed will include UV-visible, fluorescence, X-ray absorption and NMR spectroscopies. The data presented will show that our designed proteins possess the tightest Zn(II) affinities observed in any protein and the data show that the thermodynamic contribution of Zn(II) binding to Zn(II) transcription factor protein folding free energy is less than 4 kcal/mol.

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S22. INTERVENTIONS FOR COMBATING MICRONUTRIENT DEFICIENCIES IN AFRICA: PROBLEMS, PROGRESS, AND FUTURE SOLUTIONS. Rosalind S Gibson[1], Sonja Y Hess[2], Christine Hotz[3], Kenneth H Brown [2,4]
[1]Department of Human Nutrition, University of Otago, New Zealand,
[2]Department of Nutrition, University of California, Davis, USA,
[3]Harvest Plus, Washington DC, USA
[4]Helen Keller International, Senegal.
Rosalind.Gibson@Stonebow.otago.AC.NZ

Concurrent trace element deficiencies exist in many African countries with far reaching consequences on maternal, infant, and child health. Major health benefits could be achieved with cost-effective and sustainable programmes to alleviate these deficiencies. On-going programmes in Africa include therapeutic and/or preventive supplementation and fortification, both focusing on iron, zinc, iodine, vitamin A, and folate. Supplementation is targeted at vulnerable groups. Mass and targeted fortification in Africa uses condiments, cereal flours, beverages, snacks, or vegetable oils as food vehicles. Unfortunately, few intervention programmes have been formally evaluated in Africa. Results of efficacy studies have often been mixed, possibly attributed to differences in age, nutritional and health status of the target population, presence or absence of malaria, study duration, design, and methodological limitations, outcomes measured, dose, form, and combinations of micronutrients and interactions. More research is needed on the optimal safe and effective doses for supplementation regimens especially iron in malarial areas. Information on the best way to integrate preventive and therapeutic supplementation, improve their operational effectiveness and efficiency, and enhance their sustainability by linking with ongoing health and nutrition programmes, is urgently required. For fortification, issues include the form and optimal fortificant levels, especially for young children and when fortified products are high in phytate, as well as on-going monitoring and quality assurance to avoid excessive intakes. Some innovative approaches to minimize the risk of antagonistic interactions in intervention programmes have been developed using combinations of weekly and daily supplements, microencapsulated fortificants, and home-based fortification using complementary food supplements (CFS) such crushable tablets (foodlets), powders (sprinkles), or lipid-based supplements to mix with complementary foods (CFs) (especially in malarial areas). Ready-to-use therapeutic foods for treating severely malnourished children in home-based care are also being used. Efficacy studies of these products in Africa have focused mainly on the prevention of anemia and iron deficiency. Future studies should examine their impact on other micronutrients as well as functional health outcomes, and consider adding phytase to hydrolyze phytate in the CFs. Delivery of supplementation and fortification programs in resouce poor settings in Africa is challenging. Strategies such as fortification at local village mills, dietary diversification and modification are more appropriate and sustainable in these settings. However, despite some positive effects on behaviour change and nutritional status over the short-tem, there is no information on their long-term impact on functional health outcomes or on safety issues. Before introducing dietary diversification and modification, the strategies should be designed, implemented, and evaluated using formative research, and include appropriate information, education, and communication strategies to ensure their adoption, sustainability, and effectiveness. In the future, biofortification holds promise as a

sustainable approach to improve the trace element content and/or bioavailability of staple food crops of subsistence farming households. Biofortification includes plant breeding, use of fertilizers, and genetic manipulations. Efficacy studies are planned in Africa to investigate whether biofortified staple foods have a positive effect on micronutrient status and health. Data on their economic and environmental impact are also needed before biofortification can be recommended for implementation.

S23. SELENOPROTEINS AND THEIR ROLES IN REDOX BIOLOGY. Vadim N. Gladyshev
Redox Biology Center and Department of Biochemistry, University of Nebraska, Lincoln, NE, USA
vgladyshev1@unl.edu

Selenoproteins contain a rare amino acid, selenocysteine, which occurs in all three domains of life and functions as the catalytic redox group is several classes of oxidoreductases. Full sets of selenoproteins have recently been identified in a variety of organisms, including humans, which have 25 known selenoproteins. Aquatic environments are associated with increased of use Sec, whereas many terrestrial organisms lost selenoproteins. Thioredoxin-like proteins are particularly prone to conversion into selenoprotein forms. Because dietary selenium is required for selenoprotein expression, diets differing in selenium levels provide means of regulating selenoprotein function and redox homeostasis in mammals. Selenoproteins may also be used as tools to identify proteins that contain catalytic redox-active cysteine residues and determine location of these residues in protein sequences. In addition, studies on selenoproteins provide new information about the genetic code. Being redox catalysts, selenoproteins are involved in the repair of oxidatively damaged proteins, activation and inactivation of thyroid hormone, regulation of the redox state of thioredoxin, removal of hydrogen peroxide, and other functions. In turn, these functions implicate selenoproteins in cancer prevention, regulation of the aging process, and male reproduction.

S24. HUMAN ZINC DEFICIENCY: NEW INSIGHTS INTO ZINC HOMEOSTASIS PROVIDE A QUANTITATIVE BASIS FOR PREVENTION AND TREATMENT OF A NEW PUBLIC HEALTH CHALLENGE. Michael Hambidge
Michael.Hambidge@UCHSC.edu

Individual efforts / programs rather than co-ordinated local national and international strategies for the prevention of zinc deficiency include: improved local affordable food supply with emphasis on diversity; Zn fortification of one or more food staples; Zn-containing sprinkles or Zn supplements. With the exception of the latter, for which simultaneous administration of iron supplements demands attention, there are just 2 factors that account for 86% of the quantity of Zn absorbed. These are the quantity of Zn [TDZ] and phytate [TDP] ingested daily. Mammalian absorption of Zn is a saturable process and the relationship between the quantity of Zn absorbed [TAZ] and TDZ is best and most appropriately described by saturation response modeling. This is also true for phytate-containing meals which are best fit with a trivariate model of TAZ as a function of both TDZ and TDP. Application of this model to both single meals, total diets or, with appropriate attention to regulatory changes, zinc supplements / sprinkles provides a logical basis for determining quantitatively TAZ from supplements / sprinkles, and fortified products and for better understanding of improvements needed and achieved in the local food supply. For completion, improved estimates of physiologic requirements, especially for women, are needed as are the quantitative effects of phytate in young children. To be of practical benefit, optimal application of these data will be facilitated by acquisition of reliable national and local data on dietary intakes.

S25. IRON IN PARKINSON'S DISEASE. Etienne C. Hirsch, Julio Salazar, Natalia Mena, Stephane Hunot, Annick Prigent, Daniel Alvarez-Fischer, Miguel Arredondo, Charles Duyckaerts, Veronique Sazdovitch, Lin Zhao, Laura M. Garrick, Marco T. Nuñez, Michael D. Garrick, Rita Raisman-Vozari.
INSERM, UMR_S679, Neurologie et Thérapeutique Expérimentale, Hôpital de la Salpêtrière, Paris, France;
Millennium Institute for Cell Dynamics and Biotechnology and Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, Chile;
Department of Biochemistry, SUNY at Buffalo, Buffalo, NY, USA.
etienne.hirsch@upmc.fr

Dopaminergic cell death in the substantia nigra (SN) is central to Parkinson's disease (PD) but the neurodegenerative mechanisms have not been completely elucidated. Iron accumulation in dopaminergic neurons and glial cells in the SN of PD patients may contribute to the generation of oxidative stress, protein aggregation and neuronal death. However, the mechanisms involved in iron accumulation remain unclear. Recently, we have described an increase in the expression of the divalent metal transporter 1 (DMT1/Nramp2/Slc11a2) in the SN of PD patients. Using the PD animal model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication in mice, we showed that DMT1 expression increases in the ventral mesencephalon of intoxicated animals, concomitant to iron accumulation, oxidative stress and dopaminergic cell loss. A mutation in DMT1 that impairs iron transport protected rodents against parkinsonism-inducing neurotoxins MPTP and 6-hydroxydopamine (6-OHDA). This study supports a critical role for DMT1 in iron-mediated neurodegeneration in PD.

S26. CONTRADICTORY FINDINGS ON THE INFLUENCE OF VITAMIN A ON IRON BIOAVAILABILITY IN HUMAN SUBJECTS. Richard Hurrell
Institute of Food Science and Nutrition, Swiss Federal Institute of Technology (ETH), Zurich, CH 8092 Zurich, Switzerlandrichard.
hurrell@ilw.agrl.ethz.ch

It has been known for some time that vitamin A deficiency predisposes to anaemia and that, in some subjects, anaemia will respond to vitamin A supplementation alone. What is less clear however is how vitamin A deficiency impairs iron metabolism, and whether iron deficiency influences vitamin A metabolism. Several possible interactions of vitamin A deficiency on iron metabolism have been suggested in the literature, most notably the need for vitamin A for mobilization of iron from the ferritin stores and the need of vitamin A for effective erythropoeisis. The erythropoietin gene contains a retinoic acid response element.
In recent years, the influence of vitamin A on iron absorption has been investigated however the findings have been contradictory. Several studies from Venezuela measured iron absorption in adults from the lower socioeconomic classes using the extrinsic tag radio iron technique. These studies reported that vitamin A and β-carotene added to maize, wheat and rice meals increased iron absorption 2-4 fold. However, when these studies were repeated with Swiss and Swedish students using both radio and stable isotope techniques, no influence of addition vitamin A on iron absorption from maize bread could be demonstrated. On the assumption that these contradictory findings were due to differences in the vitamin A status of the subjects, erythrocyte incorporation of iron was measured in vitamin A deficient Ivorian children fed a maize gruel with and without vitamin A. The study was repeated 3 weeks after the children had consumed a high dose vitamin A supplement. Unexpectedly, the addition of vitamin A to the maize gruel decreased erythrocyte incorporation of iron in the vitamin A deficient children, but had no influence on erythrocyte incorporation of iron in these children after their vitamin A stores had been repleted. The reasons for these contradictory findings are unknown. Other nutrient deficiencies in the Ivorian children may have impacted on their vitamin A metabolism or iron metabolism. It is clear however that iron bioavailability from foods depends on subject characteristics as well as food composition.

S27. POTENTIAL OF SUPPLEMENTAL SELENIUM IN IMPROVING MEAT QUALITY OF FOOD ANIMALS. Zongyong Jiang
Guangdong Public Laboratory of Animal Breeding and Nutrition, Institute of Animal Science Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
jiangz38@hotmail.com

Although the need of dietary selenium (Se) for animals has been well established, its potential antioxidative function in improving meat quality of food animals is still being debated. The purposes of our studies were to investigate the effects of Se on antioxidative capacity and meat quality in finishing pigs and broilers involved by feeding trial and slaughter trial. The results indicated that Se supplementation can improve meat quality by increasing water holding capacity, meat colour scores and pH values, and its action mechanism could be related to the antioxidative property, because Se addition in the diet can inhibit lipid, myoglobin peroxidation and increase antioxidative enzymes activities such as GSH-Px in pigs and broilers. Recently, we also found that the contents of metallothionein and glutathione in breast muscle were increased, the malondialdehyde and carbonyl compouds productions were decreased, the decline of superoxide dismutase and catalase activities in meat during storage were retarded, and total antioxidative capacity of meat post-slaughter was elevated by organic Se supplementation in broilers. Therefore, Se acting as a dietary antioxidant may have great benefit for prevention of meat oxidation. Despite the present data strongly indicated that Se can regulate antioxidative function in animals, further works is required to definitively establish overall antioxidative effects of Se, which may vary with age and species. Such studies will further justify the use of Se in animal nutrition and its applications in the prevention of meat food peroxidation. Key words: selenium, antioxidative property, meat quality, food animals *Corresponding Author: Zongyong Jiang. Tel: +86-20-87596262, Fax: +86-20-87503358, E-mail: jiangz38@hotmail.com.

S28. COPPER METABOLIC DISORDER IN HEART FAILURE. Y. James Kang
Department of Medicine and Department of Pharmacology and Toxicology, University of Louisville School of Medicine
yjkang01@louisville.edu

Progression of heart hypertrophy leads to heart failure and suppression of myocardial angiogenesis is involved in this transition. In recent studies, we have found that pressure overload-induced heart hypertrophy is associated with a decrease in copper (Cu) concentrations in the heart. Dietary supplementation with physiologically relevant levels of Cu prevents the loss of Cu from the heart and suppresses the progression of heart hypertrophy in response to pressure overload. Further studies have demonstrated that Cu stimulates vascular endothelial growth factor (VEGF) expression in the heart, which is responsible for the promotion of myocardial angiogenesis. Conversely, anti-VEGF antibody blocks Cu prevention of heart failure developed from heart hypertrophy. Cu is required for activation of hypoxia-inducible factor-1 (HIF-1), a transcription factor involved in the regulation of VEGF expression. Specifically, Cu interacts with HIF-1a, the activity determinant component of HIF-1. Although excess Cu causes cellular accumulation of HIF-1a leading to enhanced activity of this transcription factor, the requirement of Cu in the physiological function of HIF-1a is related to Cu regulation of HIF-1a interaction with its target gene sequences and the formation of HIF-1 transcriptional complex. The effect of Cu on HIF-1a transcriptional activity in the nucleus appears to be mediated by a Cu chaperon for superoxide dismutase (CCS), as evidenced by the fact that deletion of CCS using siRNA targeting CCS blocks Cu promotion of HIF-1a activation. Therefore, Cu supplementation prevents heart failure developed from heart hypertrophy through at least in part activation of molecular pathways leading to enhanced myocardial angiogenesis. These studies were supported in part by US-NIH grants HL59225 and HL63760.

S29. WHY ARE INDICATORS OF ZINC STATUS SO ELUSIVE? Janet C King, Emily Ho, David W Killilea, Daren Knoell, Jung Suh.
Children's Hospital Oakland Research Institute, Oakland, CA, USA
Oregon State University, Corvallis, OR, USA
Ohio State University, Columbus, OH, USA.
jking@chori.org

Although human zinc deficiency was identified nearly 50 years ago, sensitive indicators of human zinc status, or the ratio of tissue zinc need to supply, remain unknown. The complexity of systems involved in sustaining tissue zinc steady-state levels with changes in intake has made it difficult to determine actual zinc status among individuals, especially during modest deficiency. Changes in gastrointestinal zinc absorption and excretion quickly re-establish whole body zinc balance with moderate decreases or increases in zinc intake. Subtle changes in tissue zinc content may also occur. Nevertheless, plasma zinc concentrations, the usual marker of zinc status, typically do not change, and there is little clinical evidence of any impairment in zinc status. However, severe zinc depletion, with intakes less than 1 mg/d, does cause plasma and extracellular pool zinc levels to decline and, in some cases, the onset of clinical signs of zinc deficiency. In addition to robust systems for maintaining whole body zinc balance with changes in zinc intake, the emerging literature has revealed a second level of regulation at the cellular level. This system is extremely important since 95% of the whole body zinc is intracellular and involved in numerous metabolic functions. The complexity of cellular zinc homeostasis is reflected by the large number of proteins dedicated to zinc transport and buffering, including at least 10 members of the ZnT (SLC30A) family, 14 members of the ZIP (SLC39A) family, and 2 of the 4 isoforms of metallothionein (MT). The zinc transporter proteins modulate cellular zinc influx, efflux, and vesicular sequestration. ZnT transporters reduce cytosolic zinc bioavailability by promoting zinc export, while ZIP transporters import zinc to the cytosol. Studies in humans show that the expression of some of these transporters in specific cell types changes with moderate zinc inadequacy or supplementation. Furthermore, the induction of MT expression rapidly adjusts to cellular zinc imbalances to buffer the amount of cellular labile zinc. Although these 2 separate systems couple zinc homeostasis tightly with changes in dietary zinc supply, preliminary data suggest that zinc-dependent functions at the cellular level may still be altered. For example, the incidence of DNA strand breaks increased significantly in young men fed a diet providing 4.5 mg zinc/d for 6 weeks, while other measures of whole body zinc regulation were not altered. Furthermore, evidence is accumulating that the normal aging process alters the capacity of the zinc homeostasic regulatory systems to adequately respond to changes in zinc intake. The increased demand for zinc during growth may also affect the response of the cellular and whole body systems to inadequate or excessive intakes. Comprehensive studies of zinc homeostasis are needed, therefore, in growing children, the elderly, and in mature adults to identify the biomarkers most sensitive to changes in zinc status throughout the lifecycle. Components of both the cellular and whole body systems will likely need to be assessed to fully evaluate an individual’s zinc nutrition.

S30. COPPER HOMEOSTASIS DISORDERS; A TALE OF DOGS, MICE AND MEN. Leo WJ Klomp
Department of metabolic and endocrine diseases, UMC Utrecht, and Netherlands metabolomicscenter, The Netherlands
L.Klomp@umcutrecht.nl

Menkes disease and Wilson disease provide the human genetic illustration of the copper homeostasis paradox: this transition metal is essential but potentially toxic. Menkes and Wilson disease are characterized by systemic copper deficiency and hepatic copper overload, respectively, and are caused by mutations affecting homologous copper transporting P-type ATPases. The cloning and characterization of the Menkes and Wilson disease genes in 1993 has enabled detailed molecular studies of the cell biology of copper homeostasis. The past decade has significantly increased our knowledge on the mechanisms of copper import, intracellular distribution, utilization, sequestration and excretion, and have unraveled new concepts of metal handling that appeared relevant for the homeostasis of other biomedically relevant metals.
Studies in our own laboratory have focused on the mechanisms of copper homeostasis in the liver. Initially, a genetic approach was undertaken to characterize the gene that is mutated in a genetic copper overload disorder in dogs. These studies identified COMMD1, a protein with unknown function, as a new important player in the regulation of copper homeostasis in the liver. To further characterize the function of COMMD1 in liver copper homeostasis, a conditional Commd1 knock-out mouse that lacks Commd1 expression exclusively in the liver has been generated by using a LoxP-Cre approach in which Cre recombinase expression is regulated by the albumin promoter. Preliminary results displayed a significant twofold increase in liver copper concentrations compared to wild-type mice indicating the importance of proper Commd1 functioning in copper excretion into the bile canaliculus. Additionally, the effects of different patient-derived mutations on the Wilson disease protein were systematically investigated using a combination of biochemical and functional assays. These studies revealed that some of the mutant proteins displayed decreased protein expression due to impaired protein stability. Most recent data revealed that COMMD1 interacts with the Wilson disease protein, and that mutations in the Wilson disease protein resulted in a marked increase of this interaction. COMMD1 was thus found to function in the transition of protein folding and degradation. Taken together, our data provided a possible biochemical explanation for the similarities between copper overload in patients with Wilson disease and dogs with hepatic copper overload.

S31. ZINC HOMEOSTASIS: TAKING CURRENT KNOWLEDGE TO THE FIELD. Nancy F. Krebs, Leland V. Miller, K. Michael Hambidge
Section of Nutrition, Dept. of Pediatrics, University of Colorado School of Medicine, Denver, CO USA
Nancy.Krebs@UCHSC.edu

The last five years have brought a steady expansion of our understanding of human zinc homeostasis. These findings have direct implications for design of intervention studies. The objectives of this session are to review current understanding of human zinc homeostasis and the major effectors of Zn absorption; to discuss implications of the trivariate model on prominent interventions for prevention and treatment of Zn deficiency; and to briefly describe applications of isotope methodology to obtain maximal information with least intensive metabolic collections in populations of interest. The trivariate model (TM), predicts that zinc and phytate intakes account for the major portion of the variability in zinc absorption. This concept differs from previous presumptions about the influence of chronic host zinc status on absorption. What are the implications for the TM of Zn absorption for vulnerable populations? Absorption data for infants and toddlers who have started to consume complementary foods are extremely limited. Typical foods provided for infants are plant based: cereal grains and legumes, fruits and vegetables, most of which are low in zinc, and some of which also contain phytate. Strategies to enhance zinc status in this population must account for the relatively high physiologic requirements and the effects of diet composition on zinc absorption. Target values for biofortification of grains must also account for the interaction of higher zinc content with phytate content. The efficacy of home fortification strategies, e.g. using Sprinkles™, likewise will be impacted by the composition of the foods to which they are added. Obtaining absorption adequate to meet physiological requirements necessitates studies in the population of interest, and ideally measurement of daily absorbed zinc, not only fractional absorption. The effect of high doses of iron in the formulations, e.g. Sprinkles,™ may be another factor affecting zinc absorption. The effect of phytate on endogenous losses remains unclear, with extremely limited available data. Thus, study designs to quantify all variables of zinc homeostasis are desirable. Recent modifications to traditional study design and collections to simplify metabolic collections will be discussed. These greatly facilitate field studies in infants and young children. Until more sensitive, minimally invasive biomarkers of zinc status become available to evaluate efficacy of interventions, stable isotope methodologies, combined with mathematical modeling of critical homeostasis and dietary variables, provide uniquely important information.

S32. HIDDEN ROLES OF SE-GPX1 AND CU ZN-SOD. Xingen G. Lei.
Department of Animal Science, Cornell University, Ithaca, New York, USA
XL20@cornell.edu

Role of NF-κB and Parkin in Regulating DMT1 Expression and Manganese Toxicity
Selenium-dependent glutathione peroxidase-1 (Se-GPX1) and copper,zinc-superoxide dismutase (Cu,Zn-SOD) are considered to be two major intracellular antioxidant enzymes. However, our recent research has illustrated intriguing metabolic roles for these enzymes. First, we have demonstrated a spontaneous development of hyperglycemia, hyperinsulinemia, insulin resistance, and obesity in the Se-GPX1 overexpressing mice. Further characterization of these mice with diet restriction indicates that hyperinsulinemia is a primary effect of the Se-GPX1 transgene. These Se-GPX1 transgenic mice have greater pancreatic beta cell mass (> 2-fold) and pancreatic insulin content (> 40%) than their wild-type, along with an enhanced mitochondrial membrane potential and glucose-stimulated insulin secretion in islets. With diminished reactive oxygen species production, islets from the transgenic mice display hyperacetylation of H3 and H4 histone in the Pdx1 (pancreatic duodenal homeobox 1, a key transcriptional factor for beta cell differentiation and function) promoter, elevated PDX1 and decreased UCP2 (uncoupling protein-2, a key regulator of insulin secretion). Thus, overproduction of Se-GPX1 causes seemingly beneficial changes in pancreatic PDX1 and UCP2, but eventually leads to chronic hyperinsulinemia by dysregulating islet insulin production and secretion. Second, we have shown that knockout of Cu,Zn-SOD alone or with Se-GPX1 diminishes hepatotoxcity of acetaminophen. Block of the acetaminophen-mediated hepatic protein nitration represents one of the major protective mechanisms for the gene knockout. This block of hepatic protein nitration is due to the knockout of Cu,Zn-SOD per se because adding the hole, but not apo-enzyme into the liver homogenates enhances the reaction in an activity-dependent fashion and nearly eliminates the genotype difference at the high doses. Mass Spectrometry shows four more nitrotyrosine residues in bovine serum albumin and ten more nitrated proteins in the liver homogenates of the knockout mice by peroxynitrite in the presence of the added enzyme. In summary, our data unveil a novel pro-oxidant role of Cu,Zn-SOD in catalyzing the peroxynitrite-mediated nitrotyrosine formation in vivo. This feature of Cu,Zn-SOD may potentiate toxicities of peroxynitrite-inducing drugs. (Supported by a NIH grant DK53018)

S33. ZINC RESEARCH: CURRENT STATUS AND FUTURE PERSPECTIVES. Juan P Liuzzi
Department of Dietetics and Nutrition, Florida International University, Miami, Florida, USA
jpliuzzi@ufl.ifas.edu

Zinc biology has a remarkable relevance and diversity. The characterization of the function and regulation of zinc transporter(s) is contributing to a clearer understanding of zinc biological functions and homeostatic control. Findings from loss-of-function, gene knockdown and over-expression studies of zinc transporters suggest that zinc acts as an intracellular signaling molecule, regulating immune response, synapses, development and cell cycle. Additionally, aberrant expression levels and polymorphisms of zinc transporters have been implicated in the pathogenesis and progression of chronic diseases such as cancer, diabetes and cardiovascular disease. New transcription factors regulated by zinc have also been discovered. Of particular interest is the Kruppel-like factor 4 (KLF4), since this factor is involved in inflammatory response, cell differentiation, tumor suppression, and in reprogramming somatic cells into pluripotent stem cells. Zinc supplementation in diseases such as diarrhea, chronic hepatitis, leprosy and tuberculosis seems beneficial. In contrast, the results for AIDS, diabetes, malaria, age related macular degeneration and common cold are inconclusive. There appears to be agreement among researchers that intracellular zinc has a narrow range for its beneficial effects in inflammatory/immune response. Inconsistencies in the outcomes of zinc supplementation studies with the elderly can now be partly explained by the polymorphisms of proinflammatory cytokines and metallothionein. Gestational or perinatal zinc deficiency can result in behavioral, immunological and biochemical abnormalities that persist into adulthood. Future research will address the mechanisms underlying epigenetic modifications caused by inadequate zinc intake during early development. At least one zinc transporter has been found to be involved in iron metabolism. The study of the roles of zinc transporters in iron metabolism and their regulation by iron is a new and exciting challenge.

S34. IRON-ZINC INTERACTIONS: EFFECT OF MATERNAL FE AND ZN SUPPLEMENTATION ON OFFSPRING FE AND ZN HOMEOSTASIS. Bo Lönnerdal
Department of Nutrition, University of California, Davis, USA
bllonnerdal@ucdavis.edu

Deficiencies of Fe and Zn are common during pregnancy, particularly in less developed countries. Supplementation with Fe during pregnancy is therefore often recommended and, more recently, Zn supplementation has become increasingly common. While the effects on the pregnant woman are evaluated, the lasting effects on the offspring are rarely assessed. We have therefore developed a rat model to investigate effects of maternal Fe and/or Zn supplementation during pregnancy on Fe and Zn status and homeostasis of the offspring at 21 days of age. Although positive effects of Fe and Zn supplementation of the pregnant dam on offspring Fe and Zn status were observed, adverse effects of Fe and Zn supplementation of control dams were observed. These adverse effects were manifested in offspring body weight as well as in Fe and Zn status. Effects on Fe and Zn transporters were assessed and it was apparent that Fe and Zn status and supplementation during pregnancy had long lasting effects on offspring ability to regulate Fe and Zn homeostasis. These outcomes as well as possible molecular mechanisms behind these observations will be discussed. In conclusion, Fe and/or Zn supplementation of Fe and Zn adequate dams during pregnancy may adversely affect the ability of the offspring to regulate Fe and Zn metabolism. Therefore, Fe and/or Zn supplementation of pregnant women without considering their Fe and Zn status may warrant some caution.

S35. RECENT TRACE ELEMENTS INTERVENTIONS IN LATIN AMERICA. Daniel López de Romaña, Ph.D.
Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile.
dromana@inta.cl

Nutritional and health problems associated to trace elements, especially their deficiencies, remain a concern in Latin America, as evidenced in the past 4-5 years by continuous efforts in better understanding the effect of provision of key trace elements in susceptible populations via clinical trials or community-based interventions. Most of the interventions completed in the Region have focused on the administration of iron, zinc or multiple trace elements to children, pregnant women or women of childbearing age. Five trials focusing on iron nutriture have been conducted in 4 different countries, all with different objectives, but all targeted to infants and/or children. Results of one study in Honduras showed that exclusive breast-feeding for 6 months plus provision of iron supplements maintains an adequate micronutrient status in term, low birth weight infants. Trials in Mexico and Argentina have demonstrated that delayed clamping of the umbilical chord reduces anemia at 6 months post birth. Fifteen studies focusing on zinc nutriture have been conducted in 6 different countries. A trial in Chile showed that provision of zinc supplements for 2 months has no effect on iron absorption or the iron status of women. In Ecuador a dose-response trial of zinc supplementation found positive effects in plasma zinc concentrations and in the reduction of the prevalence of acute diarrhea as well as no negative effects on either the copper or iron status of children. Studies in Guatemala and Peru focusing on improving zinc nutrition via genetic modification or fortification of staple foods failed to show an effect on total zinc absorption and on plasma zinc concentrations, anthropometric or morbidity parameters, respectively. Two trials in Mexico have looked at the relationship between zinc supplementation and hormones or biochemical parameters associated to obesity or obesity-related chronic diseases. A study in Peru concluded that prenatal zinc supplementation has a positive effect on fetal bone growth and that presumably this effect is maintained post-birth until 12 months of age. Eleven trials focusing on provision of multiple trace elements have been conducted in 4 countries. Assessment of national targeted food fortification programs in Chile, Ecuador and Mexico have found significant reductions in the prevalence of anemia, but show mixed results with regard to zinc deficiency. A nutrition education intervention trial in Peru, which was delivered via the health services, demonstrated an increase in the intake of iron and zinc in beneficiary infants that presumably was translated into better weight and length gains. Scaling-up of a weekly multi-micronutrient program in Peru showed a protective effect of supplementation on the prevalence of anemia of children under 5 and women and adolescent girls. Finally, two trials in Mexico failed to show an effect of providing iron and zinc supplements in the reduction of plasma lead concentrations or behavior of children exposed to lead. In conclusion, continuous efforts have been implemented in Latin America to better understand how to control nutritional and health problems related to trace elements as evidenced by the number of interventions trials conducted in the Region in the past 4-5 years.

S36. SUBSTRATE PROFILE AND SELECTIVITY OF THE HUMAN DIVALENT METAL-ION TRANSPORTER DMT1. Anthony C Illing, Ali Shawki, Christopher L Cunningham, and Bryan Mackenzie
University of Cincinnati College of Medicine, Department of Molecular & Cellular Physiology, PO Box 670576, Cincinnati, OH, 45267-0576, USA
mackenb@UCMAIL.UC.EDU

DMT1 (SLC11A2) is essential for intestinal iron absorption and erythroid iron utilization. Iron deficiency is a serious risk factor for cadmium intoxication, especially in children, suggesting that cadmium and iron share a common absorptive pathway. Whereas DMT1 exhibits reactivity (based on evoked currents) with a broad range of transition metal ions including Cd2+, questions have arisen as to which of these are actually transported. We used a fluorescence-based approach for live imaging of metal-ion transport in Xenopus oocytes and provide here a comprehensive substrate-profile analysis for human DMT1. We first established the reactivity of the fluorophore PhenGreen SK (PGSK) with a range of metal ions in a cell-free system. We injected control oocytes and oocytes expressing DMT1 with PGSK and used the confocal laser-scanning microscope (excitation at 514nm and detection in the band 530-600 nm) to monitor fluorescence changes during 10-min superfusion with metal ions. We also measured radiotracer metal-ion uptake and metal-ion-evoked currents using the two-microelectrode voltage clamp. We took the first-order rate constant of quenching as an index of metal-ion uptake and validated our fluorescence approach by comparing the findings with radiotracer data. The half-maximal concentration (K0.5) for Fe2+ (4.2 ± 2.2 μM, SEE) determined from PGSK quenching at pH 5.5 matched that determined from 55Fe2+ uptake (5.9 ± 0.9 μM), and Fe2+-induced PGSK-quenching rates were pH-dependent in the same manner as 55Fe2+ uptake. Neither Cr2+ nor Cr3+ quenched PGSK fluorescence, consistent with 51Cr data revealing that Cr(II) and (III) are excluded by DMT1. DMT1 also excluded Cu1+, Cu2+, Fe3+, Pb2+, Sn2+, Sr2+ and VO+ (or V2+). Several metal ions resulted in more rapid quenching in DMT1 oocytes than in control oocytes. We determined for these the order of substrate selectivity (using the ratio Imax/K0.5) from evoked currents (I): Fe2+, Cd2+ > Co2+, Mn2+ > Ni2+, VO2+ (or V3+), Zn2+ (where “>” ≈ 0.5 log10 units). 55Fe2+ transport was inhibited by Cd2+, Mn2+ and Co2+ in a competitive manner. Notably, whereas Zn2+ evoked large currents, 65Zn2+ was very poorly transported and Zn2+ only weakly inhibited 55Fe2+ transport (Ki ≈ 50 μM). Our data reveal that DMT1 is a ferrous iron-preferring transporter that efficiently transports Cd2+ but our data do not support DMT1-mediated transport of Cu either in its 1+ or 2+ state. We speculate that DMT1 will serve as a route of entry for the toxic heavy metal Cd, especially in iron deficiency, and contribute to the absorption of Co and Mn (and trace metals Ni and V) but DMT1 is unlikely to be physiologically relevant to the absorption of Zn or Cu. We expect that our PGSK fluorescence-based metal-ion transport assay will have considerable utility in the live imaging of metal-ion transport in intact tissues.

S37. IDENTIFICATION OF BIOMARKERS FOR MICRONUTRIENT STATUS – A COMPARISON OF GENOMICS AND PROTEOMICS APPROACHES.
Timothy Miller[1], Lorraine Gambling[1], David Brown[1], Magdalena Araya [2] and Harry J. McArdle[1].
[1] Rowett Institute of Nutrition and Health, University of Aberdeen AB21 9SB UK
[2] INTA, University of Chile, Santiago, Chile.
h.mcardle@abdn.ac.uk

Over the years, there have been many attempts to try and identify parameters that can be used as an indicator of copper status in humans. None of these have been successful. To some extent, ceruloplasmin levels in serum are valuable in that levels drop in marked copper deficiency. However, levels can also change in many other conditions, such as infection, other inflammatory disorders and contraceptive use. Superoxide dismutase, PAM, BMAO and other enzymes have also been tested, with limited success. For a biomarker to be valuable, it must fulfil several conditions. It must be sensitive to status, specific to that nutrient and vary in a correlative, rather than a stochastic, fashion. We have suggested that an approach using multiple markers may have more potential than just using one single one. In this abstract, we examine this hypothesis and demonstrate some of the pitfalls in trying to identify a robust suite of biomarkers for copper status. We use several different approaches. In the first, we use DNA arrays taken from rats given copper deficient diets, and compare the results with animals given iron deficient diets, testing the hypothesis that we can identify genes that are altered specifically as a result of micronutrient deficiency. We can then test whether the changes are specific to that nutrient or not by comparing the results with those taken from animals undergoing a related but different nutrient stress. We can also extend these results to different pathways using any one of a variety of pathway analysis software packages. We find some of the results are predictable, but changes in lipid metabolism in iron deficiency are significant, with the results being informative of the phenotype of micronutrient deficiency. In the second series of experiments, we compare proteomics approaches. In one set of animal experiments, we carry out 2D gel electrophoresis, again comparing copper and iron deficiency. In a further set of experiments we use SELDI and iTraq to identify proteins differentially affected in serum taken from human volunteers. Again, we can use the comparative approach to identify specific and sensitive potential biomarkers. In the serum proteomics experiments, we endured a series of unexpected problems, which clearly helped us to define the parameters needed to design an effective biomarker experiment. These problems will be outlined, but include changes occurring as a result of shipping samples from the field to the analytical laboratory, setting up of instruments and the use of relevant and appropriate analytical techniques.

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S38. PREBIOTICS AND IRON BIOAVAILABILITY: USING THE PIGLET AS A MODEL TO STUDY EFFECTS AND MECHANISMS. Dennis D. Miller, Koji Yasuda, Jannine K. Patterson, and Xingen G. Lei.
Departments of Food Science and Animal Science, Cornell University, Ithaca, New York, USA
ddm2@cornell.edu

Pigs are recognized as useful experimental models for studying the digestion and metabolism of nutrients and other dietary factors important to human nutrition. Pigs are truly omnivorous and their nutrient requirements, gastrointestinal anatomy and physiology, and metabolic processes are similar to humans. Recently, we used weanling piglets to study the impact of supplemental inulin, a prebiotic, on the absorption and utilization of iron in maize-soybean diets. Iron bioavailability, assessed as the percentage of ingested iron incorporated into hemoglobin, was increased by 28% compared to controls in piglets fed a diet containing 4% inulin ($P<0.01$). Furthermore, feeding inulin increased relative numbers of lactobacilli and bifidobacteria with concomitant reductions in clostridia in the colon, confirming that inulin is functioning as a prebiotic in pigs. Since inulin is a prebiotic and, therefore, presumably exerts its primary action in the colon, we hypothesized that the increase in iron bioavailability we observed was due to enhanced iron absorption in the colon. We conducted a dual stable isotope study to test this hypothesis. Enriched 54Fe was administered in the feed and 58Fe was infused directly into the cecum through an incision made in the abdomen. After two weeks, the amount of each isotope present in circulating hemoglobin was measured using inductively coupled plasma mass spectrometry. Absorption of the orally administered isotope was 23.2 ± 2.7 and 20.7 ± 3.5 % in the inulin-fed and control-fed pigs respectively. Absorption of the isotope infused directly into the cecum was only 0.4 ±0.1 and 1.0 ± 0.2 %, respectively. This clearly shows that inulin does not enhance iron absorption in the colon. Our results suggest that prebiotics have potential for reducing the prevalence of iron deficiency by enhancing iron bioavailability in diets high in iron absorption inhibitors. Further studies will be required to understand th

S39. ZINC ABSORPTION: MODELING THE IMPACT OF DIETARY FACTORS. Leland V. Miller, Nancy F. Krebs and K. Michael Hambidge
Section of Nutrition, Dept. of Pediatrics, University of Colorado School of Medicine, Denver, CO USA
leland.miller@uchsc.edu

Numerous dietary factors have been demonstrated or hypothesized to affect the absorption of zinc. The two known to have the greatest impact are the zinc and phytate content of the diet. The mathematical modeling of absorption data is a means of characterizing quantitatively the interacting effects of these dietary factors, thereby providing the valuable capability of predicting zinc absorption from various kinds of diets and, perhaps, enhancing our understanding of the absorption process. We have developed a mathematical model of zinc absorption as a function of dietary zinc and phytate (J Nutr 137:135-141, 2007), one of several reported in recent years. The model is derived from a basic conception of transporter-mediated absorption and the competition for binding zinc between transporters and dietary phytate, and has three parameters: AMAX, KR and KP, which represent the maximum quantity of zinc which can be absorbed daily and the equilibrium dissociation constants of the zinc-transporter and zinc-phytate binding reactions, respectively. The model has been fit to data from isotope tracer studies of adults wherein true zinc absorption was measured for a complete daily diet and total daily dietary zinc and phytate were measured. Initially, only 21 published data were found that met the selection criteria. Since then additional data acquired by our lab or reported by others have been incorporated into the model, roughly tripling the number of data now fit by the model. As would be hoped, the process of adding more data to the model has generally improved the quality of the parameter estimates, thereby increasing confidence in the model's predictions. Furthermore, the new data have supported the validity of the model. Nonetheless, the still limited number and range of currently available data preclude the realization of the model's potential utility. As more data become available, it is reasonable to expect that the influence of other dietary factors, or other biological processes, on zinc absorption may become discernable. Among the latter is the possibility of observing evidence of transporter regulation in response to changing dietary zinc. In fact, an examination of modeling data from studies where absorption was measured shortly after commencement of the study diet compared to data from studies conducted over longer periods does suggest behavior consistent with a hypothetical transporter adaptation. While the primary application of the model, where its value as a predictive tool can immediately be put to use, is the modeling of total daily zinc data from adults, it has been fit to other kinds of data, including absorption data from single test meal studies and infant studies. These applications have also proved to be informative and supportive of the model's validity.

S40. AN IRON-CALCIUM CONNECTION IN NMDA RECEPTOR SIGNALING AND HIPPOCAMPAL SYNAPTIC PLASTICITY. Pablo Muñoz-Carvajal[1], Alexis Humeres[2,3], Cecilia Hidalgo[3] and Marco T. Núñez[4].
[1]Centro de Neurociencias, Universidad de Valparaíso
[2]Programa de Doctorado en Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile
[3]Centro FONDAP de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile
[4]Departamento de Biología, Facultad de Ciencias and Institute for Cell Dynamics and Biotechnology, Universidad de Chile.
mnunez@uchile.cl

Iron deficiency during early life is associated with significantly lower cognitive and behavioral development in infants, indicating that iron is essential for neural development. In particular, nutritional iron deficiency interferes with hippocampus-depending learning and affects synaptic plasticity in animal models. Yet, current understanding of the relationship between neuronal function and brain iron status is sparse. Sustained hippocampal CA1 long-term potentiation (LTP) involves calcium-dependent stimulation of the extracellular regulated kinase (ERK) pathway. In this work, we investigated the participation of iron on a) calcium release, b) ERK1/2 stimulation induced by the glutamate agonist N-methyl D-aspartate (NMDA), and c) hippocampal synaptic plasticity. Incubation of PC12 cells or hippocampal neurons with NMDA or iron promoted reactive oxygen species generation, enhanced ryanodine receptor-mediated calcium release and activated the ERK1/2 pathway, as determined by increased ERK1/2 phosphorylation and nuclear translocation of the phosphorylated proteins. Selective iron chelation with desferrioxamine, intracellular calcium chelation with BAPTA-AM or specific RyR inhibition with ryanodine inhibited the ERK1/2 activation induced by either NMDA or iron. Pre-incubation of hippocampal slices with desferrioxamine decreased basal synaptic transmission; this inhibitory effect required NMDA receptor activation. Pre-incubation with desferrioxamine also prevented sustained (1 h) LTP induction in CA1 neurons produced by 4 cycles of theta burst stimulation. The present results suggest that hippocampal neurons require iron-derived reactive oxygen species for NMDA receptor-dependent stimulation of the ERK1/2 pathway, a requisite step of sustained LTP.
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S41. UNTUNING OF PROTEIN-METAL INTERACTIONS IN ALZHEIMER DISEASE. Carlos Opazo
Laboratorio de Neurobiometales, Departamento de Fisiología, Universidad de Concepción, Chile
carlosopazo@udec.cl

Brain regions of Alzheimer's disease (AD) patients show (i) extensive synaptic failure, (ii) an increase in oxidative damage products and (iii) an accumulation of proteinaceous oligomers/aggregates, mainly formed by the amyloid β-peptide (Aβ), which has metal binding properties. In fact, Aβ aggregates in AD are enriched in transition metals that may mediate its assembly. The genetic, cellular and biochemical evidence obtained so far suggest that oligomeric species of Aβ peptide may be responsible for neuronal damage in AD, and it has been proposed that some accessory molecules could modulate Aβ synaptic effects. In this context, it has been shown that transition metals such as copper, iron and zinc can interact with the Aβ peptide promoting amyloid aggregation and formation of reactive oxygen species. Therefore, the interaction between transition metals and the Aβ peptide may explain, at least in part, the neuronal and oxidative damage detected in the brain of patients suffering AD, opening a pharmacological avenue for intervention of this disease. Currently, the physiological function of Aβ is still unknown, but a considerable body of data suggests that Aβ could have an important role in Cu2+/Cu+ homeostasis, which may impact the synaptic vicinity where Aβ is generated and apparently released under electrical stimulation.

S42. EVIDENCE OF INCREASED COPPER AND IRON BUT NOT ZINC IN ALZHEIMER'S DISEASE: ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDS) ELEMENTAL MICROANALYSIS.
Gjumrakch Aliev[1,3], Paula Moreira[5], Sandra L. Siedlak[6], Mark A. Smith[6], Celia J. Cobb[1,7], Domingo Ferrer[2], Miguel Jose Yacaman[2], and George Perry[4,6]
[1]Department of Biology, [2]Department of Physics & Astronomy, [3]Electron Microscopy Research Center, and [4]College of Sciences, The University of Texas at San Antonio, San Antonio, Texas 78249, USA; [5]Center for Neuroscience and Cell Biology of Coimbra, University of Coimbra, 3004-517 Coimbra, Portugal; [6]Department of Pathology, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106 USA; [7]Microelectronics Research Center, The University of Texas at Austin 10100 Burnet Road, MER 1.606J /R9900, Austin, TX 78758-4445, USA
george.perry@case.edu

Alzheimer's disease (AD) is a progressive chronic neurodegenerative disorder and despite extensive research into its pathogenesis, the mechanisms underlying the earliest and most critical of these processes remain elusive. The current dominant theory regarding the etiology of this disease attributes are the toxic effects of reactive oxygen species (ROS) produced in the brain based on evidence correlating metal-bound Aβ peptides (i.e., Aβ-Zn2+ and Aβ-Cu2+) to the formation of fibrillar and/or amorphous amyloid plaques, or alternatively, the decline in brain function could result from ROS produced by mitochondrial dysfunction. Previous studies have linked abnormal mitochondrial turnover triggered by mitochondrial dysfunction to increased levels of redox-active iron in AD brains. However, the role of dysfunctional mitochondria in the production of other metal ion imbalances remains less clear. The current study analyzed the concentrations of certain metal ions, namely, Fe, Cu, and Zn, within neuronal mitochondria and mitochondria-derived autophagosomal structures (including mitochondria-derived vacuoles and associated lipofuscin) within AD and control brains utilizating the Energy Dispersive X-ray Spectroscopy (EDS) probe on a FEI TECNAI G2 F20 X-TWIN Transmission Electron Microscope (TEM).
In controls, intact neuronal mitochondria comprise a higher percentage of the total amount of mitochondria when compared to AD brains, and showed no abnormalities in their ultrastructure, displayed no positive signals for mtDNA with the 5kb common deletion, and contained only trace levels of Cu, Fe, and Zn ions. The AD samples however, even in intact mitochondria without visible ultrastructural damage, were positive for 5kb-deleted mtDNA and had higher concentrations for all three metal ions. Damaged mitochondria from both age-matched control and AD brains, displaying either broken cristae or as structures such as autophagolysosomes and lipofuscin granules, showed increases in the concentrations of Fe and Cu ions, but contained a decreased amount of Zn when compared to their intact counterparts. Even more striking, a 4-5 fold increase in Fe and almost 70-fold increase in Cu levels within lipofuscin granules were found in AD brains relative to age-matched control samples. These results further confirm a strong spatial correlation between excessive lipofuscin formation triggered by elevated amounts of damaged mitochondria and very high levels of accumulated Cu ions. This finding critically links metal ion dyshomeostasis to oxidative stress-induced mitochondrial damage during the development and maturation of AD. This work supported by NIH and Alzheimer's Association.

S43. A CYTOSOLIC IRON CHAPERONE THAT DELIVERS IRON TO FERRITIN. Haifeng Shi[1], Krisztina Z. Bencze[2], Timothy L. Stemmler[2], and Caroline C. Philpott[1]
[1]Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD
[2]Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI
carolinep@intra.niddk.nih.gov

Ferritins are the main iron storage proteins found in animals, plants, and bacteria. The capacity to store iron in ferritin is essential for life in mammals, but the mechanism by which cytosolic iron is delivered to ferritin is unknown. Human ferritins expressed in yeast contain little iron. We identified Poly r(C)-Binding Protein 1 (PCBP1) as a human protein that increased the amount of iron loaded into ferritin when expressed in yeast. PCBP1 bound to ferritin in vivo, and bound iron and facilitated iron loading into ferritin in vitro. Depletion of PCBP1 in human cells inhibited ferritin iron loading and increased cytosolic iron pools. Thus, PCBP1 can function as a cytosolic iron chaperone in the delivery of iron to ferritin.

S44. IMPACT OF COPPER DEFICIENCY ON BRAIN ENERGY METABOLISM. Joseph R Prohaska, Anna A Gybina.
University of Minnesota Medical School Duluth
jprohask@d.umn.edu

*Copper is esse*ntial for proper brain development, particularly the cerebellum, where copper deficiency attenuates biosynthetic processes including myelination and synaptogenesis. Copper is a cofactor for mitochondrial complex IV, cytochrome c oxidase (CCO). Copper deficiency severely limits CCO activity and leads to signs of mitochondrial inhibition in brain. Impaired energy metabolism in copper deficiency, due to limitation in CCO, is thought to cause developmental delays but aspects of energy metabolism in copper-deficient cerebella remain unexplored including mechanisms connecting energetic deficits with delayed development. Characterization of cerebellar glucose metabolism and assessment of mitochondrial functional changes were explored in a series of experiments with copper-deficient (Cu-) rat pups. These pups were derived from dams that began treatment on embryonic day 7 and continued throughout lactation. Cerebella from copper-adequate (Cu+) control pups and Cu- rat pups were analyzed. Cerebella were removed from unanaesthetized pups in about 12 seconds and frozen in liquid nitrogen. AMP activated protein kinase (AMPK) is a metabolic 'master switch' that is activated by energetic stress. AMPK shuts down biosynthetic processes to conserve cellular ATP levels and regulates glucose metabolism in some tissues. Analyses of Cu- cerebella revealed elevated AMPK activation (enhanced levels of phospho-AMPK). Furthermore, enhanced levels of phospho-acetyl CoA carboxylase (ACC), the first enzyme in fatty acid biosynthesis and a target of AMPK, were also detected, consistent with enhanced pAMPK function. However, activation of Cu- cerebellar AMPK did not appear to regulate cerebellar anaerobic glycolysis. Activity of AMPK's glycolytic target phosphofructokinase 2 (PFK2) remained unaltered and concentrations of the PFK2 product, the glycolytic stimulator fructose-2,6-bisphosphate (F2,6BP), were markedly lower in Cu- cerebella. This data and higher levels of glucose and glucose-6-phosphate in Cu- cerebella suggest potential glycolytic inhibition, possibly a consequence of higher cerebellar citrate and/or lactate levels that were also detected. Further analyses revealed that high lactate levels in Cu- cerebella were likely due, in part, to elevated blood lactate entering the cerebella. However, higher cerebellar lactate in Cu- tissue was also found to be generated by cerebella itself, suggesting mitochondrial inhibition. Analysis of rats treated with dichloroacetate (DCA) found evidence of pyruvate dehydrogenase complex (PDC) inhibition in Cu- cerebellar mitochondria consistent with higher NADH/NAD+, perhaps due to limiting CCO. Activation of AMPK provides a plausible mechanism in which biosynthetic processes are attenuated in copper deficiency rather than augmentation of glycolysis when mitochondria are inhibited. Further research will be necessary to determine the mechanism for AMPK activation since no changes in adenine nucleotides were detected despite the solid evidence for mitochondrial inhibition. Research supported by NIH R01 HD-39708

S45. ROLE OF NF-KB AND PARKIN IN REGULATING DMT1 EXPRESSION AND MANGANESE TOXICITY. Jerome Roth, Prasad Paradkar, Steven Singleton, Suma Das
Department of Pharmacology and Toxicology, University at Buffalo, Buffalo, NY 14214
jaroth@buffalo.edu

Chronic exposure to manganese (Mn) has been linked to development of a severe, irreversible neurological disorder known as manganism consisting, in the initial stages of the disorder, of reduced response speed, intellectual deficits, mood changes and compulsive behaviors to more prominent and irreversible extrapyramidal dysfunction resembling Parkinson's disease upon protracted exposure. Differences in the response to Mn overexposure, most likely, are due to underlying genetic variability which ultimately manifests in deviations in both susceptibility as well as the characteristics of the neurological lesions and symptoms expressed. Although there is not always a clear distinction in the expressed symptoms between manganism and Parkinson's disease, the initial injury in manganism is the globus pallidus in contrast to the substantia nigra pars compacta identified in Parkinsonism yet Mn accumulates in both areas of the basal ganglia. This becomes relevant based on recent studies demonstrating that the gene associated with early onset of Parkinson's disease, parkin, may prevent Mn toxicity. Parkin, one of over 600 protein-ubiquitin E3 ligases, is part of the ubiquitin/proteasomal pathway responsible for the degradation of a multitude of proteins controlling normal homeostatic function. The mechanism by which parkin prevents Mn toxicity is not known but may relate to our findings indicating that the major protein for the cellular uptake of Mn, divalent metal transporter 1 (DMT1) is degraded via the proteasomal pathway. Four isoforms of DMT1 are present in mammalian cells resulting from alternate splicing of a single gene transcript. All are putative 12 membrane-spanning domain proteins but differ both in their N- and carboxy-terminal residues with two of the forms possessing an iron response element (IRE) motif in the 3' UTR of the message. These isoforms are referred to as either the -IRE or +IRE isoform of DMT1 depending on whether the mRNA contains the IRE element or not. Transcriptionally regulated splice variants, IRE forms□exon 1A and 1B, are found on the proximal N-terminal end for both the of DMT1 mRNA. Transfected human SH-SY5Y neuroblastoma cells overexpressing parkin exhibit decreased levels of the 1B species of DMT1 as well as decreased transport and toxicity upon exposure to Mn. Inhibition of the proteasomal pathway in these cells results in increased levels of 1B-DMT1. In contrast, human lymphocytes expressing mutations in the parkin gene express elevated levels of the transporter. These data suggest that parkin is the E3 ligase responsible for degradation of the 1B isoforms of DMT1 and imply that development of manganism and Parkinson's disease may share a similar biochemical defect. Our studies also reveal that that the 1B species of DMT1, responsive to parkin, are also regulated at the level of gene transcription by NF-κB which is induced upon exposure to excess Mn and other pro-inflammatory cytokines. Based on these finding, we hypothesize that individuals expressing mutations in the parkin gene have a greater propensity to accumulate Mn by having diminished capacity to degrade newly synthesized DMT1. Thus, parkin plays an integral role in a complex series of events that ultimately potentiates Mn toxicity.

S46. DETECTION OF TRACE ELEMENT-CONTAINING PROTEINS. Dirk Schaumlöffel
CNRS, LCABIE, UMR 5254, Pau, France
dirk.schaumloeffel@univ-pau.fr

In recent years analytical chemistry has made significant contributions to the progress of research in life sciences by the systematic analysis of genes, proteins, and metabolites. However, the elucidation of their functional connections with metals and metalloids remains difficult and is regarded as one of the greatest biology challenges in the post-genomic era. Consequently, many novel analytical developments are focused on the analysis of metallo- and metalloid-proteins in tissues and body fluids. The detection of trace element-containing proteins in a complex biological matrix is a great challenge for the analytical chemist. Recent impressive progress toward lower detection limits in inductively coupled plasma mass spectrometry (ICP-MS), and higher sensitivity and reduced matrix interferences in electrospray and MALDI mass spectrometry for molecule specific detection at trace levels in complex matrices allows new frontiers to be crossed. This lecture highlights recent analytical approaches for the detection of trace element-containing proteins. Special emphasis will be made on hyphenated (coupling) techniques which combine high resolution separation with sensitive element- and molecule-specific detection. ICP-MS is a valuable alternative molecular MS for sensitive protein detection and quantification via the element signal under the condition that the protein structure contains a detectable heteroelement such as phosphorus, sulfur, selenium, iodine or a metal. For illustration purposes, recent studies from the author's laboratory will be discussed concerning selenium-, sulfur-, and metal-containing protein detection and quantification.

S47. SELENOPROTEIN P AND DISEASE IN HUMANS AND ANIMALS. Lutz Schomburg
Institute for Experimental Endocrinology, Charité University Hospital, Berlin, Germany
lutz.schomburg@charite.de

Selenoprotein P (SePP) is the key protein for transport and storage of Se in humans and animals. Genetic inactivation of SePP in mice (SePP-KO) impairs regular development, neurological function, male fertility and overall health. Interestingly, it was possible to prevent those explicit phenotypes in a dose-dependent manner by dietary Se supplementation or hepatocyte-specific expression of a human SePP transgene. These findings indicate that SePP appears to be indispensable to mediate efficient hepatic Se organification, transport and distribution when the dietary Se supply is limiting, though it does not have an essential life-sustaining function. Hemizygous SePP inactivation in SePP+/- mice affected Se metabolism only moderately and caused no obvious phenotype. However, SePP+/- mice turned out to be more susceptible to cancer when tested in an established intestinal tumorigenesis model. These findings are in agreement with recent observations in humans. Here, single nucleotide polymorphisms of the SePP gene correlate with an enhanced risk for advanced colon tumours. Some reports show that circulating SePP concentrations may serve as a suitable indicator for tumour risk and cancer progression. These findings support the claim that Se supplementation may reduce cancer risk, as SePP biosynthesis directly depends on the Se status and thus on Se intake. Therapeutic Se supplementation has also been shown to be effective in a second major disease, i.e., for the treatment of sepsis patients on the intensive care units. In general, Se decreases as a negative acute phase reactant in humans and experimental animals during inflammation. In fact, low circulating Se concentrations even indicate poor survival prognosis in these patients. Accordingly, Se supplementation in critically ill patients turned out to be effective in some clinical trials. These effects are best mirrored by circulating SePP concentrations which have proven to be a useful biomarker for Se status under pathological conditions. Using the model of LPS-induced acute phase response in mice, we have now analyzed the molecular basis for the decreasing circulating Se and SePP concentrations. Our results point to efficient hepatocyte-specific downregulation of SePP translation and indicate new therapeutic targets that might be useful to restore regular Se metabolism in critically ill patients and to increase their survival odds. Thus, its presence in serum renders SePP an ideal biomarker in cancer and sepsis as well as for the other health issues that have been correlated to Se status and dietary Se intake.
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S48. INTERVENTIONS WITH TRACE ELEMENTS (TES) IN SUSCEPTIBLE POPULATIONS: WHEN DOES SAFETY TRUMP EFFICACY? Noel W. Solomons[1] and Klaus Schümann[2]
[1]Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), Guatemala City, Guatemala
[2]Technical University of Munich, Freising, Germany
cessiam@guate.net.gt

The topic of the symposium, 'Interventions with trace elements in susceptible populations,' provides an ideal opportunity to frame the terminology and underlying assumptions in an area that has important implications for public health, especially of children in developing countries. To some, the research campaign that has brought on the widespread proliferation of TE interventions has the character of a 'one trick pony,' with a stereotyped paradigm: Let us see if giving more of a TE produces a beneficial effect -- and, if so, replicate the trial as public intervention policy. If, indeed, this has been the origin of the policies regarding fluorine, iodine, iron, zinc or selenium, it merits a comprehensive reexamination that weaves safety concerns into the tapestry of efficacy and effectiveness. Probing the semantics of 'susceptible populations,' moreover, allows deeper nuance in considerations. Do we merely mean vulnerable to micronutrient deficiency (or excess)? Or is it susceptibility to benefiting from a given intervention? Or has it the context of prone to clinical situations, e.g. malaria or HIV, with implications regarding TE exposures? Inherently, we sit on the horns of a dilemma between the public policy guideline 'the greatest good for the greatest number' and the Hippocratic principle 'first do no harm.' The distinction between a TE intervention to re-balance adequate uptake (physiological) and one to provide a supra-dietary exposure (pharmacological) is real. Intervention 'with' trace elements, in the title, explicitly connotes the TEs as the tool for manipulation. Among intervention formats, the two that bring more TEs to the table are: 1) supplementation and 2) fortification, including biofortification. Interventions 'concerning' TEs widens the consideration to health measures (reducing dysentery) and dietary diversity (increasing meat intake), as well as situations of correcting excess exposure (e.g. reducing arsenic intake in Bangladesh), and not only under-consumption. The safety microscope, moreover, should not be too tightly focused on the interaction of a supplement or fortificant with the host. Possible, untoward consequences upstream or downstream of the intervention must be considered. Theoretical examples include: 1) agricultural dislocations in introducing a biofortified crop or a phytate-reduced variety; or 2) distortion of usual consumption patterns (attraction or rejection) of the food vehicle used in TE fortification. For example, either increasing salt, sugar or oil consumption to seek greater nutrient exposure, or reducing grain consumption due to organoleptic factors imparted by hybridization or anxiety deriving from genetic modification could have disastrous consequence on the health of intervened populations. Ethical issues intercede at many levels, including: 1) testing hypotheses regarding adverse effects in children; 2) the analytical imperative to include a no-treatment (placebo) group in order to examine safety; and 3) issues of assent, consent and individual will. In the clinical domain, e.g. Zn for HIV+ children, the intervention entails implied consent within the practitioner-patient relations. Mass public health intervention with a TE, however, implies delicate ethical issues of autonomy, as the potentially benefited, unaffected and adversely-affected alike are exposed without distinction, and not necessarily with consideration of popular or individual will.

S49. TRACE MINERAL TRANSPORTERS: IMPLICATIONS FOR ANIMAL NUTRITION.
Jerry W Spears, Stephanie L Hansen, Robert S Fry
North Carolina State University, Department of Animal Science, Raleigh, NC, USA
jerry_spears@ncsu.edu

Recently a number of metal transport proteins have been identified in rodents that play critical roles in cellular trace mineral homeostatis. More recently, many of these transporters have also been studied in domestic animals. The intestinal ferrous iron (Fe) import protein, divalent metal transporter 1 (DMT1), has been found in cattle, swine, and poultry, and the intestinal basal membrane Fe exporter, ferroportin, has been identified in cattle and swine. In addition to Fe, DMT1 can also transport manganese (Mn) and perhaps copper (Cu). Animal diets are frequently high in Fe and high dietary Fe in ruminants has been shown to reduce bioavailability of Cu and Mn. Increased competition for DMT1 by Fe as well as down regulation of DMT1 by high dietary Fe may explain the antagonism between Fe and Mn and Cu. Copper transporters are of interest in ruminants because both Cu deficiency and toxicity are of concern in cattle and sheep. Major differences in Cu metabolism occur between ruminants and nonruminants. Copper absorption is much lower in ruminants (largely because of interaction that occur in the rumen), while Cu concentrations are considerably higher in ruminants than in nonruminants. Within ruminant species, genetic differences in Cu metabolism among breeds can be large, especially in sheep, suggesting possible differences in Cu transporters in the intestine and/or liver. Copper transporter 1 (Ctr1) involved in cellular import of Cu, and the Cu-transporting ATPases (ATP7A and ATP7B) involved in the secretory pathways for Cu have been shown to be expressed in liver of cattle. Copper-deficient cattle had reduced expression of ATP7A and ATP7B in liver compared to Cu-adequate cattle. ATP7B is critical for export of Cu from the liver via biliary excretion or incorporation into ceruloplasmin for secretion into the blood. Two forms of ATP7B have been isolated and sequenced from liver of Merino sheep, a sheep breed that is one of the least susceptible to Cu toxicosis. One form was similar in chain length and molecular mass to that previously found in mouse, rat, and human. An alternate form of the ATP7B gene was isolated from sheep liver that differed from the normal ATP7B at the amino-terminal region. Both forms of ATP7B isolated from sheep liver appeared to exhibit normal Cu transport properties when transfected into fibroblast cell lines. Copper chaperone proteins, responsible for binding and delivering Cu to specific intraceullular sites have been examined in cattle. Consistent with studies in rodents, levels of the chaperone protein (CCS) that delivers Cu to cytosolic superoxide dismutase have been found to be increased during Cu deficiency in cattle. Gene expression of COX17, the chaperone protein that delivers Cu to cytochrome c oxidase in the mitochondria, was decreased in liver by Cu deficiency in cattle. Increased understanding of trace mineral transporters and their regulation in animals should enhance our understanding of mineral interactions, and allow animal nutritionist to more adequately balance diets for trace minerals.

S50. Cu(I)-GLUTATHIONE COMPLEX: A NEW BIOLOGICAL SOURCE OF SUPEROXIDE RADICALS?. Hernán Speisky, Maritza Gómez, Catalina Carrasco-Pozo, Francesca Burgos and Margarita Aliaga.
Nutrition and Food Technology Institute, University of Chile, Macul 5540, Santiago, Chile. hspeisky@inta.cl

Reduced glutathione (GSH), is the single most abundant intracellular thiol. The metal-reducing capacity and nucleophile character of GSH endows it with the ability to react with copper ions in a reaction which involves, initially, the reduction of Cu2+ ions, and subsequently, the chelation of Cu+ to form a Cu(I)-[GSH]2 complex. The occurrence of such a complex in copper-overloaded cells is believed to serve as a cell protective mechanism since it prevents the occurrence of the metal in its free reduced, and thereby in its redox-active state. In the present study we provide evidences that, rather than preventing free radical formation, the Cu(I)-[GSH]2 complex would behave as a biologically-significant source of free radicals. We assessed the ability of this complex to generate such specie in aqueous solutions by means of polarographic (changes in oxygen concentration; Clark-electrode), ESR (changes in paramagnetic properties) and spectroscopic techniques. The interaction between the Cu(I)-[GSH]2 complex and molecular O2 was found to lead to a steady decline in the concentration of O2 in the solution in the presence but not absence of added superoxide dismutase (SOD). The latter was inferred to be result from a Cu(I)-[GSH]2-dependent reduction of O2 into O2•-. Formation of the latter radicals was confirmed through by the generation of a SOD-inhibitable ESR-DMPO-OOH adduct. Further evidence on the ability of the complex to generate O2•- was attained through the demonstration of its capacity to reduce cytochrome c and to oxidize dyhydroethidium (two superoxide-suscptible probes); having been both effects SOD-inhibitable. The Cu(I)-[GSH]2 complex was, in turn, unable to induce the oxidation of fluorescein, a hydroxyl radical-sensitive probe. From these experiments we conclude that in solutions containing the complex, oxygen would be continually reduced into O2•- anions, and that –in absence of interceptors- the latter radicals would be quantitatively re-oxidized into molecular O2. We suggest that coupled to the re-oxidation of O2•-, the Cu(I)-[GSH]2 complex would be re-generated from an “oxidized form of the complex”.
Experiments based on the use of NMR and EPR were then employed to identify the above-referred putative “oxidized complex form”. Cu(I)-[GSH]2-containing solutions added Tempol (another O2•--interceptor) generated EPR and NMR spectra which were coincident with those featured by a pre-formed Cu(II)-GSSG complex. The biological occurrence of latter complex has not been established yet. In complementary experiments, we observed that the incubation of the Cu(I)-[GSH]2 with Tempol led to the emergence of a band at 625 nm which had also been previously reported as characteristic of the Cu(II)-GSSG complex. Interestingly, the latter band disappeared time- and concentration-dependently upon addition of increasing concentrations of GSH. The addition of ascorbate failed, however, to induce such reversal. According to these results, removal of the superoxide radicals generated during the Cu(I)-[GSH]2 /molecular oxygen interaction, results in the oxidation of Cu(I) into Cu(II) and that of GSH into GSSG molecules. Based on these data, on the EPR and NMR spectra, and on the appearance of a band at 625 nm data, we postulate that Cu(II)-GSSG molecules are formed when the superoxide anions generated by the Cu(I)-

[GSH]2/O2 interaction are intercepted.
Prompted by the ability of the Cu(I)-[GSH]2 complex to generate O2-. radicals, we also undertook experiments aimed to assess the ability of the later complex to induce the reduction of certain O2-.-susceptible target molecules. Previous in vitro evidence has revealed that superoxide anions are capable of inducing the reduction of iron in ferritin and favouring its subsequent release as Fe2+ into the media. Considering the latter, we evaluated the ability of the Cu(I)-[GSH]2 complex to induce in vitro the reduction of Fe3+, both, as free- and bound-to-ferritin metal; ferritin is the major intracellular iron-storing protein. Superoxide radicals generated by the Cu(I)-[GSH]2 complex were found to reduce free Fe3+ ions (using batophenantroline- and triazine as chelators) and to induce the reduction and the release of Fe2+ from iron-loaded ferritine. Noteworthy, when H2O2 was also added to the ferritin/Cu(I)-[GSH]2 containing solution, the Fe2+ ions generated by the complex were found to be capable of catalyzing the generation of hydroxyl radicals in the medium (using fluorescein as probe). These results indicate that during its interaction with iron atoms (whether in its free or bound forms), the Cu(I)-[GSH]2 complex would generate redox-active Fe2+ ions which would favour the occurrence of superoxide-driven Fenton reactions. On the basis of its pro-oxidant activity, it is suggested that the Cu(I)-[GSH]2 complex could exacerbate iron-dependent oxidative damage in copper over-loaded cells.
The ability of the Cu(I)-[GSH]2 complex to generate superoxide anions and, thereby, to deleteriously affect superoxide-susceptible biological targets warrants further studies.
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S51. MOLECULAR SELENIUM NUTRITION – FROM DISCOVERY TO MOLECULAR BIOLOGY BIOMARKERS FOR SELENIUM STATUS AND REQUIREMENTS. Roger A Sunde.Nutritional Sciences, Univ. of Wisconsin, Madison, WI 53706 USAsunde@nutrisci.wisc.edu

Immediately after the discovery in 1971 that glutathione peroxidase-1 (Gpx1) was a selenoenzyme, Se supplementation studies revealed that Gpx1 activity was profoundly regulated by Se status. This discovery provided a biochemical role for Se, and provided a most useful biomarker for assessment of Se status and requirements in humans and animals. Over the next 15 years, a handful of additional selenoproteins were grudgingly identified, until rudimentary bioinformatics in 1986 revealed that the Se moiety of Gpx1 is encoded by a stop codon. With this discovery, Se research became molecular. Over the following 15 years, this research uncovered the elaborate and rich biology used for Se metabolism and Se incorporation into proteins, and it revealed a dozen or so additional selenoproteins. This work set the stage for the elegant genome-wide screens reported by Dr. Gladyshev and colleagues in 2003, resulting in the complete enumeration of the selenoproteomes in a number of species, and the identification of a number of new selenoproteins. Since the discovery of Gpx1, I have been fascinated by the use of selenoproteins to assess Se requirements. We found that Gpx1 protein levels are regulated similar to enzyme activity, and then discovered that Gpx1 mRNA levels also decrease dramatically in Se deficiency in rats and can be used to determine Se requirements. The uncovering of the complete selenoproteome now has given us the tools to examine the full selenoproteome for mRNA biomarkers that could be used to assess Se status. In mice and rats, we found that Selh, Sepw1, and Gpx3 as well as Gpx1 mRNAs decrease in Se deficiency to <40% of Se-adequate levels. The minimal dietary Se requirements of these Se-regulated mRNAs are similar, between 0.04-0.07 µg Se/g, and all are less than the requirement (0.1 µg Se/g) for Gpx1 activity, suggesting a common mechanism for this regulation. The majority of selenoprotein mRNAs (12 in rat liver; 15 in kidney; 20 in muscle), however, are not significantly down regulated by Se deficiency. These same Se-regulated mRNAs are present in total RNA isolated from human blood, and we are beginning studies to determine if these molecular biomarkers can also be used to assess human Se status and requirements. (Supported by NIH DK74184 & T32-DK07665, UW WIS04909)

S52. FRONTIERS IN IRON NUTRITION AND ANTIOXIDANT METABOLISM. Elizabeth C. Theil,
CHORI and UC-Berkeley,Oakland, CA. USA
etheil@chori.org

Iron is an essential metal nutrient that functions in man and in animals as the active center of proteins important in respiration, oxygen transport, cell division, fatty acid desaturation and detoxification. Diagnosed 500 years ago, iron deficiency still affects large numbers of humans and is the most common nutrient deficiency worldwide. Deficiency symptoms are associated with anemia, fatigue, work losses, and developmental lags in motor and cognitive development. The reaction of free oxygen with iron that produces radical reactions and oxidative damage in living tissues has led to the classification of iron and some iron - proteins as sources of free radicals, and as prooxidants. However, recent genetic evidence links the regulation of some iron proteins to cell oxygen levels and to antioxidant responses. The antioxidant activity of the protective iron proteins would be diminished during iron deficiency.

S53. TRACE ELEMENTS INTERVENTIONS IN ASIA: KNOWLEDGE vs CHALLENGES.

Emorn Wasantwisut
Senior Advisor, Institute of Nutrition, Mahidol University, Salaya, Phuttamonthon, Nakhon Pathom 73170, Thailand
numdk@mahidol.ac.th

The prevalence of child undernutrition (underweight, stunting and wasting) in south-central Asia contributes significantly to the burden of disease and child mortality. Amidst this backdrop are deficiencies of key trace elements: iron, zinc and iodine, leading to compromised growth, development, cognition and resistance to infection. Both 2008 Lancet series and Copenhagen Consensus cited micronutrient supplementation and forfification, as having strong impact and cost effective to reduce child morbidity and mortality. Efficacy trials in Asian mothers and children have generated knowledge for policy and program implementation. Major strategies include food fortification, biofortification and supplementation. Examples of successful interventions at scale are iron/folic acid supplementation during pregnancy in Nepal, universal salt iodization in China, multi-micronutrient powder added to meals in West Bengal-India. Many others show potentials for scaling up and must overcome constraints to get there. Compelling evidence from efficacy trials demonstrates the benefit of zinc supplementation for the treatment and prevention of diarrhea and still, mainstreaming zinc into the national diarrhea control programs is needed to accelerate progress. Improvement in iron status has been shown with iron-fortified fish sauce in Vietnam as well as fortified soy sauce in China and both interventions are in the process of scaling up. Efficacy study of multiple fortification of seasoning sachet in Thailand showed improvement of zinc, iron and iodine status as well as cognition. Evidence is accumulating on the efficacy of in home fortification of multiple micronutrient powders in complementary foods in Bangladesh, Vietnam and Mongolia. Progress is also being made in plant breeding of bio-fortified rice high in iron and wheat high in zinc in Asia. While scientific knowledge points to benefits of the forementioned trace element interventions, the challenges of bringing them to scale are many. Key factors include national commitment; government leadership; cooperation among key stakeholders; effective monitoring and evaluation system; programmatic and technical capacity development.

S54. COPPER AND ZINC METABOLIC CHANGES AND MANIPULATION IN HYPERTENSION. Huiqi Xie
Division of Stem Cell and Tissue Engineering, National Key Laboratory of Biotherapy, West China Hospital, Sichuan University
xiehuiqi@163.com

Human studies have shown that the ratio of copper (Cu) to zinc (Zn) concentrations decreases in the blood samples of hypertension population. The consequence of this change is unknown. We have used a spontaneous hypertension rat (SHR) model to examine the effect of increasing the ratio of Cu to Zn by dietary Cu supplementation on the development of hypertension. Male SHR rats develop hypertension starting at 6 weeks old of age and reaching a stable peak high blood pressure at 12 to 16 weeks old. Cu supplementation through drinking water at 25 mg Cu/L in addition to dietary Cu of 6 to 7 mg Cu/kg diet starting at 6 weeks old significantly suppressed the development of hypertension at 8, 10, 12, and 16 weeks old. Cu is required for vascularization and we hypothesize that Cu improvement of microcirculation through angiogenesis is responsible for the observed prevention of hypertension. In cultured human umbilical vein endothelial cells, Cu promoted cell proliferation and stimulated expression of genes involved in endothelial cell differentiation, including endothelin-1, endothelial nitric oxide synthase, PECAM-1, ICAM-2, Tiel, and CD144. In addition, Cu enhanced the proliferative response of the cultured human umbilical vein endothelial cells to vascular endothelial growth factor (VEGF). Taking together, Cu promotion of endothelial cell proliferation and differentiation would significantly promote vascularization and improve microcirculation. Therefore, Cu supplementation-induced prevention of hypertension in the SHRs would result at least in part from Cu promotion of microcirculation.

S55. MULTIFUNCTIONAL IRON CHELATORS NEUROPROTECTIVE AND NEURORESTORATIVE DRUGS FOR ALZHEIMER'S DISEASE. Moussa B.H. Youdim
Eve Topf Center of Excellence for Neurodegenerative Diseases Research
and Department of Pharmacology,Technion-Rappaport Faculty of Medicine,
Haifa, Israel
Moussa.Youdim@inet.polyu.edu.hk

Alzheimer's disorders (AD), and Parkinson's disease (PD) are initiated by cascade of neurotoxic events, that includes oxidative stress, brain iron dysregulation, glutamate excitotoxicity, inflammatory process, neurotxic processing of APP misfolding of proteins Aβpeptide and α-synuclein. Significant percentage of AD subjects also suffer from extrapyramidal symptoms (Lewy Body disease) and depression and PD with dementia and dpression.These subjects are benefiting from drugs developed to act on a single molecular target. Such drugs have limited symptomatic activities and current pharmacological approaches are highly limited in their ability to modify the course of the disease, offering incomplete and transient benefit to patients. However, the new therapeutic strategies for neurodegenerative diseases, such as AD, PD, Huntington disease and ALS (amyotrophic lateral sclerosis) are those in which drug candidates are designed expressly to act on multiple neuronal and biochemical targets involved in the neurodegenerative process and neurotransmission. Monoamine oxidase (MAO) B activity, iron, and glutamate excitotoxicity increase in ageing brain AD and PD. They are thought to contribute to oxidative stress dependent neuronal death. The iron deposition in AD hippocampus and substantia nigra of PD, can induce oxidative stress via interaction with hydrogen peroxide produced by MAO-B and other oxidative processes to promote the Fenton chemistry and generate the neurotoxic reactive hydroxyl radical. Furthermore such radical cause aggregation of iron responsive amyloid precursor protein (APP) and α-synuclein to highly toxic aggregates that inhbit both mitochondrial function and ubiquitin-proteasome system (UPS). Thus we have developed molecular entities that combine two or more of cholinesterase inhibition, brain selective MAO inhibition, iron chelation, inhibitors of glutamate release, anti apoptotic-neurorescue and neurorestorative activities. These iron chelator drugs are also inhibitors of iron dependent prolyl-4-hydroxylase, which inactivates of hypoxia inducing factor (HIF) via ubiquitination .They increased release of neuroprotective and neurotrophic erythropoietin and cause differentiation of neurons. This has been attributed toprevntion of entry in to cell cycle as a consequence of Cyclin D inhibition. Two of such multimodal compounds presently under development are ladostigil, a cholinesterase inhibitor derivative of rasagiline (azilect) and iron chelator-MAO B inhibitor M30 series. These drugs possess iron chelating-radical scavenging, brain selective MAO-A and B inhibitory activity, acetylcholine and butyrylcholinesterase inhibitory moieties. Animal behavioral and neurophramacological studies have shown their anti Alzheimer, anti Parkinson and anti depressant activities. Both drugs also have neuroprotective and neurorestorative activities in neuronal cell cultures and in vivo. These properties indicate that multimodal drugs rather than "magic bullets" might serve as an ideal drug for treatment of PD and AD, for which they are being developed.

S56. INTERACTIONS OF VITAMIN A AND IODINE DEFICIENCIES: EFFECTS ON THE PITUITARY TSHB-GENE AND THE THYROID AXIS. Ralf Biebinger[1] and Michael B. Zimmermann[2]
[1]DSM Nutritional Products, Kaiseraugst, Switzerland.
[2]Human Nutrition Laboratory, ETH Zürich, Switzerland
Ralf.Biebinger@dsm.com

Vitamin A (VA) deficiency (VAD) and the iodine deficiency disorders (IDD) affect > 30% of the global population and these deficiencies often coexist in vulnerable groups. VAD has multiple effects on the pituitary- thyroid axis. Recent VA and iodine depletion studies in rats indicate moderate VAD alone has no measurable effect on the pituitary-thyroid axis; however, concurrent iodine deficiency (ID) and VAD produce more severe primary hypothyroidism than ID alone. Repletion studies in VA- and iodine-deficient animals suggest: 1) primary hypothyroidism in animals with concurrent moderate VAD and ID does not reduce the efficacy of high doses of oral VA; 2) VAD does not reduce the efficacy of dietary iodine to correct pituitary-thyroid axis dysfunction due to iodine deficiency; and 3) given alone, without iodine repletion, high-dose VA supplementation in combined VAD and ID may reduce thyroid hyperstimulation and reduce risk for goiter. Human studies indicate that VAD in severely- IDD-affected children increases TSH stimulation and thyroid size, and reduces risk for hypothyroidism. In children with VAD, the higher TSH concentrations in the face of higher circulating total thyroxine suggest central resistance to normal TSH suppression by thyroid hormone. In IDD- and VAD-affected children receiving iodized salt, concurrent VA supplementation improves iodine efficacy. A recent intervention trial in African children with VAD and IDD that gave VA and/or iodine supplements showed iodine prophylaxis is effective in controlling ID in areas of poor vitamin A status. VA supplements are effective in treating VAD in areas of mild ID and have an additional benefit— through suppression of the pituitary TSHβ gene, VAS can decrease excess TSH stimulation of the thyroid and thereby reduce the risk of goiter and its sequelae.

S57. FERRYING CU+ ACROSS MEMBRANES: MOLECULAR MECHANISM OF CU+-ATPASES. José M. Argüello, Manuel González-Guerrero, Elif Eren
Department of Chemistry and Biochemistry, Worcester Polytechnic Institute
Worcester, MA, 01609, USA
arguello@wpi.edu

Cu+-ATPases such as the Menkes and Wilson disease proteins drive metal efflux from the cell cytoplasm. Paramount to this function is the binding of Cu+ within the transmembrane region and its coupled translocation across the permeability barrier. By characterizing the Archaeoglobus fulgidus Cu+-ATPase (CopA), a model thermophilic archaeal ATPase, we established that these enzymes have two Cu+ binding sites in the transmembrane region. These can be independently loaded with Cu+; however, their simultaneous occupation is associated with enzyme turnover. Both sites bind Cu+ with high affinity (Site I Ka = 1.3 fM¬-1, Site II Ka = 2.6 fM-1) in the absence of other enzyme ligands. Site I is constituted by two Cys in transmembrane six and a Tyr in transmembrane seven. An Asn in transmembrane seven, and Met and Ser in transmembrane eight form Site II. Single site X-ray spectroscopic analysis indicates a planar trigonal coordination in both sites. This architecture is distinct of that observed in Cu+ trafficking chaperones and classical cuproproteins where the ion remains bound during the functional life of the protein. Although it is an essential micronutrient, free Cu is toxic to the cell. Consequently, Cu+ is not free (hydrated) in the cell cytoplasm but bound to specific Cu+ chaperone proteins. This generates interesting questions: How does Cu+ reach the transmembrane transport sites? How do these bind the substrate such as the backward release of free Cu+ is prevented? We have characterized Cu+ access the transport sites by studying the functional interaction between Archaeoglobus fulgidus CopA and the corresponding Cu+-chaperone, CopZ. As expected, the Cu+-loaded chaperone interacts with and delivers the metal to regulatory cytoplasmic metal-binding domains (MBDs) present in the Cu+-ATPases. Cu+-loaded MBDs, acting as metal donors, are unable to activate CopA. Conversely, Cu+-loaded chaperone activates the ATPase independently of the presence of MBDs. These data are consistent with a model where the chaperone delivers Cu+ directly to the two transmembrane transport sites present in Cu+-ATPases via protein-protein interaction, while the MBDs serve a regulatory function without participating in metal transport. The high affinity Cu+ binding to transport sites in conjunction with the observed reversible direct Cu+ transfer from chaperones, points to a transport mechanism where backward release of free Cu+ to the cytoplasm is largely prevented by tight binding independent of transported substrate “occlusion”.

O01. THE ZINC TRANSPORTER 3 (ZNT3) SORTING AND TRANSPORT ACTIVITY ARE REGULATED BY COVALENT ZNT3-OLIGOMERS.
Gloria Salazar and Victor Faundez. Department of Cell Biology, Emory University. Atlanta, USA.
gsalazar@cellbio.emory.edu

Synaptic vesicles store ionic zinc in their lumen at high concentrations. This process requires the presence of the zinc transporter 3 (ZnT3) in the synaptic vesicle membrane. Deficiencies of either ZnT3 (ZnT3-/-) or the membrane trafficking machinery interacting with ZnT3 (the adaptor complex AP-3, Ap3dmh/mh) severely hinder ionic zinc accumulation in synaptic vesicles. In an effort to identify novel ZnT3 structural elements required for its zinc transport function, we found that ZnT3 exist as monomers, dimers, and other high molecular species. Dimers were stable in reducing agents and strong denaturants suggesting di-tyrosine bond formation. Consistently, the basal levels of dimeric ZnT3 forms were increased by oxidative stress and prevented by free radical scavengers. We identified three tyrosines in the cytosolic C-terminal domain of ZnT3 responsible for ZnT3-dimer formation. Mutagenesis of these tyrosines to phenylalanine (Y>F) identified dimerization gain- and loss-of-function mutants. Mutants that abrogated dimer formation (loss-of-function) impaired ZnT3-dependent zinc transport, ZnT3 interaction with AP-3, and ZnT3 targeting to synaptic-like microvesicles. Conversely, Y>F gain-of-function mutants increased ZnT3 interaction with AP-3 and its targeting to vesicles. These results indicate that the sorting and function of a synaptic vesicle membrane protein are regulated by oxidative modification of tyrosine residues in physiological conditions.

O02. DIETARY IRON AFFECTS PROTEINS INVOLVED IN IRON METABOLISM IN WEANLING PIGS. Stephanie L Hansen and Jerry W Spears.
North Carolina State University, Raleigh, NC, USA
slhansen@unity.ncsu.edu

Twenty-four weanling male pigs averaging 21 days of age were blocked by litter and weight and randomly assigned to 1 of 3 dietary treatments: 1) no supplemental Fe (-Fe); 2) 100 mg supplemental Fe/kg DM (CON); and 3) 500 mg supplemental Fe/kg DM (+Fe). The basal diet contained 20 mg Fe/kg DM and supplemental Fe was provided as iron sulfate. All pigs received an injection of 100 mg Fe from iron dextran shortly after birth. Pigs were group fed in pens of 2 for 32 days, after which they were humanely euthanized for tissue collection. Average daily gain was greater (P<0.01) in CON pigs (328 g/day) compared to –Fe pigs (224 g/day), and did not differ between CON pigs and +Fe pigs (290 g/day). Liver Fe increased (P<0.01) in a linear fashion with increasing dietary Fe (72, 227, and 411 mg Fe/kg DM for -Fe, CON and +Fe pigs, respectively). Day 32 serum Fe concentrations were lower (P<0.01) in –Fe (0.34 mg/L) compared to CON (2.47 mg/L) and tended (P=0.10) to be greater in +Fe (2.78 mg/L) compared to CON. Hemoglobin concentrations on day 32 were lower (P<0.05) in –Fe pigs (6.2 mg/dL) compared to CON (12.8 mg/dL), but did not differ between CON and +Fe (13.3 mg/dL). Duodenal concentrations of divalent metal transporter 1 (DMT1), the protein responsible for Fe and Mn import into enterocytes, tended (P=0.12) to be lower in +Fe pigs compared to –Fe pigs. Intestinal levels of the Fe export protein ferroportin also tended (P=0.09) to be lower in +Fe pigs compared to –Fe pigs. Because DMT1 is important in the absorption of Mn, liver Mn concentrations were determined. Liver Mn concentrations were lower (P<0.05) in +Fe pigs (8.1 mg/kg DM) than in CON (12.7 mg/kg DM) or –Fe pigs (11.7 mg/kg DM). This suggests that absorption of Mn may have been negatively affected by feeding a diet high in Fe, likely because of reduced amounts of intestinal DMT1. Excessive dietary Fe has also been shown to negatively impact Cu absorption. To determine if dietary Fe level affected cellular Cu status, CCS, the Cu chaperone protein for superoxide dismutase, was measured. Duodenal CCS concentrations were increased (P<0.01) in +Fe compared to –Fe and did not differ between CON and +Fe pigs. Increased CCS concentrations may reflect an increased need for antioxidant protection from oxidative damage caused by Fe. Alternately, it has been demonstrated that CCS protein is generally greater in situations of Cu deficiency; therefore supplementation of Fe in the present study may have negatively affected intestinal Cu status. Interestingly, liver Cu was greater (P<0.05) in –Fe (35.8 mg/kg DM) and +Fe (37.1 mg/kg DM) compared to CON pigs (26.7 mg/kg DM). In conclusion, supplementation of 500 mg Fe/kg DM to weanling pigs resulted in decreased amounts of proteins involved in intestinal Fe acquisition, which may have resulted in reduced absorption of dietary Mn.

O03. EFFECT OF ZINC ON HEPATIC COPPER AND IRON CONCENTRATIONS IN EXPERIMENTALLY INDUCED COPPER TOXICITY IN RATS. Carmen Fuentealba[1], Susan Haywood[2], Kent G. Hecker[1] and Jim Trafford[2]
[1]University of Calgary, Calgary, AB, Canada
[2]University of Liverpool, Liverpool, England.
cfuentea@ucalgary.ca

Copper (Cu)-associated diseases are increasingly reported in humans and animals. Zinc (Zn) supplementation is used in the management of Wilson's disease and other chronic liver diseases in humans. Zn supplementation diminishes the severity of spontaneous Cu-associated hepatitis in the LEC rat, an animal model of Wilson's disease, and similar beneficial effects are reported in the treatment of some Cu-associated diseases in dogs. However, the exact mechanism(s) of protection by Zn supplementation are unclear. Copper toxicity due to accidental exposure to excess dietary Cu is often diagnosed in farm animals. The pathogenesis and pattern of liver injury in Cu toxicity of dietary origin is different to that observed in spontaneous Cu-associated diseases. The objective of this study was to study the effect of Zn on the prevention of experimentally induced Cu toxicosis in Wistar rats fed excess copper (Cu-loaded). Methods: One group of rats was fed a custom research diet containing excess Cu (1,500 mg/kg), according to established protocols to induce Cu toxicity at about 4 weeks. A second group was fed a diet with excess Cu (1,500 mg/kg) and received Zn as ZnCO3, given by subcutaneous depot, at a dose rate 110 mg/Kg. Rats fed a normal rodent diet and given subcutaneous depot with and without Zn were included as controls. Rats (4 per group) were euthanized after 4 and 6 weeks and their livers collected for morphological examination and determination of hepatic Cu, Zn and Fe concentration. Analysis of Cu and Zn in the feces was also performed. Results: Significant differences in hepatic copper concentration were detected between Cu-loaded (2,860±950 µg/g dry weight) and control rats (20±9 µg/g). In Cu-loaded rats, hepatic Cu concentration rose from 1187±642 µg/g (dry weight) after 4 weeks to 3,959±563 µg/g after 6 weeks. Zn injection did not have a statistically significant effect on hepatic Cu concentration in Cu-loaded rats (2,509±205 µg/g after 4 weeks and 3,187±917 µg/g after 6 weeks). Morphologic changes were observed in the livers of Cu-loaded rats. Strong hepatocellular Cu accumulation was detected with Rhodanine stain in all Cu-loaded rats with and without Zn injection. Fe staining was observed adjacent to areas of hepatocellular injury in Cu-loaded rats with and without Zn injection. Significant differences in hepatic Zn were not detected (109±33 µg/g in Cu-loaded and 131±37 µg/g in control rats). Similarly hepatic Fe concentrations were comparable (360±60 µg/g in Cu-loaded and 416±116 µg/g in controls). Conclusions: These results indicate that Zn injection does not reduce hepatic Cu concentration or the severity of hepatocellular injury in rats fed excess Cu. The ability of Zn to confer protection in Cu-induced hepatotoxicity may be dependant on the intracellular target of Cu-induced damage, and the route of Zn administration. Since there are numerous possible mechanisms of Cu toxicity at the intracellular and molecular level, unique therapeutic approaches need to be developed taking into consideration the uniqueness of the specie affected.
Supported by a grant from the Wellcome Trust.

O04. LIVE IMAGING OF METAL-ION TRANSPORT IN OOCYTES EXPRESSING THE HUMAN DIVALENT METAL-ION TRANSPORTER DMT1: SUBSTRATE PROFILE AND SELECTIVITY OF DMT1. Anthony C Illing, Ali Shawki, Christopher L Cunningham, and Bryan Mackenzie
University of Cincinnati College of Medicine, Cincinnati, OH, USA
bryan.mackenzie@uc.edu

DMT1 (SLC11A2) is essential for intestinal iron absorption and erythroid iron utilization. Iron deficiency is a serious risk factor for cadmium intoxication, especially in children, suggesting that cadmium and iron share a common absorptive pathway. Whereas DMT1 exhibits reactivity (based on evoked currents) with a broad range of transition metal ions including Cd2+, questions have arisen as to which of these are actually transported. We used a fluorescence-based approach for live imaging of metal-ion transport in Xenopus oocytes and provide here a comprehensive substrate-profile analysis for human DMT1. We first established the reactivity of the fluorophore PhenGreen SK (PGSK) with a range of metal ions in a cell-free system. We injected control oocytes and oocytes expressing DMT1 with PGSK and used the confocal laser-scanning microscope (excitation at 514nm and detection in the band 530-600 nm) to monitor fluorescence changes during 10-min superfusion with metal ions. We also measured radiotracer metal-ion uptake and metal-ion-evoked currents using the two-microelectrode voltage clamp. We took the first-order rate constant of quenching as an index of metal-ion uptake and validated our fluorescence approach by comparing the findings with radiotracer data. The half-maximal concentration (K0.5) for $Fe2^{+}$ (4.2±2.2 µM, SEE) determined from PGSK quenching at pH 5.5 matched that determined from $55Fe^{2+}$ uptake (5.9±0.9 µM), and Fe^{2+}-induced PGSK-quenching rates were pH-dependent in the same manner as $55Fe^{2+}$ uptake. Neither Cr^{2+} nor Cr^{3+} quenched PGSK fluorescence, consistent with ^{51}Cr data revealing that Cr(II) and (III) are excluded by DMT1. DMT1 also excluded Cu^{1+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Sn^{2+}, Sr^{2+} and VO^{+} (or V^{2+}). Several metal ions resulted in more rapid quenching in DMT1 oocytes than in control oocytes. We determined for these the order of substrate selectivity (using the ratio Imax/K0.5) from evoked currents (I): Fe^{2+}, Cd^{2+}>Co^{2+}, Mn^{2+}>Ni^{2+}, VO^{2+} (or V^{3}+), Zn^{2+} (where “>” ≈ 0.5 log10 units). $^{55}Fe^{2+}$ transport was inhibited by Cd^{2+}, Mn^{2+} and Co^{2+} in a competitive manner. Notably, whereas Zn^{2+} evoked large currents, $^{65}Zn^{2+}$ was very poorly transported and Zn^{2+} only weakly inhibited $^{55}Fe^{2+}$ transport (Ki ≈ 50 µM). Our data reveal that DMT1 is a ferrous iron-preferring transporter that efficiently transports Cd^{2+} but our data do not support DMT1-mediated transport of Cu either in its 1+ or 2+ state. We speculate that DMT1 will serve as a route of entry for the toxic heavy metal Cd, especially in iron deficiency, and contribute to the absorption of Co and Mn (and trace metals Ni and V) but DMT1 is unlikely to be physiologically relevant to the absorption of Zn or Cu. We expect that our PGSK fluorescence-based metal-ion transport assay will have considerable utility in the live imaging of metal-ion transport in intact tissues.

O05. DUAL-LOCALIZATION OF ZNT2 TO MITOCHONDRIA AND EXOCYTOTIC VESICLES REDISTRIBUTES ZINC POOLS IN MAMMARY CELLS. Young Ah Seo, Veronica Lopez and Shannon L Kelleher
Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA
slk39@psu.edu

Expression of the zinc transporter ZnT2 is restricted to several highly specialized tissues including mammary and prostate glands. These tissues have unique zinc requirements due to the extraordinary amount of zinc secreted into milk and seminal fluid. Several studies have determined that ZnT2 facilitates zinc sequestration into an intracellular compartment. In mammary gland, we have determined that ZnT2 plays a major role in zinc secretion. Thus, our aim is to explore the role and regulation of ZnT2 in the mammary gland to provide important insight as to how zinc metabolism is regulated in highly specialized secretory tissues. To do so, we utilized cultured mammary cells (HC11) which can be hormonally manipulated to a pseudo-secretory phenotype. We first examined the localization and function of endogenous ZnT2 in HC11 cells and confirmed our observations in HC11 cells generated to express a ZnT2-HA fusion protein. Secondly, we explored the physiological implications of ZnT2 localization and function in non-secreting and secreting cells. Abundant endogenous expression of ZnT2 was detected in HC11 cells. Transfection of cells with ZnT2 siRNA significantly reduced zinc export (~40%) in cells pre-loaded with 65Zn. To define mechanisms through which ZnT2 mediates zinc efflux in secreting mammary cells, confocal imaging determined that ZnT2 was localized to vesicles primarily associated with the late endosomal/secretory compartment. FluoZin-3 (a labile zinc fluorophore) was used to quantify changes in vesicular zinc pools. Our data indicated that fluorescence was 2-fold higher in cells over-expressing ZnT2 clearly implicating ZnT2 in zinc vesicularization. Importantly, confocal imaging of cells expressing a ZnT2-HA fusion protein specifically localized ZnT2 to exocytotic vesicles and zinc secretion was significantly higher (~16%) in ZnT2-overexpressing cells. Thus our data clearly indicate that ZnT2 facilitates both zinc vesicularization followed by secretion in secreting mammary cells. Intriguingly, in non-secreting cells, confocal imaging indicated that endogenous ZnT2 was primarily co-localized with the mitochondrial protein COX IV. We verified mitochondrial localization by detecting ZnT2 in proteins isolated from purified, protease-digested mitochondria by immunoblotting. Importantly, ZnT2 gene attenuation significantly reduced mitochondrial ZnT2 abundance, 65Zn uptake (~20%) and mitochondrial zinc pools (~15%) as quantified by the mitochondria-specific zinc fluorophore RhodZin-3. Importantly, over-expression of ZnT2 increased RhodZin-3 fluorescence (~20%) further indicating that ZnT2 modulates mitochondrial zinc pools. To our knowledge, these studies identify the first zinc transporter associated specifically with mitochondria. Functional studies suggest that mitochondrial and vesicular zinc pools are reciprocally regulated and reflect cellular needs such that mitochondrial ZnT2 abundance, zinc uptake and zinc pools are higher in non-secreting cells while vesicular ZnT2 abundance, zinc pools and zinc secretion are higher in secreting cells. In summary, our data suggest that ZnT2 is a dual-localized protein which resides in both mitochondria and intracellular vesicles and modulates sub-cellular zinc pools dependent upon cellular requirements. Studies are underway to identify the factors which regulate the sub-cellular targeting of ZnT2 and determine metabolic consequences of modulating mitochondrial zinc pools.

O06. IS COPPER CHAPERONE FOR CU/ZN SUPEROXIDE DISMUTASE A POTENTIAL BIOMARKER OF MILD COPPER SUPPLEMENTATION?.

Miriam Suazo[1,4], Talía del Pozo[1], Marco Méndez[2], Mauricio González[1] and Magdalena Araya[3]

[1]Laboratorio de Bioinformática y Expresión Génica, INTA-Universidad de Chile.
[2]Laboratorio de Microminerales, INTA-Universidad de Chile.
[3]Laboratorio de Genómica Evolutiva, INTA-Universidad de Chile.
[4]Departamento de Nutrición, Facultad de Medicina, Universidad de Chile.
msuazo@med.uchile.cl

Copper has a physiological duality, on the one hand is an essential micronutrient, and other is potentially toxic. The effects of the copper deficiency and excess have been identified in genetic diseases of Menkes and Wilson respectively. In contrast, there are no indicators to detect early effects of marginal deficiency or moderate copper excess in normal population, because the traditional clinical indicators are not sensitive to mild changes in the metal status. In a recent study, we found that Cu-Zn superoxide dismutase (SOD1) and its chaperone (CCS) mRNA were reduced in peripheral mononuclear cells (PMNCs) of individuals exposed to 10 mg/day Cu/day (as copper sulfate) given as a single dose for 2 months. The aim of the present study was to further explore the changes in the expression of these transcripts to assess them as potential sensitive indicators of early adaptation to copper excess. We studied the gene expression changes associated with the copper metabolism in PMNCs obtained from healthy men (n=30) supplemented with 8 mg Cu/d (as copper gluconate) or with placebo (n=30), for 6 months. PMNCs gene expression of encoding proteins associated with copper transport (divalent metal transporter or DMT1), storage (metallothionein or MT2A) and the intracellular distribution CCS and SOD1were measured through real-time RT-PCR; also, copper, iron and zinc content in PMNCs and serum copper were determined, before and after copper supplementation. Results indicate that copper in serum did not change, suggesting that in normal individuals intestinal and/or hepatic regulation maintain metal homeostasis, at moderately high copper exposure. PMNCs copper content and the relative abundance of SOD1 and MT2A transcripts significantly changed both in supplemented and placebo individuals, suggesting that they were not specifically associated with supplementation. Only CCS, the copper chaperone for SOD1, showed a significant and specific change after copper supplementation, while there were no significant changes in serum levels of copper, iron and zinc. In order to explore the temporal pattern of CCS expression in response to copper, ongoing studies are assessing THP-1 and Jurkat human mononuclear cell lines exposed to physiological concentrations of copper (2 - 20 µM). Results of these later studies plus others of CCS responses in animals and Cu deficient cell lines will clarify whether CCS expression would be a biomarker of early changes in copper status.

O07. LONG TERM EFFECTS OF CADMIUM ON FOREARM BONE DENSITY IN A CHINESE POPULATION.
Xiao Chen[1], Guoying Zhu[1], Taiyi Jin[2,4], Agneta Åkesson[3] , Ingvar A. Bergdahl[4] , Lijian Lei[2], Shifang Weng[1] and Yihuai Liang[2,4]
[1]Department of Bone Metabolism, Institute of Radiation Medicine, Fudan University, Shanghai, China
[2] Department of Occupation Medicine, School of Public Health, Fudan University, Shanghai, China
[3] Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden
[4] Department of Occupational and Environmental Medicine, Umeå University, Umeå, Sweden
chxwin@yahoo.com.cn

The main focus of this study was to evaluate long term effects of cadmium on forearm bone mineral density after stopping ingestion of cadmium-polluted rice for ten years. A total of 532 persons (338 females and 194 males) participated. The residents in low exposed area ingested the rice containing about 0.072mg cadmium/kg, while those living in the highly exposure areas stopped ingesting of cadmium-polluted rice in 1996 (ten years). The participants completed a questionnaire and the bone mineral density was measured by DXA at the proximal radius and ulna. Samples of urine and blood were collected for determination of urinary (UCd) and blood (BCd).The BMD in heavily and polluted area was significantly lower than that in control area both in male and female, $p<0.01$. But in moderately polluted area, only the females' BMD was greatly lower than that in the control area, $p<0.05$. BMD declined with increasing BCd and UCd both in male and female, especially in high level group, $p<0.01$. Also, there were significant differences in the prevalence of osteoporosis among the different areas ($\chi^2=13.0$, $p=0.0003$) and different urinary cadmium group ($\chi^2=4.5$, $p<0.04$) in females, but not in males ($\chi^2=0.962$, N.S. and $\chi^2=1.906$, N.S.). However, differences were significant among by BCd groups ($\chi^2=9.3$, $p<0.0025$, in female and $\chi^2=4.6$, $p=0.031$, in male). This study demonstrates that cadmium may play a long term role on bone health; more attention should pay on cadmium effects even when exposure is no longer present.

O08. GLUTATHIONE MODULATION INFLUENCES METHYL MERCURY INDUCED TOXICITY IN ALBINO RATS. Varsha Singh[1], Deepmala Joshi[1], Sadhana Shrivastava[1], Sangeeta Shukla[1] and Mohammed Abdullah[2]
[1]Reproductive Biology and Toxicology Laboratory, School of Studies in Zoology, Jiwaji University, Gwalior, India
[2]Trace Element- Institute for UNESCO, Lyon, France.
singh_varsha80@rediffmail.com

The overall global increase of mercury in various forms in the environment has generated a serious toxicological problem and thus there is a need for effective therapy for human beings. Methyl mercury interferes with a number of body functions such as the liver, kidneys and brain. Chronic exposure of mercurial compounds could be pathogenetically relevant as co-factor in several neurodegenerative diseases worldwide. In this respect, chelation therapy is the most commonly used and seen as the least invasive. Chelating agents compete with the in vivo binding site for metal ion through the process of ligand exchange. In the present studies, we have evaluated the efficacy of thiol containing chelating agents in reducing CH_3Hg^+ from liver, brain and blood. Thus, the aim of the present study was to develop a companion formula of a chelator along with an antioxidant as an intervention strategy that may be a practical solution to mercury intoxication. Induction of CYP 2E1 by mercury (Hg) is one of the central pathway by which mercury generates oxidative stress. Experimental injury was induced in rats by methyl mercury (5 days/week, p.o., for 12 weeks) to determine toxicological action on CYP 2E1 enzymes. In this report GSH at a dose of (0.3 mM/kg, i.p.) supplemented with magnesium (10 mg/kg, p.o.) was used for 12 weeks (2 days/week) to validate its protective potential. Male albino rats were exposed to mercury for 12 weeks. This was associated with elevated malondialdehyde, transaminases, lactate dehydrogenases and γ-glutamyl-transpeptidase. Hepatic microsomal drug metabolizing enzymes of CYP 2E1 showed sharp depletion as assessed by estimating aniline hydroxylase and amidopyrine –N-emethylase activity after mercury exposure. The enzymatic activities of GSH cycle (GPx, GR), G-6-Pase, G-6PDH, SDH, ATPase and acetyl cholinesterase (brain) were also altered due to oxidative damage. Chelating agents supplemented with magnesium showed significant improvement in the activity of AH and AND. Histopathological evaluation of the liver revealed that the combination used restored the liver lesions including hepatocyte swelling and lymphocytic infiltrations. Scattered Purkinje neurons, large perivascular spaces and cytoplasmic vacuolation in granular cell layer of brain were induced by Hg. These results lead us to speculate that GSH + Mg may play an hepato and neuro protective role. CYP 2E1 activity and reduced oxidative stress were improved as confirmed by recovery of endoplasmic reticulum, mitochondrial assembly in liver and recouped dendrites, myelinsheath, vesicles and significant loss of vacuolation in brain.

O09. EFFECTS OF COMPLEX I INHIBITION ON MITOCHONDRIAL IRON HOMEOSTASIS, IN AN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE. Natalia Mena[1], Julio Salazar[2], Enrique Armijo[1], H. Stephen[2], Etienne Hirsch[2] and Marco Tulio Núñez[1].
[1]Biology Department, Faculty of Science, University of Chile, Santiago, Chile.
[2]INSERM, UMR679, Neurology and experimental Therapeutics. Paris, France.
npaz81@hotmail.com

Parkinson's disease (PD) is characterized by dopaminergic neurons degeneration at the substance nigra pars compact (SNPC) and the presence of Lewy bodies in the remaining neurons (Rij k et al., 1997, 2000). Although the etiology of PD is unknown, studies in animal models and postmortem tissue, suggest the existence of four events involved in neurodegeneration: (1) Mitochondrial Dysfunction, (2) Increased iron contents (thus increased oxidative stress and reduced glutathione), (3) Alteration of the ubiquitin proteasome system, (4) Inflammation. Mitochondrial dysfunction in PD is supported multiples evidences, particularly: specific inhibition of mitochondrial complex I in monkeys and mice in response to 1-methyl-4-phenyl-1, 2,3,6 - tetrahidropiridina (MPTP), an experimental model of PD (He et al., 2003), and studies mitochondrial enzymes in postmortem tissue from PD patients (Schapira and col., 1990). The increase in cell iron content is another of the events characteristic of PD. This increase has been determined in postmortem tissue of patients with PD (Sofic et al., 1988; Dexter et al., 1989; Hirsch et al., 1991) and confirmed by ultrasound and Nuclear Magnetic Resonance studies (Gorell et al., 1995; Berg et al., 1999). Therefore, both mitochondrial dysfunction and iron metabolism alterations are characteristic events of PD. We studied the relation between mitochondrial dysfunction and the iron metabolism using an in vitro experimental model of PD, thus, determined the effects of complex I inhibition on the expression of proteins involved in mitochondrial iron metabolism: Mitoferritin, an iron transporter, Frataxin; a putative chaperone protein; and mitochondrial Ferritin, responsible of iron storage. Our results indicate that complex I inhibition induces an increase in Mitoferrin and Ferritin expression, while Frataxin presents a biphasic behavior. These results are obtained by Western Blot and RT-PCR in the human neuroblastoma cell line SHSY-5Y, and by immunocytological detection of these proteins in primary cultures of rat mesencephalic dopaminergic neurons. Additionally, Iron level distribution was determined by fluorescent sensors: Calcein and RPA and while mitochondrial and cytosolic iron content was determined by radioactive iron uptake. The results indicate a possible redistribution of cytosolic iron (from various sources) to mitochondria in a time and complex I inhibition level dependent manner. These results have been confirmed in an animal model through the intoxication with MPTP, and in postmortem tissue of patients with PD. Finally, show the effects Mitoferrin knock down and its potentially protective effects.

O10. COMPETITION BETWEEN OLIGOMERIC SILICIC ACID AND TRANSFERRIN FOR ALUMINIUM BINDING AND IMPLICATIONS FOR ALUMINIUM TOXICITY. Sylvaine FA Bruggraber, Ravin Jugdaohsingh, William Cook and Jonathan J. Powell
MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, UK
Sylvaine.bruggraber@mrc-hnr.cam.ac.uk

Studies have shown that silica (as aquated silicic acid) reduces aluminium (Al) availability and ameliorates toxicity in biological systems. It was suggested for a long time that soluble monomeric silicic acid (Si(OH)4) was responsible for this effect by reducing Al absorption. But monomeric silicic acid only has low affinity for Al (log K_{eff} of 4.70±0.05 at pH 7.2; Farmer and Lumsdon 1994). Work by Taylor *et al* (1997) showed that a small polymer of silicic acid termed "oligomeric silica" has a much higher affinity for Al (minimum log K_{eff} of 11.7 at pH 7.2). Oligomeric silica, under physiological conditions, forms a stable complex with Al *ex vivo* that resists degradation in the gastrointestinal tract and therefore significantly reduces Al absorption (Jugdaohsingh *et al* 2000). However a systemic *in vivo* role for silicic acid in the amelioration of Al toxicity has not been ruled out. Indeed a recent study in freshwater snails demonstrated evidence of intracellular silica-aluminium interactions (White *et al* 2008). In the blood Al binds mostly to transferrin, suggesting that silicic acid would have to compete with transferrin to exert its "Al protective effect", at least in the circulation. The binding affinity of apo-transferrin for Al has been determined in a number of studies and has log K_{eff} of 11.7 and 12.2 at pH 7.4 (Fatemi *et al* 1991) which may be comparable to Al-oligomeric silica affinity. Here we studied the competition between silicic acid (monomeric and oligomeric) and apo-transferrin for Al binding under simulated physiological conditions. Titration of the $(Al)_2$-transferrin complex with an increasing concentration of oligomeric silica showed that oligomeric silica competes effectively with apo-transferrin (1.28 mg/mL) for Al (40 µM), and at 2 mM total silicon (Si) it completely displaces Al from the Al-transferrin complex (50% at 0.75mM and 25% at 0.35mM). As expected, weak affinity between monomeric silica and Al was observed at Si concentrations below 2 mM although this increased at higher concentrations of Si (>2mM) and was related to the onset of silica polymerisation and, thus, some formation of the high-Al-affinity form of silicic acid.
In conclusion, "oligomeric silica" competes effectively with human apo-transferrin for Al binding and it may have an in vivo role in binding and sequestering the toxic Al ion. Work characterizing "oligomeric silica" is in progress.
Farmer and Lumsdon (1994), *Geochimica et Cosmochimica Acta*, 58(16), 3331-34; Taylor *et al* (1997), *J. Am. Chem. Soc.*, 119 (38), 8852-56; Jugdaohsingh *et al* (2000), *Am. J. Clin. Nutr.*, 71(4), 944-9; White *et al* (2008), *Environ. Sci. Technol.*, 42(6):2189-94; Fatemi *et al* (1991), *Biochem. J.*, 280:527

O11. COMPARISON OF PRENATAL BIOMARKERS OF LOW-LEVEL METHYL MERCURY EXPOSURE. Janja Tratnik, Irena Rupnik and Milena Horvat
Jožef Stefan' Institute, Ljubljana, Slovenia
Janja.Tratnik@ijs.si

The objective of the study was to examine and compare different biomarkers of low-level prenatal exposure to methyl mercury (MeHg), and in particular to estimate whether the difference between MeHg levels in different types of cord blood is significant. Umbilical cord blood, cord tissue, meconium, and mother's hair were collected from 11 mother-child pairs following the parturitions in Medical Centre Ljubljana, Slovenia. Arterial and venous blood were collected separately and as a mixture of both types (total cord blood), and each blood type was subsequently divided into three aliquots – whole cord blood (whole blood), plasma, and red blood cells (RBC). MeHg was determined using isolation of MeHg by evaporation onto cystein-impregnated paper in micro-diffusion cells, extraction into toluene and detection by gas chromatography. Total mercury (THg) was determined by thermal decomposition, reduction, amalgamation and atomic absorbance spectrometry using Direct Mercury Analyser (DMA-80). Concentration of MeHg in whole blood ranged from 0.07 to 6.45 ng / g, and concentration of THg from 0.49 to 8.11 ng / g blood. MeHg was found to be correlated positively and significantly to THg in WB and RBC of all blood types, while not significantly in plasma. Since MeHg to THg ratio in whole cord blood was close to 1, THg alone could be determined to assess MeHg exposure, thus avoiding complicated sample preparation prior to MeHg analysis. Although a difference was observed between arterial and venous cord blood MeHg, probably resulting from retention of MeHg in fetus, MeHg as well as THg values did not differ significantly between arterial and total cord blood, neither in whole blood, plasma nor RBC fraction. Therefore, a mixture of both types could be collected, when the quantity of arterial blood alone is insufficient and thus make sampling easier. Furthermore, cord blood MeHg as well as THg levels were comparable to MeHg / THg levels in cord tissue, suggesting cord tissue as a potential biomarker of prenatal MeHg exposure. In contrast, MeHg levels observed in meconium were significantly lower than MeHg levels in cord blood and cord tissue; besides, the percent of MeHg in meconium was less than 0.6 %. Although the role of biliary excretion and demethylation by microflora in suckling human infants is still unknown, very low percent of MeHg in meconium indicated a potential demethylation of MeHg in fetal intestine and excretion of the inorganic form in the feces. As already demonstrated by different epidemiologic studies, cord blood showed a good relationship with maternal hair also in the present study, regardless of the type of the blood type. To conclude, all examined biomarkers of prenatal MeHg exposure were highly and positively correlated, except meconium, which is suggested not to be used as an indicator of MeHg exposure. The observation that either arterial or a mixture of arterial and venous cord blood or even cord tissue could be collected to assess prenatal MeHg exposure can make further sampling procedures in epidemiologic studies more feasible.

O12. RAPID SCREENING OF TOXIC ELEMENTS VIA X-RAY FLUORESCENCE SPECTROMETRY. Richard Jacobs, Janet McDonald and Peter Palmer.
U.S. Food and Drug Administration; Department of Chemistry and Biochemistry, San Francisco State University, San Francisco, CA. USA.
janet.mcdonald@fda.hhs.gov

Energy-dispersive X-ray fluorescence spectrometry (XRF) has been routinely used for alloy testing, determination of Pb in paint, and determination of Cd in plastic. Its use to screen for toxic elements in food and medicinal products has been surprisingly limited to date. While XRF is less sensitive than atomic spectrometry methods such as ICP-AES and ICP-MS, it offers a number of significant advantages including minimal sample preparation, rapid analysis times, multi-element detection, and true field use using hand-held analyzers. The focus of this presentation is to review various analytical figures of merit associated with several different hand-held field portable XRF analyzers and describe the use of this technology in various FDA activities focusing on protecting consumers from contaminated foods and drugs. XRF will be shown to provide positive detection of a wide variety of elements from Al through U, detection limits in the 1-10 ppm range for many elements, and analysis times as short as a minute or less. Collectively, these capabilities make XRF a powerful tool for screening of toxic elements and rapidly responding to emergency situations that require identification and quantitation of toxic elements. Several case studies will be described to illustrate the utility of XRF in various FDA activities including the determination of toxic elements in foods, dietary supplements, Asian patent medicines, ceramicware, and other types of tableware.

O13. EFFECT OF SELENIUM SUPPLEMENTATION ON SOMATIC CELL COUNTS IN GRAZING DAIRY CATTLE. Alejandro Ceballos[1], Juan Kruze[2], Daniel Uribe[2], Javier Sanchez[3], Ian Dohoo[1], Herman Barkema[4], Jeff Wichtel[1], Javier Neumann[5] and Fernando Wittwer.
[1]Dept. of Health Management, UPEI. Charlottetown, PE, Canada
[2]Inst. Microbiology, Universidad Austral de Chile, Valdivia, Chile
[3]Dept. of Production Animal Health, University of Calgary.Calgary, AB, Canada
[4]CFIA, Charlottetown, PE, Canada;
[5]Inst. Vet. Clin. Sciences, Universidad Austral de Chile. Valdivia, Chile.
aceballos@upei.ca

Introduction. Selenium (Se) deficiency has been linked to several economically important diseases in dairy cattle, such as retained placenta, altered immune responses and reduced disease resistance leading to mastitis. Supplementing dairy cattle with specific micronutrients (e.g. Se) and its effect on udder health has already been described. However, it is unknown how organic oral supplements compare to long-acting inorganic supplements regarding to somatic cell counts (SCC) in milk around calving and in lactating cows. Accordingly, the objective of the study was to evaluate the effect of two sources of Se on somatic cell count around calving in primiparous cows (Trial 1), and across lactation in multiparous cows (Trial 2). **Material and methods.** Two trials were conducted in commercial dairy farms. Primiparous cows (Trial 1, n=140), and multiparous cows (Trial 2, n=48) were fed a suboptimal Se diet. In Trial 1, one group of cows (n=45) was orally supplemented with 3 mg of Se/animal/day using an organic form of Se (Sel-Plex®, Alltech, Nicholasville, KY, USA) one month before calving; a second group (n=46) received a single s.c. injection (1 mg Se/kg BW) of a long-acting Se form (Deposel®, Young's Animal Health, Auckland, New Zealand) 30 days before calving; and the third group (n=49) remained unsupplemented. In Trial 2, 23 cows received a similar dose of the same long-acting Se source 45 days before calving, the rest of the cows (n=25) were unsupplemented. Blood samples were collected from all animals before supplementation, 2 and 4 weeks after calving in primiparous cows, and at 30, 90, 180 and 270 days after injection in Trial 2. The blood activity of glutathione peroxidase (GPx; EC 1.11.1.9) was used as an indicator of Se status of the animals. To evaluate SCCs, in Trial 1 milk samples from individual quarters were collected on a weekly basis until 28 days in milk (DIM), and milk composite samples were monthly collected for the entire lactation from cows of Trial 2. Data were analyzed using a linear mixed model in Stata release 10.0 (Stata Corp., College Station, TX, USA). **Results.** In both trials the blood activity of GPx was higher in cows treated with barium selenate ($P<0.05$), while no differences were found between Se-yeast supplemented and unsupplemented cows ($P>0.05$). The mean of SCC in cows of Trial 1 decreased in all three groups after parturition ($P<0.05$) reaching the lowest level 28 days after calving. Treated cows showed lower SCCs than did unsupplemented cows ($P<0.05$) but there were no differences between treated groups ($P>0.05$). In Trial 2, SCC were lower in Se-supplemented cows ($P<0.05$) but there was a negative interaction with milk yield ($P=0.06$), which suggests a reduction of SCC in those cows with higher milk production. **Conclusion.** Selenium supplementation resulted in low SCCs in milk of primiparous cows than did in unsupplemented ones around calving. In multiparous cows the SCC was lower across lactation depending upon the milk yield of the cow.

O14. PERSISTENCE OF BLOOD CHANGES ASSOCIATED WITH ALTERATION OF THE DIETARY ELECTROLYTE BALANCE FOLLOWING FEED WITHDRAWAL, TRANSPORTATION AND LAIRAGE IN MARKET WEIGHT SWINE. Lily N. Edwards, Terry E. Engle, Temple Grandina and David B. Anderson.
Colorado State University, Fort Collins, CO, USA
Lily.Murray@colostate.edu

While the number of in-transit losses (i.e. dead and non-ambulatory animals) in the swine industry has decreased in the past several years, there is still potential to reduce the number of animals lost. Previous work has shown that fatigued pigs (i.e. non-ambulatory, non-injured animals) at commercial pork packing plants were characterized with metabolic acidosis. By providing pigs with a cation-rich electrolyte diet prior to transportation, it is hypothesized that pigs will have the ability to more successfully buffer the metabolic acidosis associated with periods of high stress and subsequently prevent the onset of the fatigued pig syndrome and in-transit losses. Animal handling studies in a laboratory environment showed that increasing dietary electrolyte balance (dEB) reduced the incidence of fatigued pigs by fifty percent. Due to the variation in feed withdrawal protocols, transportation times and lairage lengths used in the swine industry during the marketing process, the current trial was designed to test the persistence of dEB treatment on blood pH, bicarbonate and base excess. In addition, the current trial was designed to determine if there were any negative effects of dEB treatment on growth performance, meat quality or carcass yield and to determine if the treatment reduced gastric ulcers. Sixteen pens of eight cross-bred barrows were assigned to either a Low (121 meq/kg) or High (375 meq/kg) dEB diet. Diets were formulated to meet or exceed NRC (1996) requirements for energy, protein, vitamins, and minerals. Treatments were balanced by location in the barn and previous diet regimen. Animals were provided with ad libitum feed and water for three days prior to slaughter at which time feed was withdrawn. Animals were fasted in the barn for approximately 10 h after which they were shipped to the packing plant, rested for 8 h and slaughtered. Initial and final weights of the animals were obtained. Blood was sampled at baseline (five days before administration of diets), at the time of feed withdrawal, 3 d after treatment (0 h), 10 h post-feed withdrawal (10 h) and at exsanguination (20 h). Administration of the High dEB diet for 3 d resulted in an increase in blood TCO2 (P=0.001), bicarbonate (P=0.001) and base excess (P=0.0003) and a decrease in blood chloride (P=0.0002) and anion gap (P=0.01). These differences, however, were not maintained for any of the parameters after the 10 h feed withdrawal (P>0.22). Increasing dEB had no adverse effects on growth performance, meat quality or carcass yield and did not decrease pars esophageal ulcer scores. In conclusion, this study demonstrated that the effect of dEB on blood parameters was not maintained following a 10 h feed withdrawal. It is therefore likely that the animal's ability to withstand any increased metabolic acid load associated with the stress of transport was lost following feed withdrawal. Further research is needed to determine the effects of dEB alteration in animals that have not been fasted prior to shipment and using diets with a larger difference in dEB.

O15. EFFECTS OF COPPER ON RUMINAL FERMENTATION AND BIOHYDROGENATION OF UNSATURATED FATTY ACIDS IN VITRO. Jennifer S. Schutz and Terry E. Engle.
Department of Animal Sciences, Colorado State University, Fort Collins, CO, USA
jschutz@lamar.colostate.edu

The extensive biohydrogenation of unsaturated fatty acids by rumen microorganisms results in primarily saturated fatty acids being absorbed from the small intestine and incorporated into adipose tissue regardless of diet composition (Dawson and Kemp, 1970). Limited research suggests that dietary Cu at physiological concentrations may affect lipid metabolism in ruminants. Adding 20 or 40 ppm Cu to growing and finishing diets increased polyunsaturated fatty acid proportions of total fat in longissimus muscle (Engle and Spears, 2000). The C18:1 trans isomer, an intermediate of biohydrogenation (Kepler et al., 1966; Christie, 1981), was reduced in longissimus muscle of Cu-supplemented steers, suggesting an effect of Cu on biohydrogenation. Therefore, the objective of the experiment was to investigate the effects of Cu on microbial biohydrogenation of soybean oil using in vitro fermentation techniques. Heifers fitted with ruminal fistulas were fed a high concentrate diet for 14 d. On d 15 ruminal samples were obtained 2 h post feeding. Immediately after collection, pH was recorded and ruminal fluid was combined. Treatments were assigned to digestion tubes utilizing a 2x3 factorial arrangement with factors being; 0 or 3.5% soybean oil and 0, 10, or 20 mg of Cu/kg dry matter (DM). Each treatment was run in sextuplet. Gas samples were obtained at 0, 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h post inoculation and immediately analyzed for methane and CO2. In vitro DM disappearance, CO2 production, volatile fatty acid production, and pH were unaffected by treatment. Methane production was decreased ($P<0.05$) by soybean oil supplementation. The addition of Cu tended ($P<0.11$) to increase methane production in soybean oil supplemented tubes which suggests that Cu may alter biohydrogenation of unsaturated fatty acids. As expected, the addition of soybean oil to digestion tubes increased C16:0, C18:0, C18:1, C18:2 and C18:3 fatty acids at 48 h post incubation. However, Cu supplementation tended ($P<0.15$) to decrease C18:0 and increase ($P<0.14$) C18:1 and C18:3 fatty acids which indicate that Cu may interfere with biohydrogenation of unsaturated fatty acids. However, the absence of a Cu x soybean oil interaction for C18:2 make this data difficult to interpret. Funding This research was supported by grants from the Colorado State University Agricultural Experiment Station References Christie, W. W. 1981. Lipid metabolism in ruminant animals. Peryamon Press, Oxford, England. pp 37-43. Dawson, R. M., and P. Kemp. 1970. Biohydrogenation of dietary fats in ruminants. In: T. A. Phillipson, (Ed.) Physiology of Digestion and Metabolism in the Ruminant. pp 501. Oriel Press, Newcastle-upon-Tyne, England. Engle, T. E., and J. W. Spears. 2000. Dietary copper effects on lipid metabolism, performance, and ruminal fermentation in finishing steers. J. Anim. Sci.78:2452-2458. Kepler, C.R., K. P. Hirons, J. J. McNeill, and S. B. Tove. 1966. Intermediates and Products of the biohydrogenation of linoleic acid by Butyrivibrio fibrisolvens .J. Biol. Chem. 241:1350-1354.

O16. DIETARY SILICON: A BENEFICIAL MEDIATOR OF IMMUNOREGULATING AND STRESS PROTEINS IN MAMMALS? Sarah Ratcliffe[1,2,] Ravin Jugdaohsingh[1,3] and Jonathan J Powell[1]
[1]MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, UK
[2]Hughes Hall College, Cambridge, UK
[3]Gastrointestinal Laboratory, The Rayne Institute, St Thomas' Hospital, London, UK
sarah.ratcliffe@mrc-hnr.cam.ac.uk

Silicon (as silicic acid [Si(OH)4] has long been linked to the optimal development and maintenance of connective tissues. However, there is also some evidence for its beneficial role in the innate immune system through improved responsiveness to biological stresses (e.g. pathogens). Animal data is lacking, with just broad observations on wound healing and a suggested effect on splenic lymphocyte proliferation (both improved by silicon). In plants, multiple studies have shown a positive correlation between the presence of silicon and the alleviation of both biotic and abiotic stresses. This has been most frequently described in the action of silicon against two common phyta pathogens, namely rice blast and powdery mildew. Originally it was hypothesised that hydrated silica accumulates in the leaves, and forms a mechanical barrier against invading pathogens. This theory has recently been challenged though by the finding of silicon-induced protection against root pathogens, as well as those targeting the leaves, suggesting that silicon is required to stimulate the natural defence mechanisms of the plant[1]. In this study, we took advantage of tissue availability from a recently published silicon-deprivation study in rats. In that work, over 26 weeks, 20 rats were fed a low silicon diet (3.2 µg Si/g feed) and a further 10 rats were fed the same diet but had silicon added into their drinking water (53.2 µg Si/g water) as monomeric silicic acid[2]. Despite such a low dietary intake of silicon, the silicon-deprived animals managed to maintain silicon in their bone and soft tissues at the same levels as the silicon-supplemented animals, and had marginally lower serum silicon levels, therefore not appearing to be silicon deplete. This may have been possible due to the dramatic decrease in urinary silicon excretion observed in the silicon-deprived animals suggesting a mechanism of renal silicon conservation (i.e. re-absorption). Kidneys harvested from the animals at sacrifice (four from both groups), were used for gene-array analyses to isolate differentially expressed genes between the silicon-deprived and the silicon-supplemented animals. Each sample was hybridised on a Rat Genome 230 2.0 array chip (Affymetrix), and after intra-group comparison of the four individual gene-arrays for consistency, inter-group comparison (silicon-deprived vs. silicon-supplemented) was conducted. Potential candidate genes were confirmed using qPCR (R=0.98). The results highlighted a large number of up-regulated genes (> 50) that are linked to stress response and immunoregulation in the silicon-deprived animals. Interleukin-24 (IL-24) for example was consistently up-regulated in the animals on the silicon-deprived diets. IL-24 is a cytokine known to be of importance for wound healing and also for fibroblast proliferation and its up-regulation may be a physiological adaptation to low silicon environments. Why these genes should be up-regulated with silicon deprivation, when gross tissue silicon levels do not seem affected, is puzzling but may be a knock-on effect of over-expressing the renal silicon re-absorption machinery or could be linked to differences in tissue and cellular distributions of silicon with the differing dietary intakes.

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O17. EFFECTS OF LONG-TERM COPPER DEFICIENCY ON GENE EXPRESSION PROFILES OF COPPER TRANSPORTERS AND CHAPERONES IN THE LIVER OF CATTLE.
Robert S. Fry[1], Melissa S. Ashwell[1], Stephanie L. Hansen[1], Terry E. Engle[2], Hyungchul Han[2] and Jerry W. Spears[1]
[1]North Carolina State University, Department of Animal Science, Raleigh, NC, USA
[2]Colorado State University, Department of Animal Science, Fort Collins, CO, USA
rsfry@unity.ncsu.edu

An experiment was conducted to evaluate the gene expression profiles of copper transporters and chaperones in the liver of cattle in response to long-term, induced copper (Cu) deficiency. Copper deficiency was induced by supplementing the Cu antagonist, molybdenum (Mo). Seven Angus calves (males) were born to cows fed one of the following dietary treatments for 410 days prior to calving: 1) 10 mg supplemental Cu/kg DM (+Cu) or 2) no supplemental Cu and 2 mg supplemental Mo/kg DM (-Cu). Calves (steers) remained on the same dietary treatments as their dams after weaning at 183 days of age. Steers were then individually fed their treatment diet for an additional 310 days. Jugular blood samples were taken on day 490 of the study for determination of plasma Cu concentration and ceruloplasmin activity. Steers were harvested on day 493 of the study at a commercial abattoir. Immediately following euthanasia, liver samples were collected and flash frozen for liver Cu analysis and gene expression analysis of Ctr1, Cox17, Atox1, Atp7a, and Atp7b. These Cu transporters and chaperones have been shown in rodents to regulate Cu entry into the cell (CTR1), deliver Cu to cytochrome c oxidase (COX17) and to the trans-golgi network (ATOX1), and export Cu from the trans-golgi network for incorporation into cuproenzymes (ATP7A and ATP7B). Real-time PCR primers were designed with Beacon Designer software to be compatible with SYBR Green I and to amplify across one predicted exon-exon boundary for detection of possible genomic DNA contamination. Specificity of the PCR reaction was assessed and one amplicon generated by each primer pair was sequenced to confirm the identity of the product. Plasma Cu concentration (0.2 vs. 1.3 mg/L), ceruloplasmin activity (5.4 vs. 32.4 mg/100 mL), and liver Cu concentration (6.3 vs. 217.6 mg Cu/kg) was lower ($P<0.001$) in –Cu steers compared to +Cu steers. Expression of Ctr1 and Atox1 mRNA was not different between dietary treatment groups; however, Cox17, Atp7b, Atp7a were differentially expressed due to level of dietary Cu. Relative expression of Cox17 was markedly decreased ($P<0.01$) in –Cu steers compared to +Cu steers. The relative expression of Atp7b tended to be lower ($P=0.08$) in –Cu steers compared to +Cu steers. Furthermore, steers consuming the –Cu diet tended ($P=0.10$) to have less relative expression of Atp7a than +Cu steers. Not only does this study report differentiation of relative expression of Cox17, Atp7a, and Atp7b during long-term Cu deficiency, but it also reports the presence of Atp7a mRNA in the liver. Similar work in rodent models has reported Atp7a in intestine, kidney, and brain but not in liver. This discrepancy cannot be fully explained, but we suggest the presence of Atp7a mRNA in the liver may be due to species differences in Cu metabolism. It is well documented that normal liver Cu concentrations in ruminants are much higher than in nonruminants. Expression of Atp7a in hepatic tissue of cattle could possibly serve as another homeostatic mechanism to further regulate liver Cu metabolism.

O18. EFFECT OF DIETARY ANTAGONISTS ON COPPER METABOLISM OF SHEEP. Alexander M. Mackenzie, Carolyn M. Atkin, Nia Griffith, Claire L. Williams, Simon G. Edwards and Robert G. Wilkinson
Harper Adams University College, Newport, Shropshire, UK,
amackenzie@harper-adams.ac.uk

Clinical copper deficiency in ruminant livestock is an important economic constraint to UK animal production due to the infertility, depression in growth and increased disease incidence associated with the condition. Disruption of copper function and absorption is know to occur though complex interactions between molybdenum, sulphur and iron that take place in the anaerobic environment of the rumen. However, there is still debate and uncertainty of the effects on the animal and also on reliable methods to assess copper status in these animals. The aim of this study was to investigate the effects of these dietary antagonists on copper metabolism in sheep. Thirty Scottish Blackface male sheep of approximately 14 months of age were randomly allocated to one of three treatment groups. The sheep were individually housed and fed a basal diet based on straw pellets, barley and rapeseed meal which was formulated to supply 10.7 MJ ME / kg DM and 154 g CP / kg DM. The diet also contained 5.5 mg Cu / kg DM and 0.67 mg Mo / kg DM. The sheep were fed at a level to meet the requirements for 150 g / day of live weight gain. The sheep were either offered the basal diet (Control), or the basal diet supplemented with 5 mg Mo / kg DM and 2 g S / kg DM (Mo) or, the basal diet supplemented with 500 mg Fe / kg DM and 2 g S / kg DM (Fe). Blood samples were taken prior to dietary treatments and then after 12 weeks to assess plasma trace element levels by ICP-MS and serum ceruloplasmin activity. Liver samples were analysed for trace element status by ICP-MS and to assess ceruloplasmin mRNA and ATOX1 mRNA levels by RT-PCR. After 12 weeks of dietary treatment the sheep on the control diet had significantly higher ceruloplasmin activity compared with the other two treatments ($P<0.001$). However, only the Fe group had significantly lower plasma Cu compared with the controls and Mo sheep ($P<0.05$). Plasma Mo levels were significantly higher in the Mo supplemented sheep ($P<0.001$). There was no significant effect of treatment on plasma Mn, Fe, or Zn levels. Liver copper was significantly higher in the controls ($P<0.05$) and liver Mo was significantly higher in the Mo supplemented sheep ($P<0.001$). There was no significant effect on other liver mineral levels. There was no significant effect of dietary treatment on either ceruloplasmin mRNA or ATOX1 mRNA. Both dietary Mo and Fe had significant effects on Cu metabolism of sheep. Serum ceruloplasmin activity was affected by both whereas plasma Cu was not altered significantly by Mo. The alteration in ceruloplasmin activity was not at the level of mRNA or by the level of its chaperone, ATOX1 mRNA. Therefore, the nature of inhibition is likely to be at the protein / enzyme level. Further research is required to clarify the nature of inhibition to copper metabolism and the alteration in the non-ceruloplasmin plasma Cu pool.

O19. THE EFFECT OF HIGH IODINE INTAKE ON THYROID HORMONES AND SELENIUM STATUS IN OLDER PEOPLE. Christine Thomson, Jenny Campbell, Jody Miller and Sheila Skeaff.
Department of Human Nutrition, University of Otago, New Zealand
christine.thomson@otago.ac.nz

As part of a randomized, double blind trial to determine the effect of selenium (Se) and iodine on thyroid hormone status of older residents (73±4.8 years), two groups received supplements containing 5000 µg iodine as a result of a production error by the manufacturer. Supplementation was suspended immediately on discovery of this error at 8 weeks. The effect of daily intake of 5000 µg iodine for 8 weeks was determined in two groups of older people; one group also receiving 100 µg Se as selenomethionine (+Se+highI) and the other receiving no selenium (–Se+highI), and compared with groups receiving 80 µg iodine with (+Se+lowI) or without 100 µg Se (-Se+lowI), 100 µg Se only (+Se) or a placebo (Plac) daily for 8 weeks. Thyroid hormone status (TSH, free T3, free T4), Se status (plasma Se, whole blood glutathione peroxidase activity (WBGPx)) and median urinary iodine concentration (MUIC) were determined at baseline and week 8, and 4 weeks post supplementation (week 12) for the two high iodine groups. Baseline MUIC baseline of all participants was 54.5 µg/l (IQR 67.5; n=137), indicating mild iodine deficiency. In subjects who received high iodine supplements, and for whom MUIC values were available for all weeks (0, 8, 12; n=27), MUIC had increased from 85 to 8226 µg/l at 8 weeks, but had returned to 64 µg/l by week 12. Mean baseline plasma Se was 1.20 (0.29) µmol/l (n=142) and increased by 0.85, 0.89 and 0.76 µmol/l in the +Se, +Se+lowI and +Se+highI groups at 8 weeks, respectively (P<0.0001); changes were not significantly different among the three groups. Plasma Se did not change in groups not supplemented with Se. WBGPx increased by 3.1, 6.4 and 2.3 U/gHb (7.0, 6.4 and 2.3%) in the three Se-supplemented groups, respectively. The increase was smaller in the +Se+highI group than +Se+lowI (P=0.025) and +Se (P=0.067) groups. WBGPx fell by 1.95 U/gHb (-4.8%) in the –Se+highI group and by -0.38 and -0.42 U/gHb in the -Se+lowI and Plac groups, respectively (differences not significant). Some participants exposed to excess iodine showed elevated TSH at week 8, indicating that they had developed an underactive thyroid (Wolff-Chaikoff effect). In all but two, TSH had returned to normal range (0.3-5.0 mIU/l) by week 12. Only two participants developed evidence of thyrotoxicosis (Jod-Basedow effect), with TSH falling to <0.10 mIU/ml at week 8 and remaining low at 12 weeks. In a third subject, an already low TSH at baseline fell further at week 8. These results are of interest in assessing potential adverse effects of high iodine intake, particularly in older people, and in evaluating the upper tolerable intake limit for iodine. Some evidence of iodine-induced hypothyroidism and of thyrotoxicosis was observed. Most of the abnormalities had returned to normal 4 weeks after cessation of high iodine supplementation. Excess iodine intake may have placed additional oxidative stress on these subjects resulting in smaller increases in WBGPx after Se supplementation. Supported by Otago Medical Research Fund and University of Otago Research Grants.

O20. PROBIOTICS AND SELENIUM METABOLISM: DOES IT MATTER WHETHER THE BACTERIA ARE DEAD OR ALIVE? Woravimol Krittaphol[1], Philip Wescombe[3], Arlene McDowell[1], John R. Tagg[3], Christine D. Thomson[2] and J. Paul Fawcett[1]
[1]School of Pharmacy, University of Otago, Dunedin, New Zealand
[2]Department of Human Nutrition, University of Otago, Dunedin, New Zealand
[3]Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand
kriwo441@student.otago.ac.nz

Selenium (Se) is an essential trace element in humans and animals. In selenium deficient areas supplements are given either as the organic form of selenium (selenomethionine (SeMet)) or as inorganic selenium (selenite). Se metabolism occurs in the liver and kidney and may occur in the gastrointestinal tract (GIT) subject to the activity of the gut microflora and/or probiotic bacteria. Probiotics are used to promote health and are known to be involved in drug metabolism; however there is no information about the effect of probiotic consumption and selenium metabolism. The present in vitro study aimed to (a) compare the influence of live and dead probiotic bacteria and (b) evaluate the metabolic activity of gut microflora combined with either live or dead probiotics on Se metabolism. Fresh cultures of four strains (Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium lactis and Streptococcus salivarius K12) of probiotic bacteria (BLIS Technologies Ltd., Dunedin, New Zealand) containing 109-1010 colony-forming units per mL were prepared in MRS medium. Dead probiotic bacteria were prepared by heating fresh cultures at 80°C for 30 min. SeMet and selenite (both 100 µg mL-1) were incubated anaerobically in suspensions of live and dead probiotic bacteria in phosphate buffer at 37ºC for 24 h. Similarly, SeMet and selenite were incubated in suspensions of gut contents from different segments of the rat GIT mixed with live or dead probiotics. After incubation, the SeMet concentration was determined by high performance liquid chromatography with fluorescence detection. Selenite concentration was determined by hydride generation atomic absorption spectrometry. The results show that in control incubations of SeMet and selenite with gut contents alone, concentrations of SeMet decreased by up to 4% in jejunum, 24% in ileum, 69% in caecum and 30% in colon compared with decreases of 17% (jejunum), 24% (ileum) and 100% (caecum and colon) for incubations with selenite. During incubations of SeMet and selenite with live probiotic bacteria over 24 h, concentrations of SeMet and selenite decreased by up to 20% and 83% respectively, whereas with dead probiotics concentrations decreased by 2-9% and 0-11%, respectively. However, in incubations of SeMet with suspensions of gut contents from different segments of the rat GIT mixed with live probiotics, concentrations of SeMet decreased by 14-28% (jejunum), 25-97% (ileum), 45-97% (caecum) and 79-100% (colon) compared with decreases of 9-36% (jejunum), 11-35% (ileum), 35-98% (caecum) and 100% (colon) for incubations with dead probiotics. Levels of selenite in corresponding incubations with gut contents mixed with live or dead probiotics decreased by 19-40% vs. 13-100% in jejunum, 51-100% vs. 82-100% in ileum and 100% vs. 100% in caecum and colon. These results indicate that both live and dead probiotics can combine with gut microflora to metabolise selenium. In conclusion, live probiotic bacteria mediate metabolism of both organic and inorganic Se but dead probiotic bacteria do not unless they are combined with gut microflora when they exert the same effect as live bacteria. This is the first study to show that probiotics can influence Se metabolism.

O21. IODINE STATUS AND COGNITIVE FUNCTION OF WOMEN OF CHILDBEARING AGE AND THEIR FIVE YEAR-OLD CHILDREN IN SIDAMA, SOUTHERN ETHIOPIA.

Alemtsehay Bogale[1,4], Cherinet Abuye[2], Kassu Gurmu[3], Yewelsew Abebe[4], K Michael Hambidge[5] and Barbara J Stoecker[1]
[1]Nutritional Sciences, Oklahoma State Univ, Stillwater, OK, USA;
[2]Ethiopian Health & Nutr Res Inst, Addis Ababa, Ethiopia;
[3]College of Health Sci, Hawassa Univ, Awassa Ethiopia,
[4]College of Agriculture, Hawassa Univ, Awassa Ethiopia;
[5]University of Colorado School of Medicine, Denver, Colorado, USA
Barbara.Stoecker@okstate.edu

Iodine deficiency is one of the most common micronutrient deficiencies worldwide and is a major cause of preventable mental retardation. The purpose of this study was to assess the iodine status and cognitive function of women of childbearing age and their five-year-old children. A cross-sectional study was conducted from February to March, 2007, in Ethiopia. One hundred women of childbearing age and their five year-old children who fulfilled all inclusion criteria participated. The exclusion criteria were pregnancy or grade IV goiter. Demographic characteristics and iodine status were assessed. Raven's Colored Progressive Matrices (CPM) and age appropriate tests from the Kaufman ABC-II assessment were administered to both the women and their children. The use of iodized salt in the participant's households was only 2%. The mean iodine concentration of water in and around the study area was 4.46 µg/L and a water iodine value of <10 µg/L indicates iodine deficiency. Occurrence of goiter grade I to III was 85% in the women and 33% in children. Urinary iodine excretion (UIE) for all participants was <49 µg/L, the upper cutoff value for moderate iodine deficiency. Ninety-nine percent of the mothers and 95% of the children had less than 20 µg/L UIE, the cut off point for severe iodine deficiency. The median UIE was 1µg/L for both mothers and children. The majority of scores for all cognitive tests were low for both mothers and children. There were significant correlations among individual test results for both mother and child. Raven's CPM for mothers was correlated with Sequential ($r=0.37$, $p=0.0002$), Simultaneous ($r=0.45$, $p< 0.0001$) and Planning ($r=0.4$, $p<0.0001$) indices from the Kaufman ABC-II. For the children, the Sequential index was correlated with the Simultaneous ($r=0.49$, $p< 0.0001$) index. Goiter and urinary iodine excretion (UIE) were correlated to Sequential indices ($r= 0.39$, $p= 0.0011$ and $r= 0.2$, $p= 0.05$) respectively, but not to Simultaneous indices for children. Both Sequential ($r=0.21$, $p= 0.03$) and Simultaneous ($r=0.29$, $p=0.004$) indices were correlated for mothers and children. Sex difference of children was not a significant factor for any tests. The high prevalence of goiter and the low urinary iodine excretion demonstrate serious long- and short-term iodine deficiency in the study area. Low scores in cognitive test performance might be associated with iodine deficiency and its consequences. Efficient and cost effective methods to secure iodine availability in the community are urgently needed. [This study was supported by NIH Grant R21 TW06729 (Fogarty International Center & the Office of Dietary Supplements), Oklahoma State University, and Hawassa University].

O22. SELENIUM IN MILK – SELENIUM SPECIATION AND HEALTH EFFECTS. Tien Hoac, Peter Olsson, Vasileios Pagmantidis, Gitte Ravn-Haren, Jan Stagsted, Susanne Bügel, Gunilla Önning, Jacob H. Nielsen, Lars O. Dragsted and Björn Åkesson
Biomedical Nutrition, Pure and Applied Biochemistry, Lund University, Sweden; Department of Human Nutrition, University of Copenhagen, Denmark; Department of Animal Health, Welfare and Nutrition, Aarhus University, Denmark
bjorn.akesson@tbiokem.lth.se

Background. Dairy products account for approx. 20% of the Se intake. Increasing the Se content of dairy products would be a possible way of providing the consumers with an increased Se supply. Several aspects of producing Se-enriched milk were investigated, such as the occurrence of different Se forms in milk. Moreover, the mechanisms of action of Se-enriched milk on the consumer were studied. Production of Se-enriched milk. Expt 1 was a cross-over study and two groups of cows were given either 25 mg yeast Se/day or a basal feed (Hoac 2008). The Se content increased 10-fold in milk and 10-fold in whey, and the corresponding increase in plasma was 2-fold. In another experiment 16 cows assigned to two groups were given either 100 mg organic Se/day for 1 week or 0.1 ppm selenite. The increase in Se content of both whole and defatted milk was 40-50-fold, and in whey it was 20-fold. Speciation of milk Se compounds. In whey three major peaks of Se were found using SEC (Hoac 2007). More than 65% of the Se was found in non-selenoprotein protein fractions (mainly β-lactoglobulin and α-lactalbumin) and the remainder in low-molecular-weight fractions. Supplementation of cow's feed with organic Se resulted in increased Se content mainly in the β-lactoglobulin-α-lactalbumin fractions of whey (Hoac 2008). Using this approach no evidence for the presence in whey of the specific selenoproteins occurring in mammary gland was obtained (Bruzelius 2007; Bruzelius 2008). Short-term effects of Se in humans using Se-rich milk. The effects of Se-enriched milk or yeast did not differ significantly and both increased serum Se more than selenate (Ravn-Haren 2008). Conversely, platelet glutathione peroxidase (GPx) was only increased when the subjects were given selenate. No effects were found on plasma lipid resistance to oxidation and GPx activity in erythrocytes and plasma. Conclusions. The project has given new data on how the Se content of milk and whey can be manipulated by feeding and on the occurrence of different forms of Se in whey. Se-rich milk can be a good source of Se in man and its actions on consumer physiology need further study. Acknowledgements: The project is part of the FOODANTIOX program (New antioxidant strategies for consumer health and food quality) supported by Öforsk. Lund University and University of Copenhagen are members of the supporting NoE:s NuGO, ECNIS and the NCoE SYSDIET.

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O23. SELENIUM AND HUMAN CANCER. FROM EPIDEMIOLOGICAL DATA TO MOLECULAR BIOLOGY STUDY. Wojciech Wasowicz, Ewa Jablonska, Edyta Reszka and Jolanta Gromadzinska
Institute of Occupational Medicine, Department of Toxicology and Carcinogenesis, Lodz, Poland.
wojciech@imp.lodz.pl

Background. There is a growing interest in the biological role of selenium (Se) with respect to both protection of human health and prevention of diseases. Food is a major source of Se and researchers have vested interest in Se status in various populations not only because of its deficiency or toxicity symptoms, but also in view of its potential beneficial effect contributing among others to cancer prevention. The majority of studies on the relationship between selenium and cancer, focus either on the association between Se status and cancer risk or on the association between genetic polymorphism of selenoproteins' genes and cancer risk. It should be noted that in both types of studies, the results remain conflicting. Thus, combining both types of data (concerning both dietary and genetic factors) would be more informative and valuable in the assessment of cancer risk development, which was indicated in our recent study. Objective. In the case - control study conducted in our Department, lung cancer risk associated with Se status was compared between Polish individuals possessing different genetic variants of 15 kDa selenoprotein (Sep15), the protein possibly involved in cancer development due to its redox activity. Methods. The study concerned 325 cases and 287 controls. All the participants were smokers. Plasma Se concentration was determined using graphite furnace atomic absorption spectrometry, and Sep15 polymorphism (1125 G/A transition within 3'-untranslated region) was detected with polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) assay. Results. The preliminary results of the study indicated that Sep15 polymorphism significantly modified lung cancer susceptibility associated with Se status. Although there was no association between lung cancer risk and Sep15 polymorphism analyzed alone, and plasma Se concentration was statistically lower in lung cancer cases (49.4±17.4 ng/ml) compared to controls (53.3±14.0 ng/ml, $p<0.002$), the analysis of the joint effect of Sep15 polymorphism and Se status for lung cancer development revealed that the risk differed between the Sep15 genotype groups. An increasing Se concentration was associated with a decreased risk in all individuals; however, at Se concentration above 80 ng/ml, the risk started to increase in individuals possessing the Sep15 1125 GG or GA genotype. Conclusions. Sep15 polymorphism may be a genetic factor modifying lung cancer risk associated with Se status. On the basis of our findings, we conclude that studies on the relationship between diet and cancer should focus on the interactions between dietary and genetic factors rather than on the study of each factor separately. Data from such studies would be especially interested in view of the intervention trial planning. Prior to supplementation, DNA genotyping should be first performed in order to select individuals with certain genetic background. This would allow to avoid (at least to some extent) the study bias associated with genetic variation and to identify individuals who, due to the specific gene and nutrient interaction, are more or less susceptible to cancer. Acknowledgement This study was supported by ECNIS NoE.

O24. SELENOPROTEIN W MRNA EXPRESSION ANALYSIS IN HUMAN COLONIC MUCOSA USING AFFYMETRIX HGU133 PLUS 2.0 MICROARRAYS AND REAL-TIME PCR. Jeannette Molnár[1,2], Orsolya Galamb[2], Ferenc Sipos[2], Sándor Spisák[2], Kinga Tóth[2], Norbert Solymosi[2], Annamária Németh[2], Zsolt Tulassay[2], Béla Molnár[2]
[1]National Institute of Food and Nutrition Science, Budapest, Hungary
[2]2nd Department of Medicine, Semmelweis University, Medical School, Budapest, Hungary
blaiseszolgyemy@hotmail.com

Background and aims: Selenium status has been inversely associated with colorectal cancer and adenomas. However, the mechanism for selenium as an anticarcinogenic element is not known. The aim of our study was to compare mRNA expression of various selenoproteins in human normal colonic mucosa, in colonic mucosa showing an increasing amount of dyplasia, and in colorectal cancer. Materials and methods: The expression of selenoprotein W, selenoprotein P mRNA, and glutathione peroxidase 1, 2, 3, and 4 mRNA was determined in human normal colonic mucosa biopsy specimens (n=8), in human colonic adenoma with low-grade dysplasia (n=6), in human colonic adenoma with high-grade dysplasia (n=9), stage Dukes B human colonic adenocarcinoma (n=7) and stage Dukes C or D human colonic adenocarcinoma biopsies (n=8) using Affymetrix HGU133 Plus 2.0 microarrays. Results were validated for selenoprotein W using LightCycler RT-PCR. The reference housekeeping gene was beta-actin. Statistical analysis was performed by Kendall rank correlation. Results: There was a statistically significant decreasing trend of selenoprotein W mRNA expression in the samples according to the normal-adenoma-dysplasia-carcinoma sequence ($p=0.015$). Glutathione peroxidase 1 and 4 mRNA expression rates generally increased while glutathione peroxidase 3 mRNA expression levels decreased, the trend, however, was not consistent. Glutathione peroxidase 2 and selenoprotein P mRNA expression were not related to the degree of dysplasia or cancer ($p>0.05$). The significant decreasing trend for selenoprotein W mRNA expression was confirmed by RT-PCR both for the normal-adenoma-carcinoma sequence ($p=0.016$) and for the normal – low-grade dysplastic adenoma – high-grade dysplastic adenoma - early stage colorectal cancer – late stage colorectal cancer sequence ($p=0.018$). Conclusions: Our results indicate that selenoprotein W mRNA expression shows an inverse correlation with the degree of dysplasia in human colonic mucosa biopsy specimens. This may explain literature data suggesting selenoprotein W expression in the colon to be highly sensitive to Se-depletion and serum selenium levels decreasing parallel with the progression of gastrointestinal cancer. Further studies are needed to confirm a causal relationship between selenoprotein W expression in human colonic mucosa biopsies and the formation of dysplasia or cancer.

O25. ONE-DAY ZINC KINETICS, A VERSATILE TOOL FOR ANALYSIS OF HUMAN ZINC METABOLISM AND ITS APPLICATION. Katsuhiko Yokoi[1], Harold H. Sandstead[2], Norman G. Egger[3], Nancy W. Alcock[2], V.M. Sadagopa Ramanujam[2], Hari H. Dayal[2] and James G. Penland[4].
[1]Department of Human Nutrition, Seitoku University Graduate School, Chiba, Japan
[2]Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX, USA; [3]Division of General Internal Medicine, Mayo Clinic, Rochester, MN, USA;
[4]US Department of Agriculture Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND, USA
KatsuhikoY@aol.com, yokoi@seitoku.ac.jp

The co-occurrence of zinc (Zn) and iron (Fe) deficiencies in humans is a worldwide public health problem. We estimated the co-occurrence of dietary Zn and Fe deficiencies. Dietary factors include the frequency of consumption of flesh foods, especially red meat rich in bioavailable Zn and Fe, and unrefined cereals, bran and pulses rich in phytate and other indigestible Zn and Fe binding ligands. Although Fe nutriture is well defined by established chemical indices, Zn nutriture is difficult to define by common chemical indices such as plasma Zn and alkaline phosphatase activity. Zn kinetic parameters directly describe the metabolic status of Zn. Though complex Zn kinetic models can determine many parameters, the practicality of this approach is limited by the burden on subjects including time involved and the many specimens required. Therefore, we used a simpler approach. The one-day Zn kinetic model comprises of one central Zn compartment and two peripheral Zn compartments (lesser and greater) without an outlet to the outside of the kinetic system. An outlet is excluded, because the loss of tracer from the system is not large during the one-day observation period after tracer administration. The protocol of tracer administration and the following analyses are described elsewhere. Briefly, the procedure was as follows. Two mg of ^{67}Zn tracer was administered intravenously and consecutive blood samples were collected for 24 hours. Isotope ratios ^{67}Zn/^{68}Zn in plasma were measured by inductively coupled plasma-mass spectrometry and fitted to a bi-exponential function with a constant term for determining Zn kinetic parameters including Zn pool sizes and rate constants. The one-day Zn kinetic model was applied to define Zn status of 33 premenopausal women with normal or decreased Fe stores living in Texas, USA. The frequency of consumption of specific foods and a history of menorrhagia exemplified by bleeding through menstrual pads were recorded. Zn pool sizes were explained by food frequencies and a menstrual history based on the stepwise regression analysis (P=0.002). Positive predictors were frequencies of consumption of beef (P=0.02), coffee (P=0.01) and yogurt (P=0.008). Negative predictors were a presence of bleeding through menstrual pads and frequencies of consumption of bran breakfast cereals (P=0.002) and orange juice (P=0.03). Zn pool sizes and Fe stores were highly correlated. Serum ferritin concentrations less than 20 µg/l were predictive of an increased risk of low Zn nutriture shown as a small Zn pool size (odds ratio 19.0, 0.001 of Fisher's exact probability).
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O26. HEPCIDIN AND ITS ROLE IN REGULATING INTESTINAL IRON TRANSPORT. Paul Sharp, Bomee Chung, Timothy Chaston, Joanne Marks, Edward Debnam and Surjit Srai. King's College London & University College London, UK.
paul.a.sharp@kcl.ac.uk

BACKGROUND & AIM: Hepcidin is a 25 amino acid peptide produced mainly in the liver that acts as a major regulator of body iron homeostasis. Increased levels of hepcidin give rise to hypoferraemia. These events are rapid and are mediated in part by decreased expression of iron efflux protein ferroportin in iron recycling macrophages. While macrophages are central to iron turnover, recycling some 25mg iron/day from senescent red blood cells, in the long-term body iron levels are ultimately controlled by the absorption of dietary iron by duodenal enterocytes to replace endogenous daily losses (approximately 1-2mg/day). Despite much speculation, the precise role of hepcidin in regulating intestinal iron transport remains unclear. Therefore, the aim of this study was to investigate the effects of hepcidin on intestinal iron transport and the expression of the intestinal iron transport proteins DMT1 and ferroportin. METHODS: Studies were performed in vivo in male C57BL/6 mice injected with a synthetic hepcidin peptide (10µg/mouse; i.p.) and in vitro in human intestinal Caco-2 cell exposed to the same peptide. Iron transport (measured using 59Fe) and transporter protein expression (measured by confocal microscopy and Western blotting) were measured 4h and 24h post-exposure to hepcidin. RESULTS: There were no differences in iron transport or transporter expression in either mouse duodenum or Caco-2 cells exposed acutely (4h) to hepcidin. However, after 24h exposure to the peptide there was a significant decrease in iron efflux in both the in vivo and in vitro models. In mice but not Caco-2 cells this was accompanied by a significant decrease in ferroportin protein expression. CONCLUSIONS: Our data support an inhibitory action of hepcidin on intestinal iron transport. Interestingly, the effects of hepcidin on the intestinal epithelium are greatly delayed compared with those observed in macrophages. This suggests that hepcidin exerts its influence on body iron metabolism in a cell-specific fashion with the iron recycling macrophage its principal target. This is entirely appropriate given the central role that these cells play in maintaining iron homeostasis. Funding: BBSRC grant D015456/1

O27. INTAKES AND IMPACT OF DIETARY FACTORS AFFECTING IRON AVAILABILITY IN THE MEDICAL RESEARCH COUNCIL NATIONAL SURVEY OF HEALTH AND DEVELOPMENT (MRC NSHD) (1946 BRITISH BIRTH COHORT) BETWEEN 1982 AND 1999.

Anna P. Rickard[1,2], Mark D. Chatfield[1], Jonathan J. Powell[1] and Alison M. Stephen[1]
[1]MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, CB1 9NL, United Kingdom
[2]University of Cambridge, Newnham College, Cambridge, CB3 9DF, United Kingdom.
anna.rickard@mrc-hnr.cam.ac.uk

The World Health Organisation recognises iron deficiency as one of the ten greatest global health concerns. Evidence suggests when tackling iron deficiency the amount of dietary iron consumed plays a less important role than the availability of that iron. Between 85 and 100% of iron consumed is non-haem iron and the simultaneous consumption of dietary factors that inhibit or enhance its availability result in absorption efficiency ranging from 0.1 to >35%[1].NSHD is a social-class stratified random sample of 5363 singleton births in England, Scotland or Wales during the first week of March 1946. Throughout their lives medical, social, educational and other information has been collected. Dietary data collected at age 36, 43 and 53 years (1982, 1989 and 1999 respectively) provides the opportunity to examine changes over time. This study examines changes in intakes of dietary factors known to enhance or inhibit iron availability, namely the amount of non-haem iron itself, ascorbic acid, calcium, phytate, polyphenols from beverages, red meat, fish and poultry, and using an algorithm of these dietary factors, examines changes in percentage and intakes of available iron over the 17 year period. Current iron algorithms are considered to be inadequate for use on dietary survey data[2]. In this study we used a new algorithm to apply to iron intake data to adjust for the effects of the mentioned dietary factors and thus to estimate percentage available iron. Multi-level mixed-effects linear regression showed that changes in mean daily intakes were observed for all nutrients over the 17 year period. Calcium and ascorbic acid intakes dramatically increased over this time, while the intake of red meat, fish and poultry decreased, consistent with previous findings[3]. The intake of polyphenols from beverages rose significantly between 1982 and 1989. Non-haem iron intakes were highest in 1989. Changes in phytate intake were small. Predictions of percentage available iron were significantly lower in 1989 compared with 1982 and 1999. We believed this in part to be a result of the higher intake of non-haem iron in 1989 and the inhibiting effect of this amount on percentage available iron that is determined by our algorithm. Further explanations for the observed trends are being examined.

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O28. SHORT-TERM INHIBITORY EFFECT OF CALCIUM ON IRON ABSORPTION PERSISTS AFTER AN EXTENDED COURSE OF CALCIUM SUPPLEMENTATION IN YOUNG WOMEN WITH LIMITED IRON STORES.

Karima Benkhedda, Mary R. L'Abbé and Kevin A. Cockell.
Bureau of Nutritional Sciences, Food Directorate, Health Canada, Ottawa, Canada.
kevin_cockell@hc-sc.gc.ca

Calcium (Ca) is a known inhibitor of iron (Fe) absorption in short-term meal studies. Longer-term supplementation with Ca has been shown not to compromise body Fe status, leading to the supposition that metabolic adaptation in Fe absorption occurs over time to compensate for the effect of the added Ca intake. To our knowledge, no one has previously measured the short-term effects of Ca on Fe absorption both before and after a period of Ca supplementation. We sought also to investigate whether young adult women with limited baseline body Fe stores would have the capacity to adapt to the potential inhibitory effect of continued Ca consumption over several months. Iron absorption was measured in 14 healthy women with limited Fe stores (geometric mean serum ferritin concentration, SF= 24.1 µg/L) from two stable Fe isotope-labelled breakfasts on consecutive days. One of the meals was labelled with 57Fe and the other, with 58Fe, was consumed with 500 mg Ca (as CaCO3 tablet). Label incorporation into enriched blood samples was determined from measurements (by multi-collector ICP-MS) of 57Fe/56Fe and 58Fe/56Fe isotopic ratios before and after administration of the labels, assuming 80% incorporation of absorbed Fe into red blood cells. Fractional Fe absorption was calculated as the ratio of the amount of the isotope labels incorporated to the amount ingested. Group mean absorption of 57Fe was 7.9% (range 2.5-42.2%), declining to 4.2% (range 1.4-14.6%) absorption of the 58Fe label under the influence of Ca. Mean ratio of 58Fe/57Fe absorption was 0.53, indicating a 47% inhibition of Fe absorption by Ca. Subjects were divided into two groups, given CaCO3 supplements (1000 mg Ca/d) or matched placebos for six months before repeating the short-term Fe absorption measurements. After six months, geometric mean SF (20.0 µg/L) was not significantly different (P= 0.16) from the pre-supplementation value. Ca-supplemented subjects showed pre-supplementation mean absorption of 6.1% and 3.3% of the 57Fe and 58Fe labels, respectively, demonstrating 45% inhibition of Fe absorption with the co-consumption of a 500 mg Ca tablet. Post-supplementation, the corresponding values of 6.3% and 3.3% absorption and 48% inhibitory effect of Ca on Fe were not significantly different from pre-supplementation values. Placebo group subjects showed pre-supplementation mean absorption of 10.4% and 5.3% of the 57Fe and 58Fe labels, respectively, demonstrating 48% inhibition by Ca. Post-supplementation values of 10.7% and 5.7% absorption and thus 47% inhibition by Ca were not significantly different from pre-supplementation values. Thus group mean absorptions of the 57Fe label (taken without Ca), the 58Fe label (taken with Ca) and the ratio of the fractional absorptions of 58Fe/57Fe (representing the inhibitory effect of Ca in the short-term test) were unchanged after vs before the six months of supplementation, suggesting that the inhibition by Ca of Fe absorption in the short-term test is insensitive to habitual Ca intake. Large inter-individual variation in Fe absorption was found in both short-term tests (before and after supplementation), suggesting that Fe status may not be the main physiological factor determining Fe absorption under conditions of identical dietary intakes.

O29. MATERNAL DIETARY ZINC SUPPLEMENTATION PREVENTS COGNITIVE IMPAIRMENT IN ADULT OFFSPRING OF MICE EXPOSED TO INFECTION (LPS) IN EARLY PREGNANCY.

Allan M Rofe[1], Nancy Tran[1,2], Jenny N.T. Fung[1,2], Brooke L Summers[1,2], Joanne S Chua [1,2] and Peter Coyle[1]
1Hanson Institute and Institute of Medical and Veterinary Science, Adelaide, SA 5000, Australia.
2 Department of Physiology, University of Adelaide, Adelaide SA 5000 Australia
allan.rofe@imvs.sa.gov.au

Maternal infection during pregnancy is associated with an increased risk of neurodevelopmental brain damage. Accumulating evidence suggests that the maternal inflammatory response may be responsible. Metallothionein is induced in the mother's liver during the acute phase response leading to a fetal zinc (Zn) deficiency. Infection-mediated fetal zinc deficiency in early pregnancy has been shown to cause teratogenicity which can be prevented by either a single Zn injection or dietary zinc supplementation throughout pregnancy (1,2). This study examined whether cognitive impairments can be caused by lipopolysaccharide (LPS) administration early in pregnancy and whether dietary zinc supplementation (100mg Kg diet compared to 35mg/Kg) can ameliorate these changes. Maternal inflammation induced by LPS (0.3ug/g body wt sc) at gestational day GD8 did not affect spatial learning or memory of adult mice offspring when tested in a water cross maze escape task. However, in an object recognition task, where normal mice explore novel objects in preference to familiar ones, offspring from LPS-treated mothers continued to explore only the familiar object, a highly significant ($p<0.01$) and abnormal outcome. In comparison, offspring of mice given LPS in conjunction with dietary Zn supplementation displayed normal object recognition task performance. The influence of LPS exposure on the expression of embryonic activity-dependent neuroprotective peptide (ADNP), a marker of neuronal injury, was also examined. Following exposure of pregnant mice to LPS at GD9, embryos were extracted 6 and 24h later to determine embryonic ADNP mRNA expression. The increase in embryonic ADNP expression 24h after LPS administration was significantly decreased by a single Zn injection. In further studies, maternal LPS administration on GD16 induced astrogliosis accompanied by extensive cell death in the central white matter, hippocampus, and periventricular cortex in GD18 fetuses, and this was also prevented by a single Zn injection. This study demonstrates that LPS administration in early pregnancy can cause long-term behavioural changes in adult offspring. This altered behavior, which has parallels with that seen following ethanol exposure (3), can be prevented by dietary Zn supplementation thus providing a potential strategy to limit neurodevelopmental damage caused by infections in pregnancy.

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Behav Brain Res; 186: 230-238

O30. ZINC AND IRON ABSORPTION AND NUTRITIONAL STATUS IS REDUCED AFTER GASTRIC BYPASS IN MORBIDLY OBESE PATIENTS. Manuel Ruz, Fernando Carrasco, Pamela Rojas, Attila Csendes, Karin Papapietro, Jorge Inostroza, Annabella Rebolledo, Karen Basfi-fer, Fernando Pizarro, Manuel Olivares, Nancy Krebs, Jamie Westcott, Michael Hambidge.
University of Chile, Santiago, Chile, and UCHSC, Denver , Co, USA.
mruz@med.uchile.cl

Objective: To evaluate zinc and iron absorption and nutritional status in patients with morbid obesity before and after 6, 12, and 18 months of gastric bypass (GBP). **Material and Methods:** Sixty-seven women were studied previous to gastric bypass (average age 36.7±9.8 y, weight 115.1±15.6 kg, BMI 45.2±4.7 kg/m^2). In 58, 56 and 51 individuals there were results after 6, 12, and 18 months of surgery, respectively. Zinc status was assessed through the determination of plasma zinc (PLZn), plasma alkaline phosphatase activity (AP), red blood cell membrane alkaline phosphatase activity (RBCM AP) and the size of the rapidly exchangeable zinc pool (EZP). Iron status was evaluated by hemoglobin (Hb), free erythrocyte protoporphyrin (FEP), and serum ferritin (SF). Intestinal absorption of zinc and iron were evaluated in a sub-sample of 36 subjects. Zinc absorption and EZP were determined by a dual-stable isotope method and iron absorption by a dual-radioactive isotope method. Statistical comparisons were performed by repeated measures ANOVA. **Results:** A significant reduction of zinc absorption from a standard diet was observed from 32.0% before the surgery to 13.6%, 17.9% and to 22.9% after 6, 12, and 18 months of GBP, respectively ($p<0.01$). Iron absorption decreased from an initial value of 10.5% to 3.4%, 3.5%, and to 4.7% after 6, 12, and 18 of GBP, respectively ($p<0.001$). Indices of Zn and Fe before surgery were (mean±sd): PLZn 87.2±11.1 ug/dL, AP 36.3±7.0 U/L, RBCM AP 0.31±0.09 U/g Hb, EZP 256.5±48.6 mg, Hb 13.7±0.9 g/dL, FEP 61.1±19.6 ug/dL RBC, SF (geometric mean) 37.5 ug/L. After 18 months of GBP PLZn, RBCM AP, EZP, Hb, and SF were markedly decreased ($p< 0.001$). No differences were observed regarding, AP and FEP. **Conclusion:** Gastric bypass in morbidly obese patients was associated to decreased intestinal absorption of zinc and iron. Zinc and iron nutritional status were significantly impaired in the patients after this type of surgery. In an ongoing study using a similar protocol, we are also studying the potential alterations of selected intracelular zinc and iron transporters as result of GBP.
Supported by Fondecyt Grants1040765 and 1080576.

P001. THE DEVELOPMENT OF A FOOD IRON BIOAVAILABILITY INDEX (FIBI). Marcia J. Cooper, Kevin A. Cockell and Mary R. L'Abbé
Bureau of Nutritional Sciences, Food Directorate, Health Canada, Ottawa, Canada
marcia_cooper@hc-sc.gc.ca

In establishing the DRIs for iron, an average bioavailability of 18% was assumed, considering a typical mixed diet for Canada and the United States. It has recently been suggested that a diet consistent with the Dietary Guidelines for Americans 2005 may reduce the bioavailability of iron to approximately 11%. This lower bioavailability estimate suggests that the dietary iron requirement would be 60% larger than if the iron bioavailability of the total diet were 18%.
Iron is present in food in two forms, heme iron found in meat, fish, poultry and seafood and nonheme iron, found in both animal and plant foods as iron containing salts.
The Canadian Nutrient File (CNF), which is the national food composition database, is similar to databases in other countries, and lists only total iron values. Heme/nonheme levels or bioavailability data have not been included in the database. Our ongoing objective is to determine heme and non-heme values in order to approximate bioavailable iron of specific foods with consideration for enhancing and inhibiting components and to be able to determine total bioavailable iron from the Canadian diet for various age and sex groups.
In order to estimate the bioavailable dietary iron (BDI) from foods, an extensive literature search was conducted to estimate the proportion of heme and nonheme iron content of specific foods/groups of foods. Recent research suggests that the heme content of meat, fish and poultry (MFP) is not consistent for all categories of foods as was originally proposed at 40% by Monsen et al. (1978). We have, where available, used more accurate estimates to calculate bioavailable iron for MFP foods contained within the CNF. We are currently in the process of mapping these literature values, particularly for meat, fish, poultry and seafood, to the over 5500 foods in the CNF. Challenges associated with this mapping include the limited amount of literature values for the heme/nonheme content of foods. Additionally, the cooking methods associated with these literature values are often different from North American procedures, which make application to foods within the CNF difficult. In order to overcome some of these challenges, the literature heme values for a composite of meats (e.g. strip loin roast and tenderloin) were mapped to specific foods within the CNF (e.g. New York strip loin).

P002. DIETARY INTAKE MODELLING SUPPORTS A LOW LEVEL OF IRON SUPPLEMENTATION FOR PREGNANT CANADIAN WOMEN. Kevin A Cockell[1], Hélène Lowell[2], Doris C Miller[2] and George H Beaton[3]
[1]Nutrition Research Division, Food Directorate, HPFB, Health Canada, Ottawa, ON.
[2]Office of Nutrition Policy and Promotion, HPFB, Health Canada, Ottawa, ON
[3]Professor Emeritus, Department of Nutritional Sciences, University of Toronto, ON
kevin_cockell@hc-sc.gc.ca

For a substantial proportion of pregnant Canadian women, usual iron intakes from food alone appear to be inadequate, when judged against the Dietary Reference Intakes (DRI) requirement estimates. Dietary intake modelling was undertaken to determine a level of iron supplementation for pregnant women in Canada that would convey an acceptably low risk of both apparently inadequate and apparently excessive intakes, and hence could be recommended in Health Canada guidelines. The distribution of usual dietary iron intakes was estimated using 24-hour dietary recalls of pregnant women aged 19-50 from the Canadian Community Health Survey, Cycle 2.2, Nutrition (CCHS 2.2). The prevalences of usual intakes below the Estimated Average Requirement (EAR) for pregnancy (22 mg/d) or above the Tolerable Upper Intake Level (UL, 45 mg/d) were estimated. Iterative modelling with inclusion of supplemental iron in increments of 1-2 mg/d was done to determine desirable levels of supplementation. Apparent adequacy of combined intake (usual dietary intake + supplement) was judged in accord with the estimates of iron requirements and assessment methodology as presented in the DRI reports. Predicted prevalences of <3% for both apparent inadequacy of intake and apparent excessive intake were accepted as a working target. As a cross-check for conclusions drawn from the modelling for pregnant women, the process was repeated with the much larger data set available from CCHS 2.2 for dietary iron intakes by non-pregnant Canadian women, analysing these as if they were pregnant (and the DRI estimates for pregnancy would apply). This was done solely to strengthen confidence in the conclusions of the modelling for pregnant women, which are presented here. The prevalence of apparently inadequate iron intakes fell below 5% at a level of 15 mg of daily supplemental iron. The level needed to achieve a prevalence of <3% could not be defined with precision but was unlikely to be much more than 15 mg/d. The prevalence of iron intakes above the UL became detectable above a supplement level of 20 mg/d. It was concluded that, based on the DRI requirement estimates for iron, a supplement of 15-20 mg/d throughout pregnancy is justified as both effective and safe for the general population. This supplement recommendation, based on modelling of nutrient intakes at the population level to meet estimated requirements during pregnancy, does not preclude the need for health care professionals to assess the need for therapeutic levels of iron at the individual level based on iron status. This work was undertaken to inform the revision of Health Canada's National Nutrition Pregnancy Guidelines. Revising the iron supplement recommendation based on this work fits within Health Canada's mandate to implement the DRIs within its policies and guidelines.

P003. IRON AND ZINC DIALYZABILITY IN AN INFANT DIET INCLUDING BREAD WITH DIFFERENT IRON SOURCES OR ABSORPTION PROMOTERS. Maria Julieta Binaghi, Nestor Pellegrino, Patricia Ronayne and Mirta Valencia.
Cátedra de Bromatología. Facultad de Farmacia y Bioquímica. UBA. Junín 956. Buenos Aires. Argentina. jbinaghi@ffyb.uba.ar.

Mineral fortification of generally consumed foods is one of the best strategies to prevent deficiencies in the population. However, fortification sources as well as interactions with food components should be considered.
The aim of this study was to assess iron and zinc dialyzability in an infant diet including bread with different iron fortification sources or absorption promoters.
Samples subjected to analysis comprised an infant diet made up with mashes of pumpkin, potato, wheat grits and apple, mixed with bread fortified with ferrous sulphate, ferrous bisglycinate or ferric EDTA, or non-fortified bread containing one of the following absorption promoters: ascorbic acid (AA), sodium citrate or sodium EDTA. A mixture including non-fortified bread lacking any promoter was also analysed (control). All the above mixtures were combined with one of the following beverages: water, iron fortified milk, tea infusion or an orange-based drink.
Potential iron and zinc availability was assessed using an *in vitro* modified method, which measures iron (FeD%) or zinc (ZnD%) dialyzability under controlled pH conditions, after a digestion simulating physiological processes. Determinations were sextuplicates and statistical analysis was performed by ANOVA and a posteriori Tukey test.
Regarding iron, when the tested beverage was water, there were no significant differences in FeD% between fortified breads with ferrous sulphate or bisglycinate and the control bread. On the contrary, there were significant differences between diets containing the control bread and ferric EDTA fortified bread or non-fortified breads with any of the absorption promoters ($p<0.01$). FeD% values did not differ between ferric EDTA fortified bread and non-fortified breads with AA or citrate. The highest FeD% corresponded to sodium EDTA.
In the mixtures with milk or tea there were differences between the fortified and non-fortified breads, with added promoters ($p< 0.001$). The mixtures with the orange-based drink showed the most variable values, but always superior than the values obtained with the other beverages.
With regard to ZnD%, when the tested beverage was water, the main significant differences appeared when comparing the control bread with non-fortified breads containing either citrate or sodium EDTA ($p<0.001$). In the mixtures with other beverages, milk and tea behaved similarly. The orange-based drink showed again the most variable values but also the highest ones.
In conclusion, mineral dialyzability may be enhanced according to the used combination of iron sources and/or absorption promoters. The diet with ferric EDTA fortified bread and the orange-based drink showed the highest FeD% while the highest ZnD% was achieved with the diet containing non-fortified breads with sodium EDTA and the orange-based drink.
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P004. MINERAL DIALYZABILITY FROM EXPANDED PRODUCTS FORTIFIED WITH BOVINE HEMOGLOBIN. Silvina R. Drago[1], M. Julia Birocco[1], Rolando J. González [1] and Mirta E. Valencia [2]
[1]Instituto de Tecnologia de Alimentos, FIQ, UNL, Santa Fe, Argentina
[2]Fac. de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina
sdrago@fiq.unl.edu.ar

It is well known that iron deficiency anemia is the most prevalent nutritional deficiency worldwide. Zinc has also been recognized to have inadequate intakes in developing countries. In order to improve protein and iron contribution from maize based food, expanded products were developed. The objective of this work was to evaluate the mineral availability from expanded products based on corn flour added with soy flour as improver of protein quality and hemoglobin as iron source, with or without absorption promoters added. Potential iron and zinc availability was assessed using an *in vitro* method, which measures iron (FeD%) or zinc (ZnD%) dialyzability under controlled pH conditions, after a digestion simulating physiological processes. Determinations were triplicates and statistical analysis was performed by ANOVA and a posteriori LSD test. The extrusion process was carried out with a Brabender 20 DN single screw extruder, using a 4:1 screw compression ratio, a 3.5x20mm die, a screw speed of 150 rpm, and a barrel and die temperature of 160°C. Sample was prepared using corn: soy and bovine hemoglobin in 87.5:12:0.5 (W/W) ratio. A corn: soy (88:12) was used as a control. Samples were also evaluated using Na_2EDTA and sodium citrate as absorption promoters. Results show that the sample with hemoglobin added has lower Fe and Zn dialyzabilities than the control. Both EDTA and citrate increase iron dialyzability in relation to the control and the hemoglobin fortified samples. Although these promoters increase Zn availability from the non-hemoglobin fortified samples, only EDTA has an effect on the dialyzability of Zn in the sample with hemoglobin. Taking into account the potential contribution of iron from these products (content and % dialyzability) the sample with hemoglobin and EDTA provides three times the iron amount of the control and twice the iron amount of the control with promoters.
Iron fortification using hemoglobin is not easy because it impairs the color, the flavor and the texture of the product. Even thought an acceptable product was developed the contribution of iron of such product is the same than those from a ferrous sulfate fortified sample. Hemoglobin out from the natural meat matrix did not show advantages on traditional fortification. Even though the methodology used will account only for physicochemical factors influencing mineral bioavailability, most of the dietary factors in intestinal lumen influencing Fe and Zn solubility and dialyzability could be correctly evaluated through it.

P005. EFFECT OF *LUPINUS ANGUSTIFOLIUS* CHELATING PEPTIDES ON MINERAL DIALYZABILITY FROM FORTIFIED FOODS. Silvina R Drago[1], Manuel Alaiz[2] and Javier Vioque[2].
[1]Instituto de Tecnología de Alimentos, FIQ, UNL, Santa Fe, Argentina-CONICET
[2]Instituto de la Grasa (CSIC), Sevilla, España
sdrago@fiq.unl.edu.ar

There is a great interest in natural components with biological properties. Among them, peptides with different bioactive capacities include the mineral chelating capacity or promoting mineral absorption. These peptides could be release during de digestive process or can be produced from in vitro protein hydrolysis. The objective of this work was to evaluate the effect of the chelating peptides on mineral availability from fortified juice and dairy products. Hydrolysates of *Lupinus angustifolius* were obtained through sequential hydrolysis with alkaline protease and exopeptidase. Samples at different times of hydrolysis were got and evaluated respect to the capacity of block out the discoloration of β-carotene, using the assay of β-carotene copper ion-catalyzed oxidation. The sample with higher antioxidant capacity was utilized to obtain chelating peptides. For purify these peptides, a metal-affinity separation was carried out, using a column charged with Cu^{2+} and a fraction of chelating peptides/free amino acids (CP) was obtained. Orange juice was fortified with ferrous sulfate and zinc sulfate (Fe+Zn) or ferrous sulfate, zinc sulfate and calcium chloride (Fe+Zn+Ca) at 15 mg/l, 8 mg/l and 380 mg/l of Fe, Zn and Ca. The fraction of CP was added at 8 mg protein/100 ml to mineral fortified orange juice. Fortified orange juice without peptides was used as control.Milk was fortified with ferrous sulfate at 18 m/l of Fe and the fraction of (CP) was added at 19 mg protein/ 100 ml. an aliquot was fermented. Fortified milk or yogurt without peptides was used as controls. All fortifications were made 24 h prior of the determination of mineral dialyzability. Potential iron and zinc availability was assessed using an *in vitro* method, which measures mineral dialyzability (D%) under controlled pH conditions, after a digestion simulating physiological processes. Determinations were triplicates and statistical analysis was performed by ANOVA and then LSD test. Results show that chelating peptides impaired FeD% from Fe+Zn+Ca orange juice sample and increased ZnD% and CaD% from the Fe+Zn fortified orange juice. In this food the differences between samples were statistically significant but small. On the other hand, CP impaired FeD% and CaD%, from milk and yogurt. Respect to intrinsic zinc, CP did not have effect on ZnD% from milk but impaired ZnD% from yogurt. These peptides are rich in histidine (35,3 %), principal amino acid involved in the metal affinity property. Moreover, approximately 50% of this fraction is composed of free amino acids, which easily could bond minerals and form soluble and dialyzable complexes. Complex interaction among the matrix, the pH, the minerals and the CP are responsible of the inhibitory effect of this chelating fraction on mineral availability from dairy products.

P006. EFFECT OF GLOBIN, ERYTHROCYTE STROMA, COW, CHICKEN AND FISH MEAT ON HEME-IRON BIOAVAILABILITY. Valerie Weinborn, Manuel Olivares, Miguel Arredondo, Eva Hertrampf and Fernando Pizarro.
Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile,
vweinborn@inta.cl

Iron deficiency is the most common single nutrient disorder in the world and infants are at particular risk due to their rapid growth and limited dietary sources of iron. It has been demonstrated that the bioavailability of dietary iron is much more important to iron nutrition than the amount of iron ingested or the composition of meals. One example is the notable difference between the absorption of heme-iron and non–heme iron. Heme-iron is principally found in meat as hemoglobin (Hb) or myoglobin. This form of iron is easily absorbed because it is not influenced by the many ligands present in the diet; furthermore, it is directly taken up by enterocytes via an absorption pathway different from that of non–heme-iron. On the other hand, given that heme alone is less absorbed than hemoglobin, globins appear to be crucial for heme iron absorption. Nevertheless, the role of other proteins in heme-iron absorption is not fully understood. The aim of this work was to establish the role of globin, erythrocyte stroma, cow, chicken and fish meat on heme-iron bioavailability. **Subjects and methods:** Thirty healthy women (35-45 years old) were selected to participate in two iron absorption studies. Informed consent was obtained from all the volunteers prior to the absorption studies. In protocol A, subjects received 5 mg of iron as heme, intrinsically labeled with either 3 uCi 55Fe or 1 uCi 59Fe, plus 150 g of either cow, chicken or fish meat. In protocol B subjects received 5 mg of iron as heme, intrinsically labeled with either 3 uCi 55Fe or 1 uCi 59Fe, plus either globin, the whole erythrocyte (heme plus globin plus stroma) or erythrocyte plus beef. **Results:** In study A heme-iron bioavailability was significantly decreased when chicken meat or fish was added (One way ANOVA for repeated measures, post hoc Scheffé $p<0.004$), but no effect was found for the cow meat. In study B heme-iron bioavailability for heme alone, heme plus globin (Hb), Hb plus stroma and heme as erythrocyte plus beef was 13.0, 13.7, 25.0 and 21.3%, respectively (One way ANOVA for repeated measures= 9.5; $p<0.0001$; Sheffé post-hoc test Hb alone vs. Hb plus stroma, $p<0.03$). **Conclusion**: Heme-iron bioavailability is not modified by beef. Nevertheless, in presence of chicken or fish meat, heme-iron bioavailability diminishes significantly. Erythrocyte stroma was the only compound that increased heme-iron bioavailability. These results may have practical implications for dietary recommendations.
Support: Fondecyt Grant 1061060

P007. EFFECT OF PURIFIED ANIMAL AND VEGETAL PROTEINS ON HEME-IRON BIOAVAILABILITY. Valerie Weinborn, Manuel Olivares, Miguel Arredondo, Eva Hertrampf and Fernando Pizarro.
Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile.
vweinborn@inta.cl.

One of the most important problems in human nutrition is the deficiency of certain micronutrients; among them iron being the most relevant. Infants are at particular risk due to their rapid growth and limited dietary sources of iron. This nutritional deficiency affects infant's cognitive development during the first years of life and in adults reduces the productive ability. In pregnant women, iron deficiency has been associated with a greater risk of low birth weight; therefore it induces the development of nutrition-associated chronic diseases. **Aim:** The aim of this work was to establish the role of collagen, casein, albumin, zein, gliadin and glutelin (as purified proteins); and soy, pea and lentil protein isolate on heme-iron bioavailability. **Subjects and methods:** Forty four healthy women (35-55 years old) were selected to participate in three iron absorption studies. In protocol 1, subjects received 5 mg of iron as heme, intrinsically labeled with either 3 uCi 55Fe or 1 uCi 59Fe, plus either 1.7 g of collagen, casein or albumin. In protocol 2, subjects received 5 mg of iron as heme, intrinsically labeled with either 3 uCi 55Fe or 1 uCi 59Fe, plus either 1.7 g of zein, gliadin or glutelin. In protocol 3 subjects received the same heme compounds plus soy, pea or lentil isolate (equivalent to 1.7 g protein). **Results:** In study 1 heme-iron bioavailability for heme alone, heme plus collagen, heme plus casein and heme plus albumin was 9.8, 11.8, 12.9 and 11.1%, respectively (One way ANOVA for repeated measures= 2.48; $p<0.8$; N.S.). In study 2 heme-iron bioavailability for heme alone, heme plus zein, heme plus gliadin and heme plus glutelin was 6.2, 7.2, 7.5, and 5.9%, respectively (One way ANOVA for repeated measures= 3.42; $p<0.03$, Sheffé post-hoc test heme plus gliadin vs. heme plus glutelin, $p<0.03$). In study 3 heme-iron bioavailability for heme alone, heme plus soy, heme plus pea and heme plus lentil was 11.0, 7.3, 8.1 and 9.1%, respectively (One way ANOVA for repeated measures, $p<0.008$, Sheffé post-hoc test heme alone vs. heme plus soy, $p<0.02$). **Conclusion:** Heme-iron bioavailability is not modified by the animal proteins selected for the present study. Heme-iron bioavailability is not enhanced by certain vegetal proteins or by vegetal protein isolate. Nevertheless, in presence of soy protein isolate, heme-iron bioavailability diminishes significantly.
Support: Fondecyt Grant 1061060

P008. IRON SUPPLEMENTATION IN PREVIOUSLY ANEMIC BOLIVIAN CHILDREN NORMALIZED HEMATOLOGIC PARAMETERS, BUT NOT IMMUNOLOGIC PARAMETERS. Edgar A. Sejas[1], Patrick Kolsteren[2], Tom Hoeree[2], Daniel B Roberfroi[2].
[1] Instituto de Investigaciones Biomédicas, Universidad de San Simón, Cochabamba, Bolivia.
[2]Unidad de Nutrición, Instituto de Medicina Tropical de Amberes, Belgica
edgarsejas@yahoo.com.ar

Iron Supplementation in Previously Anemic Bolivian Children Normalized Hematologic Parameters, But Not Immunologic Parameters E. Sejas(a), P. Kolsteren(b), T. Hoeree(b) and D. Roberfroid(b) (a)IIBISMED, Facultad de Medicina-Universidad Mayor de San Simón, Cochabamba-Bolivia (b)Nutrition and Child Health Unit – Institute of Tropical Medicine, Antwerp, Belgium Iron deficiency anemia (IDA) is considered to be the most prevalent micronutrient deficiency in the world. Estimates indicate that 1.2 billion people suffer mild to severe forms of anemia and that up to 46% of schoolchildren in developing countries are affected. In 2003, ENDSA, the national demographic and health survey of Bolivia showed that 60% of children under five and 72% of children under 2 years old were anemic. Micronutrient deficiency has been suggested to impair cell-mediated immunity. In particular, iron, zinc and vitamin A deficiencies have an impact on the immune system. In vitro and in vivo laboratory studies indicate a link between iron deficiency and impaired T-lymphocyte proliferation. The exact effects or mechanisms of iron deficiency on maturation and proliferation of T-lymphocytes in vivo are, however, not yet known. This study investigated the effects of iron on the maturation of T-lymphocytes in anemic but otherwise healthy schoolchildren (no apparent protein-energy deficiency or other morbidity). Anemic children of a poor peri-urban school of Cochabamba city, Bolivia, were given iron treatment for three consecutive months. We chose to look at CD1a+ lymphocytes, which are immature thymocytes. The proportions of CD1a+ lymphocytes in the peripheral circulation measured at baseline and after treatment were compared with a reference group of age-matched non-anemic children controls from the same school. The immunologic parameters, although improved, did not reach the proportions of the control group. Overall, the proportion of circulating immature T-lymphocytes decreased from 18.3% to 9.2% in the treated following iron supplementation in anemic children, compared with 3.4% in non-anemic children. Key Words: iron deficiency • CD1a lymphocyte • school children.

P009. NUTRITIONAL STATUS OF ZINC IN CHILDREN WITH DOWN SINDROME. Adriana de Souza Lima, Bárbara Rita Cardoso and Silvia Maria Franciscato Cozzolino.
Department of Food Sciences in Experimental Nutrition - University of Sao Paulo - Sao Paulo – Brazil
baritacardoso@gmail.com

Down syndrome (DS) is the most common known genetic subtype of mental retardation and is characterized by an extra copy of the 21st chromosome. It has been speculated that many symptoms found in DS have direct relationship with the overexpression of the enzymes that are encoded on the extra 21st chromosome, including the increased activity of the enzyme superoxide dismutase (SOD). It is also often suggested that zinc metabolism is altered in presence of DS, and zinc seems to have a relationship with the metabolic alterations usually present in this syndrome. The aim of this work was to evaluate the nutritional status of zinc in children with DS by the determination of biochemical and dietetic parameters. The investigation was carried out on a group of children with DS (n=35) and compared with a control group (n=33), both aging between 4 and 11 years. Zinc was evaluated in plasma, erythrocytes and 24-hour urine by using the method of atomic absorption spectroscopy and the diet assessment was accomplished by using a 3-day dietary record. Comparing macro- and micronutrient intakes to Dietary Reference Intakes (DRI), the diet of both groups presented high protein content, adequate concentrations of lipids and carbohydrates, and a decrease in calories. Adequate ingest of zinc was observed in 40% in SD group and in 67% of control group. The zinc concentration in plasma and urine was significantly lower in DS group, however this same group showed higher zinc concentration in erythrocytes when compared to control group. The decreased zinc concentration in plasma and increased in erythrocytes can be occuring because there is an increase of zinc concentration in erythrocytes carbonic anhydrase since zinc is part of the structure of this enzyme. The low intake of zinc can be related with the reduced zinc urinary excretion since the body tries to mantain the homeostasis. The results of this study allowed concluding that zinc nutritional status in DS individuals is altered.

P010. ASSESSMENT OF GLUTATHIONE PEROXIDASE ACTIVITY IN ALZHEIMER'S DISEASE PATIENTS. Bárbara Rita Cardoso, Wilson Jacob Filho, Omar Jaluul, Thomas Prates Ong and Silvia Maria Franciscato Cozzolino
Pos graduate in Human Nutrition Applied at the University of Sao Paulo - Sao Paulo – Brazil
baritacardoso@gmail.com

Alzheimer's disease (AD) is characterized clinically by a progressive decline in cognitive function and neuropathologically by the presence of neuritic plaques and neurofibrillary tangles. While numerous hypotheses have been presented, the etiology of AD remains unknown. Oxidative stress has been implicated in the pathogenesis of a number of neurodegenerative disorders that involve neuronal degeneration and loss including Alzheimer's disease. This oxidative stress may be initiated by a decline of glutathione peroxidase (GPx) activity, a selenoenzyme that acts as free radical scavenger, in the maintenance of intracellular redox potential and in the detoxification processes of endogenous and exogenous compounds. A decrease in the activity of GPx would lead to neuronal cell death. Thus, the aim of this study was to compare the erythrocyte enzymatic activity of glutathione peroxidase in AD patients and normal elderly subjects. We measured erythrocyte enzymatic activity of GPx in 17 patients with AD and 20 elderly control subjects aged from 60 to 88 years. GPx activity was determined using the commercially available Randox® kit. Randox® provided standards. We observed that 88% (n=15) and 90% (n=18) from AD and control groups, respectively, showed adequate GPx activity. Using appropriate statistical procedures, significant difference was not observed in GPx activity between AD (38.42 U/gHb) and control (41.65 U/gHb) groups. The erythrocyte GPx activity in AD patients can be the result of a constant attempt to mantain homeostasis in response to an excessive free radical generation. Further studies could be useful for elucidate the role of oxidative stress and antioxidant enzymes in AD.

P011. ERRORS IN ESTIMATING ZINC REQUIREMENTS IN POPULATIONS. Farzad Amirabdollahian and R. Ash
Department of Health and Human Sciences, London Metropolitan University, London, UK
f.amirabdollahian@londonmet.ac.uk

As part of research into the zinc requirements of the UK population, we have carefully reviewed the estimates published by a number of expert committees: (e.g., COMA [1], WHO [2], FNB/IOM [3] and IZiNCG [4]). In this process, we have noted a number of methodological weaknesses in the published data. Below is the summary of them: (1) A possible statistical error was found in the regression analysis between total zinc absorption and intestinal endogenous zinc excretion [5]. (2) The effect of the increasing average weight of some populations on estimates of zinc requirements was neglected. (3) Estimates of zinc requirements for children and adolescents extrapolated from the adult data on a kilogram body weight basis may be erroneous [6]. (4) Menstrual zinc excretion is estimated by misinterpretation of data from one previous study [3, 4, 7]. (5) Estimates of average zinc absorption based on studies that used single test meals are inaccurate [2, 4]. (6) The mathematical algorithm of zinc absorption conflicts with the recent knowledge of zinc absorption requirements and dietary phytate levels [8]. (7) Dietary phytate values based on phytate contents of foods measured by ion pair high performance liquid chromatography (HPLC) are not reliable [9]. (8) Estimates of the coefficient of variations of zinc requirements are uncertain [4]. (9) Rounding and reporting decimal numbers for some values were inaccurate. (10) More results are available to estimate urinary, integumental and intestinal zinc excretion more accurately and (11) in process of estimating zinc requirement, the mean of the published data were used without considering the normal distribution of data. We have tried to exclude these weaknesses, but include the strengths of each expert committee in the development of estimates of zinc requirements for the UK population.

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P012. PHYTATE INTAKE AND MOLAR RATIO OF PHYTATE: ZINC IN THE DIET OF THE UK POPULATION. Farzad Amirabdollahian and R. Ash
Department of Health and Human Sciences, London Metropolitan University, London, UK
f.amirabdollahian@londonmet.ac.uk

Background: There have been many studies on phytate content of different foods; however, there is limited and inconclusive data on phytate intake of the UK population. In the UK, there is no extensive database of phytate content of foods. Such a database is necessary to estimate the phytate: zinc molar ratio in the UK population in order to establish reliable values for percent zinc absorption. The aim of the study was to develop a database of the phytate content of common UK foods by reviewing previous studies and to estimate the dietary phytate: zinc molar ratio of the population for the calculation of the estimated average requirement (EAR) of zinc. **Methodology:** Tables of the phytate content of foods were developed from 28 published and unpublished studies. Because these values were to be used for analysing the NDNS data, the tables were structured on the food types and food groups used in the NDNS. These phytate food tables were applied to food consumption data of the NDNS to calculate dietary phytate variables. As the dietary phytate and the phytate: zinc molar ratio was not normally distributed, non-parametric statistics were used. **Results:** The median daily intake of phytate for children, adolescents, adults and the elderly population were 496, 615, 809 and 629 mg/day respectively. The median phytate: zinc molar ratios for these age groups were 11.8, 10.4, 9.7 and 8.7 respectively. For all age groups, the median daily phytate intake for males was significantly higher than females; however, for children, adolescents and elderly populations, no variation in phytate intake between males and females was found, after adjusting for differences in energy intake. The main source of phytate was cereal and cereal products followed by vegetables, potatoes and savoury snacks. **Discussion:** Tables of the phytate content of foods are prone to errors. For example, there is variation in phytate based on varieties and genotypes; assays used for measurement; sampling procedure; and/or environmental factors. However, these tables, together with the NDNS data were adequate for estimating the phytate intake of the UK population. The average phytate intake of people in the UK is higher than those in some other developed countries but lower than in African and Asian countries. There is limited data on phytate intake of the UK population; however, current findings are mostly in agreement with those data. **Conclusions:** This study used available data on the phytate content of foods to provide an estimate of dietary phytate: zinc molar ratios for the UK population. Further research should focus on the generation of a more accurate and complete database of phytate content of UK foods.
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P013. ZINC SUPPLEMENTATION HAS DIVERGENT EFFECTS ON PLASMA HIGH-DENSITY LIPOPROTEIN CHOLESTEROL CONCENTRATIONS IN HUMANS: A META-ANALYSIS.

Meika J Foster[1], Peter Petocz[2], Samir Samman[1]
[1]Discipline of Nutrition and Metabolism, School of Molecular and Microbial Biosciences G08, University of Sydney, NSW, Australia
[2]Department of Statistics, Division of Economic and Financial Studies, Macquarie University, NSW, Australia
s.samman@mmb.usyd.edu.au

Background – High-density lipoprotein (HDL) cholesterol is inversely associated with risk of coronary heart disease (CHD). Although total cholesterol, low-density lipoprotein (LDL), and triglyceride concentrations appear unaffected by zinc supplementation in randomised controlled trials (Hughes S & Samman S, JACN 2006; 25: 285-291), the effect of zinc on HDL cholesterol concentrations requires further elucidation. Objective – To determine the effects of zinc supplementation on plasma HDL cholesterol concentrations in humans. Design – A search of electronic databases (Medline, PubMed, Web of Science, and CENTRAL) identified 20 English-language, controlled trials that investigated the effects of zinc supplementation, alone or in combination, on HDL cholesterol levels in humans. Two reviewers independently assessed risk of study bias against pre-determined criteria, including appropriateness of allocation, randomisation, blinding, and intention-to-treat protocols, and extracted data for meta-analysis. Forest plots showing random effects were generated by using endpoint values. Standard error of difference was calculated using the independence of supplement and control groups. Outcomes – The age range of participants was 18-106 years, with an average baseline HDL cholesterol concentration of 1.35 mmol/L. The duration of eligible trials ranged from 4 weeks to 7.5 years. Zinc supplementation ranged from 15-160 mg elemental zinc/d. Overall, the random effects analysis showed no effect of zinc supplementation on mean HDL cholesterol concentrations. When trials were grouped according to the participants' health status, zinc supplementation was shown to decrease HDL concentrations significantly (-0.10 mmol/L; n=13298; $P<0.001$) in apparently healthy subjects and increase HDL significantly (0.36 mmol/L; n=196; $P<0.01$) in subjects with Type 2 Diabetes Mellitus. Conclusion – Zinc supplementation decreased HDL cholesterol in healthy individuals but increased it in those with Type 2 Diabetes. The divergent effect of zinc supplementation may be mediated by differences in baseline zinc status and underlying metabolic perturbation.

P014. RATIOS OF IGF-1, IGF BINDING PROTEIN-3 AND ZINC IN CANCER AND BENIGN HUMAN PROSTATE HIPERPLASIA. Adam Daragó[1], Andrzej Sapota[1], Jan Taczalski[2] and Anna Kilanowicz[1]
[1]Department of Toxicology, Medical University of Lodz, Poland
[2]District Hospital in Zgierz, Poland
adarago@pharm.am.lodz.pl

It is believed that zinc (Zn) modulates IGF in varied types of cancer, including prostate cancer. In vitro studies show that Zn may alter IGF distribution. It is thought that Zn is responsible for the enhanced IGF-1 binding to IGF-R1 receptor and IGF BP-3 protein. IGFBP-binding proteins act as carriers and modulate IGF functions via IGF-1R receptor, which directly affects cellular growth. Zn may also exert its direct effect on IGF-1 binding protein, i.e., IGFBP-3, leading to the activation of tyrosine phosphatase, which in turn affects IGF-1. The aim of the reported study was to evaluate IGF-1 and IGFBP-3 levels in serum and the concentration of Zn and selected essential elements in prostate, depending on the nature of changes in proliferation (BPH or PCa). Altogether 64 tissues were subjected to an analysis; 15 without neoplastic changes (post mortem), 17 with hyperplasia and 32 with diagnosed prostatic adenocarcinoma. Neoplastic tissues were obtained from intraoperative segments. Before the analysis, all tissues were submitted to pathomorphological examination to confirm the diagnosis. Levels of IFG-1 and IGFBP-3 in the patients' serum were determined by employing the immunoenzymatic method with use of chemiluminescence by means of IMMULITE IGF-1 and IMMULITE IGFBP-3 kits. Zn, Cu, Ca and Mg concentrations were determined with AAS and Se with spectrofluorometric methods. The study did not show significant changes in the IGF level in patients with BPH and PCa. However, it was found that the statistically significant increase in the IGF-1 and IGFBP-3 ratio in the serum of patients with hypertrophy and prostate cancer (compared with controls) was caused by an evident downward tendency in the level of IGFBP-3-binding protein. The analysis of the relationship between IGF-1 and IGFBP-3 showed high correlation (R=0.84) only in the control group, free of histopathological changes in the prostatic gland. As regards BPH and PCa, correlation coefficients were low, which may suggest a disturbed balance between IGF and protein binding in the pathologic condition of prostate. The study revealed significantly increased concentrations of the studied elements (Zn, Cu, Ca, and Mg) in the tissues of patients with the diagnosed benign neoplasm of prostate; the highest, over twofold, increase applied to Zn and Cu. Almost completely opposite effect was found in prostate tissues with the diagnosed PCa. The levels of a great majority of trace elements (except for selenium and copper) were significantly lower, especially with respect to the BPH group. The mostly pronounced decrease was noted for Zn. A similar tendency in changes was observed for Ca and Mg. Only Cu level in tissues with PCa was significantly higher compared with the control group, and was comparable with that in tissues with BPH. The study was supported by the State Committee for Scientific Research, Poland (Grant No. 015 31/0783).

P015. EFFECT OF RED WINE EXTRACT SUPPLEMENTATION ON COPPER, POLYPHENOLS AND TOTAL ANTIOXIDANT STATUS IN ELDERLY PEOPLE.
Grzegorz Mielcarz[1], Aleksander Barinow-Wojewodzki[2]
[1]Department of Chemistry and Clinical Biochemistry, Poznan University of Medical Sciences, Poznan, Poland
[2]Physical Education University, Poland
mielcarz@neostrada.pl

Introduction: Epidemiological studies carried out in France have shown that mortality from cardiovascular disease is significantly lower, than in other countries, although consumption of saturated fatty acids is high. It has been claimed that polyphenols and copper present in red wine, protects blood vessels from formation of atherosclerotic plaques and decreases risk factors for cardiovascular diseases. Red wine is a major dietary source of polyphenols and copper. Possible mechanisms for the protective function of polyphenols are on fibrinolysis. Copper plays important role in antioxidant defence system, as cofactor for superoxide dismutase and lysyloxidase involved in cross-linking of collagen and clotting factor V. These enzymes prevent membrane damage, especially to free radicals, repair the arterial well. Decreased copper body status can be a risk factor for Ischemic Heart Disease (IHD) and Coronary Artery Disease (CHD). Risk of IHD and CHD is higher in elderly people whereas bioavailabilities of essential nutrients are decreased. Objective: We investigated the in vivo effect of supplementation of non-alcoholic red wine polyphenols extract (RWX) on copper and polyphenols status in blood by measuring plasma and leucocyte copper and plasma total polyphenols. Total antioxidant status in plasma was determined in elderly women (aged 68 – 71y). Methods: All subjects were fasted overnight. Twenty women were given 1g/day starch as a placebo and twenty women volunteers were given 1g RWX, supplemented daily for 2 weeks. The polyphenols composition of the RWX was analysed by HPLC. Plasma and leukocyte copper were measured by Atomic Absorption Spectroscopy, plasma total polyphenols determined by Folin-Ciocalteau method. FRAP (Ferric Reducing Ability of Plasma) method applied for total plasma antioxidant status determination. Results: The plasma copper concentration was not significantly different in placebo and RWX group. However, significant difference was found for leukocyte copper ($p<0.05$). Plasma total polyphenols showed a significant increase after 14 days of RWX supplementation ($p<0.001$). FRAP which can reflect total antioxidant status increased significantly ($p<0.05$) after two weeks RWX supplementation. Conclusion: Supplementation of polyphenols and copper obtain from red wine have a beneficial effect on the fibrinolytic and antioxidant system in elderly people, through the increased level of plasma polyphenols and leukocyte copper (which can better reflect copper body status than plasma copper). Another beneficial effect of red wine polyphenols is to increase plasma total antioxidant status and thus help prevent the formation of obstructive lesions in cardiovascular disease. Funding: Supported by Polish Ministry of Education, grant N N305 267834

P016. POTENCIAL INDICATORS TO DETECT EARLY EFFECTS OF COPPER EXPOSURE IN HUMANS. Miguel Arredondo, Silvia Flores, Héctor Núñez, Valeria Candia, Fernando Pizarro, Magdalena Araya. Micronutrient Laboratory, INTA, Universidad de Chile. marredon@inta.cl

Introduction: To identify risk groups in the populations has been a mayor challenge for nutrigenomics. In the area of copper (Cu) metabolism this is particularly difficult because the early effects of deficiency and of excess are poorly defined and there are no sensitive indicators to detect them. **Aim:** To evaluate potential indicators to detect early effects of excess Cu exposure. **Methods:** Using a double blind design, we studied hepatic function (transaminases), C Reactive Protein and iron nutrition in 400 subjects (20 to 25 years old). 154/400 had normal transaminases and no signs of inflammation. Based on previous studies, participants were chosen among those belonging to the 5% tails of the distribution ceruloplasmin (Cp) curve. Only 26/400 fulfilled this requisite and accepted to participate in the studied. Participants were divided into two groups: Cu supplemented (CuS), receiving 8 mg/day of copper for 2 months or the control group receiving placebo of similar appearance. Polymorphonuclear cells (PMNc) were isolated from a blood sample at time 0, 2 or 60 days after treatment. Cells were incubated with different copper concentrations in the media (1; 5; 20 µM) for 20 hours. Total Cu content and mRNA expression of transferrin receptor (TfR), superoxide dismutase (SOD) and Atox chaperone by real time PCR were measured. **Results:** PMNc incubated with low copper concentration acquire higher levels of copper than cells incubated with high levels of copper (One way ANOVA, $p<0.001$) in both CuS and controls. TfR, SOD and Atox mRNA expression increased with time of Cu supplementation (One way ANOVA, $p<0.01$) in controls with low serum Cp. However, in controls with high Cp the higher values of expression was in cells incubated with 5 µM of copper (One way ANOVA, $p<0.02$). Both supplemented group (high and low Cp) had a low mRNA expression at 5 µM of copper (One way ANOVA, NS) **Conclusion:** the in vitro pattern of response of PMNc to different copper concentrations in the media differ depending on the amount of copper supplementation received. These genes are potential candidates for of early effects to excess copper exposure.

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P017. COPPER STATUS AND THEIR RELATION TO THYROID HORMONE PROFILE IN MALE AND FEMALE GOITROUS PATIENTS.
Ghulam Abbas Kandhro[1], Tasneem Gul Kazi[1], Sirajuddin[1], Hassan Imran Afridi[1], Naveed Kazi[2], Mohammad Balal Arain[1], Raja Adil Sarfraz[1], , Abdul Qadir Shah[1], Nasreen Syed[3] and Jameel Ahmed Baig[1]
[1]National Centre of Excellence in Analytical Chemistry, Sindh University, Jamshoro, Pakistan.
[2]Liaqut University of Medical and Health Sciences, Jamshoro, Pakistan.
[3] Nuclear Institute of Medical Radiotherapy (NIMRA) Jamshoro76080, Pakistan
gakandhro@yahoo.com

Background: Both Copper (Cu) and thyroid hormones play essential roles in the human body. Methods: In this study evaluated the Cu status in biological samples and thyroid hormones concentrations in 60 goitrous male (GMPs) and 72 female patients (GFPs), and compared with non goitrous subjects of both genders (M= 106, F= 120) of age range 16-30 years. The biological samples were analyzed for Cu concentration using electrothermal atomic absorption spectrophotometer (ETAAS), following their microwave assisted acid digestion. Quality control for the methodology was established with certified samples and with those obtained by conventional wet acid digestion method on the same CRM and real samples. Results: The results showed that the significantly lower mean values of Cu in serum, while high level in urine samples of GMPs and GFPs were observed as compared to control subjects ($p < 0.005$ and 0.007) respectively. The mean values of free triiodothyronine (FT3) and free thyroxin (FT4) were found to be lower in goitrous patients of both genders than in the age matched healthy control ($p < 0.006$ and 0.002) respectively, in contrast high mean values of thyroid stimulating hormone were detected in GMPs and GFPs ($p < 0.009$). The serum and urine Cu exhibited the significantly positive correlation with FT3 and FT4 in both genders. Conclusion: It was observed that the development of thyroid hormones in GMPs and GFPs could be more or less influenced by Cu deficiency, so need of Cu supplementation will be required to improve the efficacy of thyroid metabolism in goitrous patients.

P018. CHRONIC EXPOSURE TO HIGH COPPER DOSES IN NON-HUMAN PRIMATES: A MODEL OF COPPER LOADING. Héctor Núñez, Miguel Arredondo, Marco Méndez, Fernando Pizarro, Manuel Olivares, Ricardo Uauy and Magdalena Araya.
Institute of Nutrition and Food Technology (INTA), University of Chile
hnunez@inta.cl

Chronic exposure to high copper dosing represents a challenge to the efficiency of homeostatic regulation. At present, the amounts of copper and time of exposure to trigger adverse effects in liver function of primates are unknown. **Hypothesis:** Cebus apella chronically exposed to high copper dosing (5.5–7.5 mg Cu/kg/d/3years) will increase their copper deposits in liver and will develop detectable signs of liver dysfunction and histological alterations. **Objective:** to measure clinical indicators, copper content in liver, hair and blood, liver function indicators and liver histology in young and adult Cebus apella chronically exposed to high copper dosing. **Methods:** Cebus apella (n=16) living indoors under constant care of nursery and veterinary staff at the Primate Center, Pontificia Universidad Católica de Chile remained in individual cages in a room with controlled temperature, humidity and 12 hours light-dark cycle, with water and food available ad libitum, for three years. Four groups were formed: adult experimental animals (G1E, n=4) received 7.5 mg Cu/kg/day (as copper gluconate); young experimental animals (G2E, n= 4) received 5.5 Cu/kg/day; adult control animals (G1C, n=4) and young control animals (G2C), n=4) received a placebo of same colour and aspect. Doses represented the maximal amount of copper gluconate tolerated without triggering acute adverse effects. Clinical evaluations (general conditions, hair, activity, appetite, physical examination, anthropometry) were conducted monthly; blood biochemistry (hemogram, erythrocyte zinc protoporphirin, serum Fe, Zn, Cu (ug Cu/ml) and Ceruloplasmin (activity), hair Cu (ug Cu/mg dry tissue) and aminotransferases (GTP, GOT, GGT) were measured every third month. Liver Cu content (ug Cu/mg dry tissue) and histology were assessed in biopsies obtained every 4mo (first year) or 6mo (thereafter). Biochemical measures were performed using routine techniques and mineral contents by atomic absorption spectrometer equipped with graphite furnace (SIMAA 6100, Perkin Elmer, Shelton, CT). Results were analyzed by ANOVA repeated measures and then applying post-hoc Bonferroni correction. **Results:** Animals remained clinically healthy and active during the three year assessment. Young ones grew as expected. Serum Zn, Cu, Cp activity and liver aminotransferases were not different between experimentals and controls and between experimentals and previously obtained reference values (H. Núñez et al. J Medical Primatology 2007). After three years copper exposure, iron nutrition indicators were significantly lower in both experimental groups (Anova rep measures, $P<0.05$). Liver histology remained normal. At 18 month loading Rhodamine positive granules were observed in patches; they progressively increased in time, mainly in hepatocytes and were very prominent and widely distributed after the 3-year loading. Comparison of liver Cu content showed a significant increase in the adult and young animals groups (both $P<0.05$). Hair Cu increased significantly in both experimental groups ($P<0.05$). **Discussion:** The three-year loading with high Cu doses resulted in increased deposit of copper in liver and hair, suggesting that this is an adequate model of copper loading, but it failed to induce clinical, blood and liver changes suggestive of damage associated.
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P019- EFFECTS OF BRAZILIAN NUTS INTAKE ON THE SELENIUM BLOOD CONCENTRATIONS AND ON ERYTHROCYTE GLUTATHIONE PEROXIDASE ACTIVITY IN MORBID OBESE WOMEN.

Cristiane Cominetti[1], Maritsa Carla de Bortoli[1], Thomas Prates Ong[2], Fernando Salvador Moreno[2], Arthur Belarmino Garrido Jr.[3] and Silvia Maria Franciscato Cozzolino[1]

[1]Laboratory of Nutrition-Minerals, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil

[2]Laboratory of Diet, Nutrition and Cancer, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil

[3] Surgical Division, Department of Gastroenterology, Faculty of Medicine, University of São Paulo, São Paulo, SP, Brazil

ccominet@usp.br

Brazilian nuts are a recognized dietary source of selenium (Se) and its Se bioavailability is high. On the other hand, it is known that obese subjects have altered body mineral distribution, probably because of the retention of these substances into fat tissues. The aims of this work were to verify the selenium nutritional status and to evaluate the effects of the consumption of one unit of Brazilian nut per day for two months on the selenium blood levels and on the erythrocytes glutathione peroxidase (GPx) activity, in morbid obese women living in Sao Paulo, Brazil. The mean content of selenium in the nuts was 58.1 μg/g and each mean unit weighed 5 g, thus providing 290 μg/day. These nuts were originated from the Amazon area, which has excellent selenium contents in soil. Sixteen women formed the total study population so far. All of them were in reproductive age and did not suffer from diabetes, thyroid diseases, did not ingest multivitamin tablets and weight or cholesterol-lowering medicines, and did not smoke. The selenium levels were analyzed by hydride generation-atomic absorption spectrometry and the GPx activity was measured by a commercial kit (Ransel-Randox®). The initial mean ±SD selenium concentrations were: 55.4 ±14.9 μg/L (range 27.2-77.5) in plasma; 52.8±18.4 μg/L (range 23.6-81.4) in erythrocytes. The initial mean ±SD erythrocyte GPx activity was 25.9±9.6 U/g Hb (range 7.7-39.3). After the intervention with Brazilian nuts, the concentrations changed to 152.8±37.8 μg/L (range 97.9-234.9) in plasma; 212.9±49.9 μg/L (range 153.7-347.5) in erythrocytes; GPx activity increased to 41.6±11.4 U/g Hb (range 24.6-73.2). Percentages of increase were 62.9%, 74.9%, and 39.7%, respectively. All differences were statistically significant at a $p<0.01$. Initially, there was reduced selenium in plasma, selenium in erythrocytes and GPx activity in erythrocytes in 35.3%, 52.9% and 47% of the patients, respectively. Brazilian nuts efficiently raised all the markers measured in morbid obese subjects. On the basis of these data, if carefully introduced, Brazilian nuts appear as a good alternative to complement the usual diet, even for morbid obese subjects, in order to maintain an adequate selenium nutritional status and an optimum GPx activity.

P020. MERCURY LEVEL AND SELENIUM STATUS IN WOMEN LIVING IN CUBATÃO, SÃO PAULO, BRAZIL. Maritsa C. de Bortoli[1], Cristiane Cominetti[1], Luciana A. Farias[2], Déborah I.T. Fávaro[2] and Silvia M.F. Cozzolino[1].
[1]School of Pharmaceutical Sciences, Department of Food and Experimental Nutrition
[2]Institute for Energetic and Nuclear Research, University of São Paulo, São Paulo, Brazil.
mbortoli@usp.br

Cubatão, a city at the coastland of São Paulo state, was known in the past for its severe pollution. Even nowadays heavy metals are still found in its watercourses, among the most hazardous ones is mercury. However, a protection against the risk of mercury intoxication is selenium, which besides its regular functions -mainly as part of antioxidant enzyme glutathione peroxidase (GPx)- may form strong bounds with the metal. Until now, there is no study showing the relationship between these two substances in the population of Cubatão. Hence, the aim of our research was to assess the levels of mercury and selenium, and glutathione peroxidase (GPx) activity, in women living in Cubatão. Forty eight women formed the group of study, they were all in reproductive age, did not suffer from diabetes or thyroid dysfunctions, were not pregnant or breast-feeding, did not take multivitamins tablets and lived in Cubatão for more than 20 years in average. A sample of hair from the scalp was collected and mercury levels were analyzed by cold vapor atomic absorption spectrometry. A blood sample was collected to assess selenium levels in plasma and erythrocytes by hydride generation-atomic absorption spectrometry, and GPx activity was measured by commercial kit (RANDOX). The mean±SD mercury concentration in hair was 0.201±0.189 PPM (range 0.012-0.887). Mean selenium concentration was 57.7±14.1µg/L (range 35.1-88.9) in plasma, and 72.4±17.4 µg/L (range 40.4–114.4) in erythrocytes. The medium value found for GPx activity in the erythrocyte was 38.9±9.8 U/gHb (range 22.8–66.3). According to Pearson's test (p=0.01) we found no correlation between mercury and selenium in plasma (r=-0.0189), mercury and selenium in erythrocytes (r=0.04), or mercury and GPx (r=-0.25). Although some could hypothesize the values of mercury to be higher, they were in accordance to women's statement of very low fish intake; also, the findings show low risk of intoxication by this metal. In addition, values of selenium and GPx activity were adequate in relationship to the reference values for theses parameters, denoting that this population is not at risk of selenium deficiency. It is advisable to continue these studies in larger segments of population, to assess selenium nutritional status, as well as mercury levels.
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P021. NUTRITIONAL STATUS OF SELENIUM OF PATIENTS WITH THYROID DISORDERS OF FORTALEZA/CE–BRAZIL. Carla Soraya Costa Maia[1]; Liliane Viana Pires[1]; Luciana Sigueta Nishimura[2], Alexandre Coelho Pimentel[2]; Rafael Barofaldi Bueno[2]; Renan Magalhães Montenegro Junior[3]; Virgínia de Oliveira Fernandes[3] and Sïlvia Maria Franciscato Cozzolino[1].
[1]Department of Food Science and Experimental Nutrition, School Pharmacy, University of São Paulo, Brazil.
[2]Department of Endocrinology and Metabology, Federal University of Ceará, Brazil.
csoraya@usp.br

The selenium (Se) is a trace mineral whose essential function is linked to its antioxidant, involvement in immune function and the maintenance of homeostasis of the thyroid gland. His presence in food is linked to the concentration of this mineral in soil. The soil of the state of Ceará seems to be rich in Se. The selenoenzymes are involved in thyroid homeostasis, mainly the selenodeiodinases. Studies in this area are important to elucidate the Se participation in thyroid dysfunctions in different groups. Objective: To evaluate the nutritional status related to selenium in thyroid dysfunctional patients attending a public hospital in Fortaleza/Ceará – Brazil. Material and methods: Patients were evaluated in Federal University Hospital of Ceará, divided into five groups: control group (CG), hypothyroidism post-thyroidectomy (HO), Hashimoto's thyroidites (HA), multinodular toxic goitre (HE) and Graves disease (GR). 20 ml of peripheral blood was collected from fasting patients. All material was previously desmineralyzed by nitric acid at 20%. Selenium plasma and erythrocyte concentration was measured after chemical digestion of the blood sample using Hydride Generation Atomic Absorption Spectroscopy (HGAAS). The research was approved by the Research Ethics Committee at the Faculty of Pharmaceutical Sciences of USP. Results: Concentrations of Se in plasma were (medium±standard deviation in µg/L) CG - 77,59±15,53; HO –68,61±18,41; HA – 39,08±5,57; HE – 78,34±11,38; GR – 54,58±17,08. In the red cells were observed concentrations: CG -118,94±26,42; HO – 114, 57±36,97; HA – 143,33±36,42; HE – 147, 59±11,60; GR – 131, 39±29,01. Conclusion: Only the plasmatic values of HE presented below the CG. In the red cells, only the HO was below the CG. It seems necessary to enlarge the number of participants in the study, especially in groups HA and HE to clarify the differences between plasma and erythrocyte values. Study financed by: FAPESP

P022. ASSESSMENT OF NUTRITIONAL STATUS ON THE SELENIUM OF PATIENTS WITH TURNER SYNDROME IN DIFFERENT STAGES OF DEVELOPMENT.
Liliane Viana Pires[1], José Alexandre Coelho Pimentel[1], Rafael Barofaldi Bueno[1], Luciana Sigueta Nishimura[1], Carla Soraya Costa Maia[1], Adriana Siviero-Miachon[2], Tatiana Fabbri T[2], Angela Maria Spinola-Castro[2] and Silvia Maria Franciscato Cozzolino[1].
[1] Department of Food Science and Experimental Nutrition, School of Pharmacy, University of São Paulo, Brazil.
[2] Division of Pediatric Endocrinology, Department of Pediatrics, Federal University of São Paulo-UNIFESP/EPM, Brazil.
lianenut@yahoo.com.br

Studies relating to Turner Syndrome with the nutritional status on micronutrients, in particular the selenium are practically non-existent. This study aimed to assess the nutritional status on selenium in this population, considering the different stages of life. For the evaluation of body composition were made anthropometric measures, such as weight (kg) and height (cm). The food consumption was assessed by the method of registration of food three days and analysis through software Nutwin. The determination of concentrations of selenium in plasma, red cells, urine and nails was by Hydride Generation Atomic Absorption Spectroscopy (HGAAS). Anthropometric evaluation of the children showed that 55.6% were eutrophic and 44.4% with overweight, according to the rate of weight/height. The adolescents were classified according to the percentile of BMI adjusted for age, where 73.7% were between the percentile 5 and 85 (eutrophic) and 26.3% above the percentile 85 (overweight). Regarding the group of adults, 42.9% were overweight and 14.3% with obesity, according BMI (kg/(m)²). The assessment of food consumption was made through the software Nutwin, demonstrating to be appropriate in relation to the contribution of energy of macronutrients in the diets of children, adolescents and adults. The percentage of intake of selenium by participants regarding DRI's was 100% above of the EAR. The nutritional status of selenium, 77.8% of children, 78.9% of adolescents and 85.7% of adults were found deficient in plasmatic selenium and 55.6%, 52.6% and 57.1% of children, adolescents and adults, respectively, were deficient for selenium in erythrocyte. The percentage of children, adolescents and adults with low concentrations of selenium in urine was 100%, 94.7% and 100% respectively. The determination of the concentration of selenium nail showed that 100% of children, adolescents and 93.8% from 66.7% of adults with values were reduced in this compartment. Thus, it can be concluded that the nutritional status on the selenium is deficient for most patients, because the concentrations of these micronutrients are reduced for most of the parameters used.

P023. RELATIONSHIPS BETWEEN RESPIRATORY DISEASES, NUTRITION ITEMS AND TRACE ELEMENTS IN INGESTED FOOD. Maria do Carmo Freitas[1] and Adriano M.G. Pacheco[2].
[1]Reactor, Technological and Nuclear Institute, Sacavém, Portugal;
[2]CERENA-IST, Technical University of Lisbon, Lisboa, Portugal.
cfreitas@itn.pt

In a recent study 1161 children aged 5-10 years living and studying in Lisbon, Portugal, were questioned relatively to their asthmatic and rhinitis symptoms and week frequency of ingested food items. The latter concerned meat, fish, fruit, vegetables, legumes, cereals, pasta, rice, butter, margarine, dried fruits, potato, milk, eggs, and fast food (namely hamburgers). The symptoms were divided in asthma (9% of the children), asthma precursors (26% of the children) and rhinitis (28% of the children). The health questions might have three possible answers: yes - associated to number 2, no - associated to number 1, and no answer associated to number 13. The nutrition questions might also have three possible answers: eaten more than 3 times/week – associated to number 1, eaten less than 3 times/week – associated to number 2, no answer – associated to number 13. The number 2 was attributed for instance to the following % of children: 86% and meat, 53% and fish and vegetables, 88% and fruits, 58% and butter, 13% and margarine, 65% and rice, 51% and potato, 94% and milk, 4% and fast food. Health and nutrition data were processed with Pearson correlations and the following conclusions were estimated: asthmatic precursors may be related to consumption of rice, dried fruits, milk, and eggs; asthma may be related to consumption of meat, fish, pasta, and rice, and rhinitis may be related to consumption of meat, fish, fruit, vegetables, legumes, cereals, pasta, and rice. Asthmatic precursors were found to vary positively with the amount of meat, fish, pasta, rice and butter, and negatively with legumes and potatoes. Considering previous studies on the chemical composition of the studied nutrition items, a selection of trace elements were taken in order to study possible relationships with the outcomes of the described Pearson correlations, taking into account their essentiality and role in the immunologic system, skeleton formation, blood constitution, brain needs, etc. The discussion is based on the following elements: Ca, Fe, K, Mg, Se, and Zn.

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P024. TOTAL DIET STUDY: FE, CR, ZN AND SE CONTENT ESTIMATION OF SÃO PAULO STATE (BRAZIL) DIET BY INSTRUMENTAL NEUTRON ACTIVATION. Roseane P. Avegliano[1;2] , Vera A. Maihara[2] and Fábio F. Silva[3]
[1]Divisão de Alimentação. Coordenadoria de Assistência Social/USP, São Paulo, Brazil.
[2]Instituto de Pesquisas Energéticas e Nucleares. IPEN/CNEN-SP, São Paulo, Brazil.
[3]Empresa Júnior de Informática, Matemática e Estatística - IME Jr/USP, São Paulo, Brazil.
pagliaro@usp.br

Total Diet Studies (TDS) are based on the evaluation of food samples representing a Market Basket, which shows dietary habits of a given population. World Health Organization (WHO) has encouraged countries to conduct their own TDS, which is already being carried out in different countries. This Brazilian TDS involved essential steps to establish a TDS: information about food consumption (a recent national household food budget survey, including 5440 foods); development of a Market Basket (sampling of 57 foods consumed more than 2g/day/person, grouped in 24 food groups: cereals; leguminous; leafy, fruity and tuberous vegetables; tropical fruits; other fruits; flours; pasta; breads; biscuits; prime and standard grade beef; pork meat; sausage; poultry; milk/cream; other dairy products; sugars; sweets; sauces; alcoholic beverages; coffee and ready-made dishes); kitchen preparation of foods in restaurants of University of São Paulo (preparing ready-to-consume foods, individually and mixing foods of the same food group); chemical analysis (food groups were homogenized, pulverized and analyzed by Instrumental Neutron Activation). Trace element contents were determined in the 24 food groups. Average trace element range concentrations were: Fe (mg kg-1)): 0.05 (alcoholic beverages) and 45.00 (biscuits); Zn (mg kg-1)): 0.02 (alcoholic beverages) and 88.62 (standard grade beef); Cr (µg kg-1): 2.21 (coffee) and 734.99 (sweets); Se (µg kg-1): 2.93 (coffee) and 128.98 (poultry). Additionally, individual daily trace element intake for each element was calculated by multiplying the individual trace element concentration in the sample by the weight of the analyzed food group. Individual average daily intakes ranges were: Fe (mg): 0.0008 (alcoholic beverages) and 1.89 (breads); Zn (mg): 0.0003 (alcoholic beverages) and 0.71 (cereals); Cr (µg): 0.033 (ready dishes) and 8.62 (breads); Se (µg): 0.067 (biscuits) and 1.52 (milk/cream). Total dietary intake of each trace element was calculated by adding daily intakes of the analyzed food groups: 4.9 mg/Fe; 3.7 mg/Zn; 19.8 µg/Cr; 7.5 µg/Se. According to the Dietary Reference Intakes (DRIs) for trace elements, the average daily intake was far below the Recommended Dietary Allowances for Fe, Zn, Se, and was also below the Adequate Intake for Cr for the most frequent population groups: women and men aged between 19 and 49 years. The adequacy percentage was: Fe 37.3, Zn 39.0, Cr 66.0, Se 13.5. Major food group sources of Zn, Fe and Se were consumed in low percentages. Wheat flour fortified with Fe in breads was the main source of Fe. Moreover, main food sources were not present in the Market Basket. The results as expected were below DRIs because the Market Basket contained only those foods most consumed, representing 57% of the weight of consumed foods by the population. Furthermore, the national food budget survey included meals only consumed in the household. Thus, results could be underestimated. Despite the number of foods, this TDS is well structured and likely to be increased to include more foodstuffs. This TDS from the state of São Paulo is the first of this kind in Brazil and can contribute to further research.

P025. DETERMINATION OF IRON, SELENIUN AND ZINC IN MILK FORMULAS COMMERCIALIZED IN SÃO PAULO CITY- BRAZIL. Paola Santos[1], Vera A. Maihara[1], Mitiko Saiki[1], Maria Esther Ceccon[2], Jane Oba[3], Cecília Yu[4], Roseane P. Avegliano[5]
[1]Instituto de Pesquisas Energéticas e Nucleares -São Paulo, Brazil
[2]Universidade de São Paulo, Faculdade de Medicina -- SP, Brazil
[3]Universidade de São Paulo, Instituto do Coração - SP, Brazil
[4]Hospital Estadual Sapopemba, Departamento de Pediatria – SP, Brazil
[5]Universidade de São Paulo- Coseas/USP, SP, Brazil
paolaipen@usp.br

There are biological requirements, especially from the nutritional and immune point of view, which make breast milk the most appropriate food for newborn babies and infants. However, in cases where mothers are not able to maintain breastfeeding, the best alternative to meet infant´s nutritional needs is the pediatric industrialized milks, which are produced with cow or soy-based milk and enriched with vitamins, minerals, trace elements etc, to substitute maternal milk. There are several maternal milk substitutes offered in the market. According to the Brazilian Trade Standards of Food for Breastfeeding Infants (NBCAL), the infant formulas are classified as: milk formula for newborns of high risk; formula for infants aged 0 to 6 months; formula for infant with special requirements and formula for infants aged over six months. In this study, Fe, Se and Zn were determined by Instrumental Neutron Activation Analysis (INAA) in seventeen different milk formulas: 3 samples of soy-based; 5 samples of cow milk for 0-6 aged infants; 4 cow-milk samples for infants over 6 months; 2 samples of cow milk for newborns of high risk and 3 formulas for special requirements. The samples were acquired in São Paulo city during 2005 - 2007. About 200 mg of these powdered samples and the element standards were irradiated in the IEA-R1 nuclear research reactor at IPEN / CNEN-SP for 8 hours under a thermal neutrons flux 10^{12} $cm^{-2}s^{-1}$. After the 15 day decay period, the irradiated samples and standards were measured in a gamma spectrometer coupled to an Hiperpure Ge detector, with 20% efficiency and resolution of 1.9 keV to the peak of 1332.49 keV ^{60}Co. For quality control of the results, NIST RM 8435 Whole Milk Powder and NIST SRM 1549 Non Fat Milk Powder reference materials were analyzed. The values obtained for $|Z|$ score<2 indicated that the results are satisfactory at a 95% confidence level. Most of the obtained results agreed with the certified values, resulting in relatively good accuracy, except for Se whose values were slightly higher than those presented in the certified reference materials. Fe, Se and Zn concentrations obtained in the infant milk formula samples agreed with those levels printed on the infant milk product labels. The concentration ranges varied: Fe (mg/100kcal) from 0.6 (formula with special requirement) to 1.7 (for infants over 6 months), Zn (mg/100kcal) from 0.5 (soy-based) to 1.3 (for infants over 6 months) and Se (µg/100kcal) form 1.0 (special requirements) to 13.8 (soy-based formula). The findings of this study indicated that most infant milk formulas analyzed are within the dietary recommendation by the National Health Surveillance Agency (ANVISA) and of the Codex Alimentarius. Only Fe concentration for one sample (special requirements) was lower than the ANVISA recommendation. The results showed that soy-based infant formulas generally present higher Se concentrations than those of cow-milk based formulas.

P026. MICRO DISTRIBUTION OF BIOLOGICALLY IMPORTANT METALS IN PRIMARY INVASIVE DUCTAL CARCINOMA OF BREAST. Michael Farquharson[1], Alia Al-Ebraheem[1], Russell Leek[2] and Adrian Harris[2].
[1]Department of Radiography, City Community and Health Sciences, City University, London, UK.
[2]Cancer Research UK, Oxford Cancer Centre, Molecular Oncology Laboratories, University of Oxford, Weatherall Institute of Molecular Medicine, Oxford, UK
m.j.farquharson@city.ac.uk

Studies from the Applied Radiation Research Laboratory in the Department of Radiography, City University, on breast tissue using X-Ray Fluorescence (XRF) have shown that there is an association between trace element levels and the development of breast cancer (Geraki et al. 2002, 2004). This information raises the question “where in the tissue do these trace elements accumulate?” In order to find the answer to this question we have mapped trace elements at the cellular level which enables the exact localisation and concentration of the elements within the tissues. In order to achieve this a micro beam synchrotron x-ray fluorescence (μSRXRF) technique was used to determine the localisation of metals in primary invasive ductal carcinoma of breast. The work was carried out at two facilities, namely the Hamburger Synchrotronstrahlungslabor at Deutsches Elektronen-Synchrotron, DESY, Germany and ANKA, Forschungszentrum, Karlsruhe, Germany. A number of samples were examined which were formalin fixed tissues arranged as micro arrays of 1.0 mm diameter 10 μm thickness. Maps of the elements Ca, Fe, Cu and Zn, which are of physiological importance, are presented. The distribution of these metals was obtained at approximately 18μm spatial resolution at DESY and approximately 3μm at ANKA. The distributions are compared with light transmission images of adjacent sections that are H and E stained to reveal the location of the cancer cell clusters. Correlations were found between these reference images and the elemental distributions indicating an increase in all element concentrations in the tumour regions of all samples, with the exception of Fe, which in some cases showed a reverse of this trend. On average over all samples the percentage ≈ 67%, Cu ≈difference from the normal tissue elemental concentrations are Ca 145%. Micro X-ray absorption near edge structure (μXANES) ≈64% and Zn spectroscopy was used to estimate the oxidation state of Cu in normal and tumour regions. The shape and the position of both normal and tumour Cu K-edge XANES spectra suggest that they contain mixtures of copper ions with a significant fraction of Cu (II). However, the shape of the spectra does not exclude the presence of Cu (I). Tumour regions were found to have a higher fraction of Cu (I) compared to the normal samples. This information may provide a better understanding of the role of trace elements in breast cancer and thus help lead to a possible new approach to treatment.

P027. TRACE ELEMENTS IN DIETARY SUPPLEMENTS. Tore Syversen, Marte Aurstad Aspnes, Kristin Gellein and Lars Evje. Department of Neuroscience, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway
tore.syversen@ntnu.no

We selected 54 trace element dietary supplement products which were available from pharmacies and/or health food shops in Norway. The products were both single and multi-element supplements. The aim of the study was to compare product declarations with analytical results as well as analyzing the products for undeclared essential or toxic elements. The test material was dissolved in concentrated nitric/hydrochloric acid mixture, diluted and analyzed on a High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS) recording results for 21 elements. The results show that the dietary supplements contained the elements declared on the label, but there were several differences between the declared and analyzed content – analyzed results were up to 38% higher than the specified value. These deviations may cause a higher intake than specified by the user guidance contained in the package, but not to an extent where it may represent a health hazard for the individual. For the multi-element products only one out of 15 products was according to the product specification and we observed a very large range of deviation from label specification (±100 %). In some products we found undeclared elements such as chromium, manganese, iodine, iron and molybdenum. In most cases the amount of undeclared element corresponded to the dose range of supplements for these elements as suggested by public dietary advice.

P028. LEAD AND ARSENIC LEVELS IN REPRODUCTIVE AGE WOMEN WITH DIFFERENT NUTRITIONAL STATUS LIVING IN SANTIAGO, CHILE. Yareni Gutierrez[1], L. Muñoz[2] , Gabriela Salazar[1], Miguel N Llanos[1] and Ana M Ronco[1]
[1]Institute of Nutrition and Food Technology, University of Chile
[2]Chilean Commission of Nuclear Energy, CCHEN
amronco@inta.cl

The objectives of this study were: 1) to make a Pb and As biomonitoring in young adult women living in Santiago, Chile, 2) to evaluate the relation between nutritional status and Pb (blood) and total As levels (urine) and 3) to explore a possible association between Pb and As levels and fat mass. Three groups of voluntary women of 18 to 45 years of age were recruited (n=107). The groups were selected according to the Nutritional Status measured by Body Mass Index (BMI): low weight (IMC <18.5 kg/m2) (n: 20); normal (BMI >19 <24.9 kg/m2) (n: 43); and overweight (BMI >25 kg/m2) (n: 44). The fat mass was determined by isotopic dilution method. Pb levels were determined in blood by graphite furnace atomic absorption spectrometry (AAS-GF) and urinary As levels were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In order to evaluate differences between Pb and As levels and Nutritional Status or Fat Mass, the non parametric Kruskal-Wallis test was applied. Only one woman had Pb levels higher than the maximal levels accepted by the Chilean regulations (< 40 mg/dl); 2.6% of the women had As levels above the international regulations (<50 mg/dl), although nobody has As levels, corrected by creatinine, over the normative (<220 mg/g creatinine). No significant differences between Pb levels and As and the nutritional status (c2=1.78 p=0.410 y c2=0.039 p=0.981) and either there were no differences between Pb and As and the percentage of fat (c2=3.68 p=0.159 y c2=0.471 p=0.790). Since 9.6% of the women had blood levels higher than 10 mg/dl, and, as recent studies demonstrated that levels lower than 10 mg/dl produce adverse health effects, it is suggested here that Chilean accepted limits should be re-evaluated.
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P029. TRACE ELEMENTS IN HEMOCHROMATOSIS. Bjørn J. Bolann and Rune J. Ulvik
Institute of Medicine, University of Bergen, and Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway
bjorn.bolann@med.uib.no

Hemochromatosis is a hereditary disorder characterised by increased iron uptake from the gut, which over the years may result in iron accumulation in the body and iron overload. The condition may be treated successfully by bloodletting, thus removing the excess iron. Uptake, transport and storage mechanisms used by iron are also shared with various other metals. However, there has been little focus on how trace elements are affected by iron uptake disturbances. Trace element homeostasis may be influenced by the absorption disorder itself, by iron overload in the tissues, and by the bloodletting used to remove excess iron. Whether changes in trace element metabolism or distribution participate in the pathogenesis of iron overload tissue damage is not known. **Objective:** To study the relation between iron and trace elements in hemochromatosis. **Methods:** We recruited outpatients from the Hemochromatosis Clinic at the Department of Medicine. After genotyping we measured iron status and common clinical chemistry tests, and trace element profiles were studied before, during, and after treatment for iron overload. Multi-element analysis was done with Inductive Coupled Plasma Atomic Emission Spectrometry. **Results:** The serum selenium concentrations were higher in hemochromatosis patients with high values of total iron binding capacity (TIBC), than in patients with low values ($p<0.01$, Table). This suggests a negative relation between selenium and iron status, in agreement with a negative (but not significant) correlation between selenium and serum ferritin. Chromium, zinc and strontium had a positive, and copper a negative, but non-significant relation to iron status according to corresponding changes in TIBC and ferritin values. **Conclusion:** These preliminary results indicate that the serum level of selenium, and possibly other trace elements, may be related to iron status in hemochromatosis patients. The pathophysiological mechanisms and consequences remain unknown. Further studies are going on.

Table. TIBC, ferritin and corresponding trace element values in hemochromatosis patients.

			Serum trace elements, mean (SE)					
			Se (µM)	Al (µM)	Cr (nM)	Zn (µM)	Sr (µM)	Cu (µM)
TIBC (µM)	Range	*n*						
1. quartile	40-50	13	0.920 (0.052)	1.663 (0.114)	100.5 (3.637)	11.67 (0.40)	0.348 (0.052)	15.08 (0.786)
4. quartile	62-86	13	1.171 (0.046)	2.052 (0.436)	99.0 (3.824)	11.09 (0.49)	0.281 (0.051)	16.70 (1.367)
SF (µg/L)								
1. quartile	18-54	13	1.071 (0.066)	1.348 (0.112)	96.65 (3.916)	11.66 (0.44)	0.305 (0.038)	15.55 (0.678)
4. quartile	455-1377	13	0.956 (0.046)	1.457 (0.157)	111.47 (7.441)	12.10 (0.46)	0.327 (0.049)	14.57 (0.928)

P030. IRON, ZINC, AND CALCIUM CONTENT OF COMMERCIALLY-PRODUCED CEREAL-BASED COMPLEMENTARY FOODS FROM AFRICA AND MONGOLIA FAIL TO MEET THE ESTIMATED NEEDS FOR 9-11 MONTH BREAST-FED INFANTS. Karl B Bailey[1], Rebecca Lander[1], Tserennadmid Enkhargal[2] and Rosalind S Gibson[1]
[1]Department of Human Nutrition, University of Otago, Dunedin, New Zealand
[2]Public Health Institute, Ulaanbaatar, Mongolia
karl.bailey@otago.ac.nz

Cereal-based complementary foods (CFs) are often the major sources of Fe, Zn, and Ca from non-milk foods for young children in low income countries. Unrefined cereals contain high levels of phytate which chelates Fe, Zn, and Ca in the gastrointestinal tract, making them unavailable for absorption. It also complexes endogenously secreted Zn and Ca, making them unavailable for reabsorption into the body. Moreover, there is no evidence for adaptation to the inhibitory effect of high-phytate diets on Zn or Fe absorption. In response to these concerns, manufacturers often fortify cereal-based CFs with Fe and sometimes Zn, although whether the form, level, and combination of the fortificants are optimal is not always considered. We have analyzed the Fe, Zn, Ca, and phytic acid content (as hexa (IP6)- and penta (IP5)-inositol phosphates) of nine commercially-produced CFs sold in Africa and six in Mongolia by flame Atomic Absorption Spectrophotometry and HPLC, respectively, using methods described earlier; eight were fortified with Fe, and/or Zn and Ca. Molar ratios of phytate (Phy): Zn, Phy:Fe, and Phy:Ca were then calculated; critical values considered to compromise bioavailability were>18,>1 and>0.17, respectively. Next, we used the analytical data to calculate Fe, Zn, and Ca intakes from these CFs, assuming that breast-fed infants aged 9-11 mos consume the recommended daily intake of CF (i.e., mean of 50 g/d), based on dry weight (DW) cereal flour. We then compared these calculated intakes with the corresponding estimated needs for Fe, Zn, and Ca for breast-fed infants aged 9-11 mos, based on the difference between the WHO/FAO (2004) and IZiNCG nutrient requirements, and the intake provided by breast milk of average volume and composition. The richest sources of zinc (mg/100 g DW) were two instant porridges sold in Africa, whereas the lowest were two wheat-based flours used for CFs in Mongolia. Concentrations of Fe and Ca in the CFs were variable, but in general lowest in the Mongolian wheat-based flours, as was their IP5+IP6 concentration. Molar ratios were above Phy:Zn, Phy: Fe, and Phy:Ca critical levels for 3, 15, and 11 of the CFs, respectively. Likuni Phala (LP), the national complementary food used in Malawi, based on a 80:20 mixture of high phytate unrefined maize and soya flours, had the highest Phy:Zn (36) and Phy:Fe (22) molar ratios. None of the CFs analyzed met the estimated needs for breast-fed infants aged 9-11 mos for Fe, Zn, or Ca, even though 53% (8/15) were fortified with Fe, and Zn and/or Ca. In conclusion, manufacturers need to fortify cereal-based CFs with appropriate level and form of Fe, Zn, and Ca fortificants to ensure they meet corresponding WHO estimated needs.

P031. THE CORRELATION OF ARSENIC LEVELS IN DRINKING WATER WITH THE BIOLOGICAL SAMPLES OF SKIN DISORDERS. Tasneem Gul Kaz, Muhammad Balal Arain, Ghulam Abbas Kandhro, Muhammad Khan Jamali, Hassam Imran Afridi, Nusrat Jalbani, Raja Adil Sarfraz, Jameel Ahmed Baig, Abdul Qadir Shah.
Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro
tgkazi@yahoo.com

Arsenic (As) poisoning has become a worldwide public health concern. The skin is quite sensitive to As and skin lesions are the most common and earliest nonmalignant effects associated to chronic As exposure. In this paper, a survey (2005-2007) on surface and groundwater arsenic contamination, and relationships between As exposure via the drinking water and related adverse health effects (skin lesions) in villages located on the banks of Manchar lake, southern part of Sindh, Pakistan, was carried out. The range of arsenic concentrations in lake surface water was 35.2-158 µg/L (average 78.5 µg/L), which is 3- 5 folds higher than World Health Organization (WHO, 2004). During the survey, a preliminary clinical examination in study area, 61 to 73 % population was found to be exposed with typical arsenical skin lesions. The effects of As toxicity via drinking water were estimated by biological samples (scalp hair and blood) of adults (males and females), have or have not skin problem (n=285). The referent samples of both genders were also collected from those areas having low level of As (<10 µg/L) in drinking water (n=121). Arsenic concentration in drinking water and biological samples were analyzed using electrothermal atomic absorption spectrometry. It was observed that, As concentration in the scalp hair and blood samples were above the range of permissible values 0.034-0.319 µg As/g and <0.5-4.2 µg/L, respectively. The linear regressions showed good correlations between arsenic concentrations in water versus hair and blood samples of exposed skin diseased subjects (R^2=0.852, 0.718) as compared to non-diseased subjects (R^2 =0.573, 0.351), respectively.

P032. STUDY ON DIETARY IRON INTAKE IN CHINESE ADULT MAN AND INTERACTION BETWEEN IRON AND LEAD IN RATS. Junquan Gao

National Institute for Nutrition and Food Safety, Chinese Centre for Disease Control and Prevention, Beijing 100050, P.R. China
jqgao@vip.sina.com

Purpose: To research the effect of antagonistic and interaction between iron and lead in rats. Method: The evaluation on effect of antagonistic and interaction between iron and lead in rats have been obtained through two animal experiments. All groups rats drunk freely 400mg lead acetate /L solution in for four weeks except the blank control group in two animal tests. In animal experiment one: 56 Wistar rats were randomly divided into seven groups. In the meantime, the rats were treated with the intervention factors: blank and lead mode control groups received double distilled water; positive drug group received DMSA solution; other four groups received $FeSO_4$, $Fe(C_3H_5O_3)_2$, NaFeEDTA and high dose NaFeEDTA respectively, and the effect of exclusion lead from the blood of Wistar rats had been compared, the Fe level was 3.34mg/kg bw in three iron fortifiers groups(see Table1); In animal experiment two: 60 Wistar rats were randomly divided into six groups. In the meantime, the rats were treated with the intervention factors: three dose NaFeEDTA fortifiers were used, low, middle and high dose in NaFeEDTA fortifiers group rats was fed 1.67mg, 3.34mg and 6.69mg Fe/kg bw respectively, the lead exclusion from the blood and organs of Wistar rats had been compared(see Table 2). Result: In animal experiment one, the lead level of blood in supplementing $FeSO_4$, ferrous lactate, NaFeEDTA, and High-NaFeEDTA groups were reduced 27.0±10.9, 24.0±12.2, 39.0±19.4, and 65.7±7.4 μg Pb/L respectively; Based on the animal experiment one, the lead excretion effect of different NaFeEDTA level on different organs and blood were studied in animal experiment two and it was found out that three NaFeEDTA groups can reduce the lead levels in blood, liver, kidney and tibia, the lead level in these organs and blood in low, middle and high NaFeEDTA groups compared with lead mode control group were reduced significant ($P<0.05$). Conclusion: We found that among the three kinds of iron fortifiers, using NaFeEDTA to reduce the blood lead was the best; There were significant reverse correlations between NaFeEDTA supplement and the lead levels in liver, kidney and tibia, and the correlation coefficients were -0.4432, -0.6134 and -0.3878 respectively.

Table 1 Test scheme of experiment one in rats

Group	Gastric perfusion dosage /per 100g BW/per day
Blank	1ml double distilled water
Lead mode control	1ml double distilled water
Positive drug	1ml 12.5mg /ml DSMA
$FeSO_4$	1ml 1.66g/L $FeSO_4$ (3.34mg Fe)
$Fe(C_3H_5O_3)_2$	1ml 1.72g/L $Fe(C_3H_5O_3)_2$ (3.34mg Fe)
Low-NaFeEDTA	1ml 2.51g/L NaFeEDTA (3.34mg Fe)
High- NaFeEDTA	1ml 5.03mg/L NaFeEDTA (6.69mg Fe)

Table 2 Test scheme of experiment two in rats

Group	Gastric perfusion dosage /per 100g BW/per day
Blank	1ml double distilled water
Lead mode control	1ml double distilled water
Positive drug	1ml 12.5mg /ml DSMA
Low-NaFeEDTA	1ml 1.50g/L $Fe(C_3H_5O_3)_2$ (2.00mg Fe)
Middle-NaFeEDTA	1ml 3.01g/L NaFeEDTA (4.00mg Fe)
High-NaFeEDTA	1ml 4.51mg/L NaFeEDTA (6.00mg Fe)

P033. DIETARY EXPOSURE ASSESSMENTS OF HEAVY METALS AND TRACE ELEMENTS IN CHINA. Junquan Gao and Xiaowei Li
National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing 100021, China
jqgao@vip.sina.com

In order to assess the safety and nutritional status of dietary trace elements in different areas in China, the dietary intakes of fifteen trace elements have been obtained in Chinese adult males by using the 2000 Chinese Total Diet Study approach. These elements include Fe, Zn, Se, Cu, Mn, Cr, Ni, V, Li, Mo, Pb, Cd, Hg, Al, total and inorganic arsenic. The dietary safety of these trace elements was assessed by using themselves PTWI recommended by WHO. Nutritional status was evaluated by RNI and AI as established by the Chinese Nutrition Society. Chinese dietary Pb, Cd, Hg, Al, total arsenic and inorganic arsenic intakes (% of PTWI) in adult male were 0.081 mg/d (36.1%), 0.022 mg/d (35.3%), 0.007 mg/d (15.2%), 22.9 mg/d (36.3%), 0.276 mg/d and 0.079 mg/d (58.6%), respectively. The main dietary sources of these harmful elements were cereals and vegetables. Chinese dietary intakes of Fe, Zn, Cu, Se, Mn, Cr, Mo, Ni, V and Li (% of AI or RNI) in adult males were 13.0 mg/d (87.0%), 10.4 mg/d (69.3%), 1.7 mg/d (84.5%), 0.064 mg/d (128.6%), 3.8 mg/d (127.9%), 0.138 mg/d (276.4%), 0.257 mg/d (428.9%), 0.242 mg/d, 0.034 mg/d, and 0.053 mg/d respectively. The dietary intakes of selenium were higher than RNI in most provinces, but the intakes in Ningxia (0.037 mg/d) and Jiangxi (0.040 mg/d) were lower than the average level. Harmful elements in most food groups of four regions were well below the national limited standards of China except a few samples in some areas, such as lead in eggs in South 1 region exceeded 8.1% of limited standard as well as cadmium in aquatic foods in North 1 and South 1 exceeded 49.0% and 27.6% of limited standards respectively. The results indicate that dietary lead, cadmium, total mercury, total arsenic and inorganic arsenic intakes were safe in different regions. The intakes of iron and zinc through the diet are insufficient in Chinese people. This paper was supported by Ministry of Science and Technology of the People´s Republic of China

P034. FACTORIAL DESIGN AND CCD USED AS OPTIMIZATION PROCEDURES FOR DIRECT DETERMINATION OF IRON IN CANINE SERUM SAMPLES BY GF AAS WITH IN-SITU MATRIX REMOVAL. Carolina Carvalho de Souza, Henrique José Ferraz Fabrino, Waldomiro Borges Neto, Aldair junior Woyamos Pinto, José Bento Borba da Silva and Wagner Luiz Tafuri
Federal University of Minas Gerais, Belo Horizonte, Brazil
hfabrino@yahoo.com.br

Fe is a cofactor in many enzyme and biochemical processes, such as that used by parasite Leishmania in the macrophage to ensure its survival and virulence. Evidences shows that one of the strategies of the host for eliminating Leishmania is decreasing its Fe metabolism. A defense mechanism would be redistributing trace elements, such as Fe, in serum. Thus, detecting this element in canine serum is fundamental for understanding clinical and histopathologic correlation in Canine Visceral Leishmaniasis. Trace analysis in complex matrices may require sample preparation involving several steps. Therefore, it usually demands long preparation times, which increases possibilities of loss and/or contamination. The advantages of in-situ digestion have been explored in several works using GF AAS. Univariate optimization of experimental parameters requires a large number of time-consuming and costly experiments. Moreover, the interactions between optimized variables are not evaluated. Multivariate optimization seems to be more adequate when many variables are involved. In the present work, a method for determining Fe in canine serum using GF AAS was developed, aiming at determining this element in the serum of animals with Visceral Leishmaniasis. Samples were prepared by means of a simple 1:19 dilution of the serum with 1% HNO3 and 0.1% triton X-114. Optimization of the method included studies of pyrolysis and atomization temperatures, as well as evaluation of chemical modifiers by multivariate approach. First, modifiers were evaluated (Ir, Rh, Ta, Nb, Zr, W and no modifier) using the furnace program recommended by the manufacturer, in order to select two of them to be used in the factorial design. The best results were obtained using W (5200µg) and no permanent modifier. The 23 factorial design indicated that all of the variables studied, as well as the interactions among them, had a significant effect on the response (integrated absorbance) at a 95% confidence level. A CCD was constructed in order to obtain an answer surface using a tube treated with the modifier selected in the factorial planning (no modifier) and evaluating variables pyrolysis and atomization temperatures. The answer surface equation resulted in values of 1530 and 2500 oC for pyrolysis and atomization temperatures, respectively. Matrix effect was evaluated by comparing the slopes of aqueous and matrix-matching calibration curves. Results indicated no significant difference at a 95% confidence level. Figures of merit were determined using aqueous calibration in the range between 0 and 100 ppb, which presented an average linear correlation coefficient of 0.995±0.004 (n=3). Recovery and precision studies were performed at 3 concentration levels (30, 50 and 90 ppb). The average value obtained for recovery was 101±1% (n=21). A coefficients of variation of 5.2±2.2% (n=15) was obtained for precision. Equations LD=3S and LQ=10S were used to calculate the limits of detection and quantification, respectively. S is the standard deviation of 10 independent readings of the blank. LD and LQ were 5.0 and 16.5 ppb, respectively, and the characteristic mass was 6.1±0.9 pg (n=5).

P035. COPPER DEFICIENCY IN CALVES MAY INCREASE SUSCEPTIBILITY TO INFECTIOUS KERATOCONJUNCTIVITIS. Leonardo Minatel[1], Gabriela Cintia Postma[1], Susana Cristina Underwood[1], María Elena Dallorso[2] and Julio César Carfagnini[1].
[1]Área de Patología, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Ciudad de Buenos Aires,
[2]Facultad de Ciencias Agrarias, Universidad de Lomas de Zamora, Pcia. de Buenos Aires, Argentina.
lminatel@ciudad.com.ar

Evidence shows that copper deficiency impairs immune response in ruminants, predisposing to infectious diseases. The aim of this work was to determine if copper deficiency predispose calves to get infectious keratoconjunctivitis. Twelve castrated male Holstein and Holstein x Aberdeen Angus calves, weighing approximately 68 kg, were used. Calves were fed with a diet based on corn grains, feather flour, wheat straw, and a mineral and vitamin mix without copper. This diet contained 3.8 mg de Cu/kg DM; 0.4 mg de Mo/kg DM y 0.4 g de S/kg DM. Calves were allocated by breed, weight and liver copper level into two groups of 6 animals each. One group received the diet supplemented with 9 mg of Cu/kg DM (+Cu group), while the other received the diet supplemented with molybdenum (11 mg of Mo/kg DM), to get a Cu/Mo relation of 1/3, and sulphur, to reach 3 g of S/kg DM (+Mo group). Animals were weighed and samples of blood and liver were taken every 28-35 days. Copper levels were determined in liver, plasma and erythrocytes, and superoxide dismutase activity (SOD) was determined in erythrocytes. When calves from +Mo group showed copper deficiency, animals of both groups were inoculated with a suspension of *Moraxella bovis* ($2x10^8$ CFU/ml) in their right eye, previously irradiated with an UV lamp. Samples for bacteriology were taken 48 hrs before and after challenge, and when animals showed signs of disease. Eyes were examined daily for clinical signs. Serum and tear samples were obtained for antibody (IgG and IgA) assessment at days 0, 14, and 28, after inoculation. Phagocytic, bactericidal and SOD activities were determined in polymorphonuclear leucocytes at 28 day. Induction of copper deficiency lasted 314 days. At this time, significant differences ($p<0.05$) between groups were observed in live weight, liver, plasma, and erythrocyte copper levels, and SOD activity in erythrocytes. Three of six calves in +Mo group developed infectious keratoconjunctivitis while none did in +Cu group, although differences between groups were no significant ($p=0.18$). There were no differences in polymorphonuclear phagocytic and bactericidal activities between groups, but polymorphonuclear leucocytes from +Mo group showed lower SOD activity ($p=0.03$). Calves from +Mo group showed a decrease in tear IgA ($p=0.02$) and IgG at 28 days, although differences in IgG was no significant between groups. Although Cu-deficient calves seem to have increased susceptibility to *Moraxella bovis* infection and decreased production of local antibodies, the low number of animals used in this experiment probably explains the lack of significant differences between groups. Absence of differences in bactericidal activity of polymorphonuclear leucocytes may be due to the low proportion of bacteria used in the assay (2 polymorphonuclears per bacteria); this was decided on the basis of previous experiences that showed that a higher proportion of bacterias induce leukocyte lyses. It is concluded that calves with copper deficiency may be more susceptible to infectious keratoconjunctivitis. Further investigation is needed to explain this susceptibility.

P036. COPPER AND IRON BIOAVAILABILITY IN ANEMIC RATS FED FRUCTANS-CONTAINING YACON (*SMALLANTHUS SONCHIFOLIUS*) FLOUR-SUPPLEMENTED DIETS. Alexandre Rodrigues Lobo[1], Maria Lúcia Cocato[1], Primavera Borelli[2], Amanda Crisma[2], Karina Nakajima[2] and Célia Colli[1].

[1]Departamento de Análises Clínicas e Toxicológicas; Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo,
[2]Departamento de Alimentos e Nutrição Experimental; Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brazil

cecolli@usp.br

Dietary modification in order to increase the intake of components that promote Fe absorption from low-bioavailability diets is an effective strategy for combating nutritional iron deficiency. Inulin-type fructans (ITF) are known to be fermented in the large intestine affecting the mineral absorption and its retention. ITF occur naturally in a wide variety of plant-based foods and yacon roots have been considered a functional food due to the high levels of ITF they contain. In this study, the effects of ITF-rich yacon flour (YF) on Fe bioavailability from ferric pyrophosphate (Fe4(P2O7)3; FP), a water-insoluble compound, were evaluated in Fe-deficient anemic rats using the Hb repletion assay. Weanling male Wistar rats (n=50) were fed a low Fe diet (12 mg/kg) for 15 d followed by two wk of Fe repletion with modified AIN diets providing 35 mg Fe/kg as encapsulated ferrous sulfate (FeSO4; FS) or FP, supplemented with 7.5% ITF as YF (YF+FP) or Raftilose P95 (Orafti International; RAF+FP), a purified source of ITF from chicory roots (Chicorium intybus). The animals were randomly assigned according to Hb × body weight. Fe status and hematological parameters were evaluated. Fecal (9th-14th days of repletion) and liver samples were collected for total Cu and Fe analysis by AAS. Blood Hb was significantly higher in ITF-fed rats than those fed with the FP diet. No significant differences were observed in total cellularity in bone marrow and spleen among the groups. In addition, Cu absorption was not affected by ITF consumption. Liver Cu levels in FP were higher than in FS group ($P<0.005$) whereas that ITF feeding resulted in an augmented liver Cu mobilization. The greater Fe absorption by ITF feeding was reflected in a higher overall Hb regeneration efficiency ($P<0.001$) in YF+FP (+47%) and RAF+FP (+33%) groups. The Fe bioavailability in FP groups was compared with that of the FS control group. Relative biological value in ITF groups was higher than those of FP group. These findings showed that Fe was incorporated in Hb and this was reflected in increased Fe liver stores in ITF-fed rats (+68% in YF+FP-group and +39% in RAF+FP-group; $P<0.001$) comparatively to FP rats. Moreover, recovery of liver Fe concentrations after supplementation with ITF was at least as good as recoveries after recovery with FS. In conclusion, ITF intake stimulated Fe absorption, leading to greater Fe bioavailability. These effects seem to be more pronounced when YF was the ITF source.

P037. VARIATIONS IN METALLOTHIONIEN, COPPER AND ZINC CONCENTRATIONS IN THE LIVERS AND KIDNEYS OF DOGS, CATS AND HORSES.
Carmen I Fuentealba[1], M George Cherian[2], JC Lau [2]
[1]Faculty of Veterinary Medicine, University of Calgary; Calgary, Canada
[2]Department of Pathology, University of Western Ontario, London, Ontario, Canada
cfuentea@ucalgary.ca

Metallothionein (MT) is a low-molecular weight, metal-binding protein with high affinity for metals such as copper (Cu), zinc (Zn) and cadmium (Cd). MT has a role in the metabolism of essential metals and detoxification of heavy metals in humans and animals. Indeed, domestic animals are used as sentinels for exposure to environmental chemicals, and models for human diseases. The objective of this study was to investigate the species differences in hepatic and renal MT, Cu and Zn in three common companion animals: dogs, cats and horses. Fresh liver and kidney were collected over a 1 year period from dogs (N=38), cats (N=17) and horses (N=7) that died acutely due to accidental trauma. In each case a complete port-mortem exam, including histological assessment of the liver and kidney, was carried out. Metallothionen was measured using the 109 cadmium-heme assay. Cu and Zn were quantified using atomic absorption spectrophotometry. Statistical analysis was done using Duncan's multiple range test and results are reported as Mean±SD µg/g tissue, wet weight. Dogs and cats had comparable levels of hepatic Cu (36.4±3 µg/g and 27.4±5 µg/g wet weight respectively) which were significantly higher than in horses (12±5 µg/g). In contrast, hepatic Zn was significantly higher in horses (45.7±7 µg/g) compared to dogs and cats (26.8±1 µg/g and 20.8±4 µg/g respectively). Differences in hepatic Cu and Zn seemed to be independent of hepatic MT concentration which was similar in these three species (203.8±10 µg/g in dogs, 231.9± 30 µg/g in cats and 272.5±119 µg/g in horses). Similarly, renal Zn was statistically higher in cats 22.5±3 µg/g compared to dogs 10.5±1 µg/g, but MT renal levels were not significantly different in these two species (40.9±15 µg/g and 39.6±2 µg/g respectively). Renal MT concentration was significantly higher in horses (134.8±38 µg/g) compared to dogs and cats, although statistically significant differences were not detected in the renal Cu concentration in these species (3.4±0.2 µg/g in dogs, 8.1±9 µg/g in cats, and 2.6±9 µg/g 0.4 in horses). In addition to nutritional status, inflammatory or neoplastic changes are reported to influence metal and MT tissue concentration. However, in this study morphological changes compatible with either inflammation or neoplasia were not detected in the tissues examined. The results of this study demonstrate that there are marked species differences in hepatic and renal MT, Cu and Zn concentrations in dogs, cats and horses. The finding of low levels of hepatic Cu and comparatively high levels of hepatic Zn in the horse is interesting as this particular interaction has been associated with musculoskeletal diseases reported in horses but not in other species. Conversely, copper-associated liver diseases unrelated to dietary copper levels are commonly diagnosed in dogs, but only occasionally in cats. These findings emphasize the importance of understanding idiosyncratic species differences in metal metabolism when selecting spontaneous or experimental animal models.

P038. LIVER BIOPSY IN CATTLE. A SAFE PROCEDURE TO EVALUATE COPPER ACCUMULATION IN THE LIVER. Marco García-Vaquero[1], Isabel Blanco-Penedo[1], José Luis Benedito[1], Marta López-Alonso[1], Betiana Gutiérrez[1] and Marta Miranda[2]
[1]Universidade de Santiago de Compostela, Departamento de Patoloxía Animal, Facultade de Veterinaria, Lugo, Spain; [2]Universidade de Santiago de Compostela, Departamento de Ciencias Clínicas Veterinarias, Facultade de Veterinaria, Lugo, Spain.
marta.miranda@usc.es

Hepatic copper (Cu) concentration is the most reliable indicator of animal's over-all Cu status. Although in live animals liver biopsy is recommended to evaluate chronic Cu accumulation in ruminants, there are no studies to prove the effectiveness of this technique to evaluate the Cu burden in the whole organ. The aim of this study was to determine if in live liver biopsy using a non-invasive needle procedure is appropriate to determine Cu accumulation in the liver in cattle. Liver biopsies were performed in 29 beef cattle aged 10 month, fed with 35 mg/kg DM Cu sulfate during the growing and finishing period. In order to know if Cu accumulation is homogeneous in the whole organ, samples of 6 areas of the liver were taken (right lobe visceral and parietal faces, left lobe, caudate lobe, quadrate lobe, and processus papillaris) at slaughter. Samples were acid-digested and Cu concentrations determined by ICP-AES. No statistically significant differences were found for the Cu concentrations determined in the in vivo (181 mg/kg wt. w.) and post-mortem (168 mg/kg) biopsy samples, and a strong correlation between Cu concentration in both samples was found (r=0.937, p=0.000). The mean percentage of difference between both procedures was low (9.1%, p=0.281), in all cases below 20%. No statistical significant differences were found for Cu accumulation among the hepatic lobes (F=5,173=2,057, p=0,074), so the sample obtained by in vivo biopsy was in good agreement with Cu accumulation in the whole organ. Our results indicated that non-invasive biopsy techniques are a good tool to evaluate chronic Cu accumulation in the liver in cattle.

P039. DIETARY FLUORIDE EFFECT ON LIVER WEIGHT AND BODY WEIGHT OF NEW ZEALAND WHITE RABBITS. Artur Canella Avelar and Walter Motta Ferreira.
Department of Animal Sciences - Universidade Federal de Minas Gerais, Brazil.
avelara.can@gmail.com

Recent findings have reinforced concerns that fluoride can lead to a variety of unwanted harmful effects on health. In this study, deleterious effects of fluoride from eight different phosphates were investigated using 30 to 72 days white New Zealand young rabbits *Oryctolagus cuniculus*. Eight groups of twelve 30 days-aged young New Zealand Rabbits were used. Rabbits were fed with a basic diet. Each group was fed with the basic diet and supplemented with a different phosphate source, as follows: calcinated bone meal (FAR), dicalcium phosphate (BIC), super triple phosphate (FST), super simple phosphate (FSS), monoammonium phosphate (FMA), sulphur ammonium phosphate (FSA), ammoniated calcium polyphosphate (POLI) and a bovine mineral supplement (SMB). Body weight was measured individually during the 42-day experimental period. The 96 rabbits survived until study completion. At the end of the experiment, all animals were slaughtered and livers were collected and weighed. F content in phosphates and diets were measured by ion selective potentiometry. Statistical study was performed using statistical package SAS System® - an integrated statistical package. Both body weight and liver weight were tested for significance using the Student-Newman-Keuls test (SNK test, $p>0.05$). Animals receiving fluoride high level content diet (sulfur phosphate of ammonium, and bovine mineral salt) showed the worst final body weight (different letters in the same sequence differ by test SNK, $p>0.05$) - BIC: 2136.7g^{a}; FAR: 2132.7g^{a}; FST: 2115.6g^{a}; POLI: 2047.9g^{a}; FMA: 2059,4g^{a}; FSS: 1989.0g^{a}; SMB: 1703.6g^{b}; FSA: 1696.5g^{b}- and also the lighter livers amongst all tested animals - FSS: 75.2g^{a}; BIC: 70.1g^{a}; POLI: 69.9g^{a}; FAR: 69.9g^{a}; FMA: 64.1g^{a}; FST: 60.3g^{a}; FSA: 47.0g^{b}; SMB: 46.9g^{b} . These results confirm the high toxicity that fluoride presents to this organ and to the whole animal development.

P040. EFFECT OF PLANT SPECIES, HARVEST TIME AND SOIL TYPE ON MINERAL CONTENT OF ORGANIC FORAGE CROPS. Jakob Sehested, Søren Krogh Jensen and Karen Søegaard
Faculty of Agricultural Sciences, University of Aarhus, Denmark
jakob.sehested@agrsci.dk

Organic milk production is based on principles of low input of resources and recirculation of nutrients. However, supplementation with imported inorganic minerals is common in organic dairy production in Denmark as insurance due to uncertainties regarding actual feed mineral content, mineral availability and animal requirements. The objectives of the present study is to obtain knowledge about the variation and level of minerals in grassland species and the possibility to affect forage mineral content through selection of plant species and harvest time. To study the content of minerals in forage crops experiments are carried out in two parts: a) plot experiment on the research station without mineral fertilizers and pesticides with the aim to analyse effect of plant species, morphological development, and time in the growing season; and b) registrations on organic dairy farms with the aim to examine the effect of soil type. In a) the experiment contain different species and seed mixtures with one grass and one legume in four replicates: perennial ryegrass (Lolium perenne) together with either white clover (Trifolium repens), red clover (Trifolium pratense), Lucerne (Medicargo sativa) or birds-foot trefoil (Lotus corniculatus), and white clover together with either timothy (Phleum pratense), meadow fescue (Festuca pratensis) or hybrid ryegrass (Lolium x boucheanum). There are four cuts per season and in spring growth and second regrowth there will be three harvest times with one week between each time. In b) six organic dairy farms have been selected with different soil types, ranging from coarse sandy soils to clayey sand. There has been established perennial ryegrass/white clover pastures with different herbs (chicory Cichorium intybus), caraway (Carum carvi), plantain (Plantago lanceolata), birds-foot trefoil, salad burnet (Poterium sanguisorba) and Lucerne) on the farms. Content of minerals and herbage quality in the single plant species will be determined in the pastures in June and August. In June an area will be fenced off for cutting in the pasture, at the mineral content and herbage quality will be determined. Plant and soil samples is currently being analysed by ICP-MS for content of the following minerals: Na, K, Ca, P, Mg, Mn, Cu, Zn, Co, Se. The perspective of the present study is to increase self-sufficiency with minerals in organic milk production.

P041. CADMIUM LEVELS IN EDIBLES FISH SPECIES OF GULF AND PACIFIC COASTS OF MEXICO. Marisela Mendez-Armenta[1], M Isabel Castro-González[3], Sergio Montes[2], Camilo Rios[2] and Sara Montaño[3]
[1]Laboratorio de Neuropatología Experimental. Instituto Nacional de Neurología y Neurocirugía MVS, México City, Mexico
[2]Departamento de Neuroquímica. Instituto Nacional de Neurología y Neurocirugía MVS, México City, Mexico
[3]Departamento de Nutrición Animal. Instituto Nacional de Ciencias Médicas y Nutrición SZ.México City, Mexico
isacastro55@yahoo.com.mx

Metals, particularly heavy metals such as cadmium constitute a significant potential threat to human health; the environmental persistence of metals in concert with their intensive use by modern society. Fish are a major part of the human diet and it is therefore not surprising that increased concentrations of metals in fishes has been reported shown that cadmium bioaccumulation (such as other metals) in fish tissues is influenced by biotic and abiotic factors. Several studies reported low cadmium levels on different fish species, showing that exist significant interspecific differences for all metals and no fish type had the highest levels of more than two metals, which suggests that the differences are due to geography, throphic levels, size foraging method/localization and propensity of metals to undergo biomagnification in the food chain, demonstrating the variability in concentrations of cadmium on fish species. In the present paper cadmium levels in 35 marine species of comestible fishes of Mexico coasts were determined by using atomic absorption spectroscopy. The samples were obtained from the biggest Market of fish and sea foods in Latin America “La Nueva Viga”, obtaining 15 fishes of each species (5 K of sample aprox.), at random was taken 1g of tissue/fish for digestion in Nitric Acid Suprapur. All the samples were analysed for cadmium by atomic absorption spectrometry (AAS) with graphite furnance (Perking-Elmer) with an autosampler. The cadmium content expressed in µg/g wet weight varied depending upon the specie studied. The levels of cadmium ranged from 0.00022785-0.00872675µg/g with an average of 0.00213448µg/g. No statistical differences were observed with compared Gulf and Pacific coast regions and similar results were obtained when compared the different ecologic habitats (pelagic, benthopelagic and demersal). However, cadmium concentrations founded on pelagic habitat fishes indicate that this species contained more metal than the other habitat. The fish species with lower cadmium concentrations were: common snook (Centropomus undecimalis), albacore (Thunnus alalunga), southern flounder (Paralichtys lethostigma), pot snapper (Lutjanus synagris) whereas that the fish species with higher cadmium concentrations were: pacific Spanish mackerel (Scomberomorus maculatus), atlantic croaker (Micropogonias undulatus) and angel shark (Squantina californica). The data generated in the present study were similar with other studies carried out in different parts of the world. The concentrations of cadmium in all species examined were below the maximum levels indicated by the FAO/WHO for this metal (0.05 mg/kg fw) in fish.

P042. PHOSPHORUS, POTASSIUM, CALCIUM, PROTEIN AND N-3 PUFA CONTENT IN MARINE FISHES AS AN OPTION IN PATIENTS WITH RENAL DISEASES´ DIET. María Isabel Castro-González, Daniela Miranda Becerra and Sara Montaño Benavides.
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Dirección de Nutrición. México, D.F. MEXICO
isacastro55@yahoo.com.mx

The nutritional support in the nephritic and nephrotic patients is necessary in their treatment. The diet must include limited amounts of high quality protein (Pr), phosphorus (P), potassium (K) and calcium (Ca), due that the kidneys can't eliminate them. The n-3 polyunsaturated fatty acids (PUFA) (EPA and DHA) maintains beneficial properties against the renal damage progression, and due they encountering in fishery products, a quotidian consume of some species could contribute such benefits. The aim was to quantify the content of (Pr), (n-3PUFA) (P), (K) and (Ca) in fifteen marine fish species, as a complement to its evaluation as an alimentary option in nephritic and nephrotic patients. The samples were obtained from the biggest fish and seafood market in Latin America known as "La Nueva Viga", obtaining 15 fishes of each species (about 5 K of sample), at random was taken 1g of tissue/fish for the chemical analysis. (Pr) was analysed in a Kjeltec equipment; minerals by atomic absorption spectrophotometer and n-3 PUFA was analysed by CG-FID. The results showed (Pr) values (g/100g) from 14.3 (red snapper) until 22.8% (dollar fish). The fishes with the highest (P) (mg/100g) content were: vermillion snapper (VS) and blue runner (BR) (289), followed by barred sand bass (BS), red snapper and dollar fish (DF) (273). The species with the lowest ratio of P:n-3PUFA were: (BR), gafftopsail sea catfish (CF) and spotted seatrout (ST) (0.4-0.5), and the highest were smalltail shark (SS) (9.4), (BS) (7.4) and Atlantic croaker (4.6). The fishes with a high n-3PUFA/gPr content were (BR), (CF) and (ST) (22.8-33.7). It was a tendency in the increase of the (P) as the fat in muscle was increased (mg (P)/100 g of edible portion): lean fishes (193.7), semi fatty fishes (239.) and fatty fishes (261.5). In conclusion, the fish inclusion in nephrotic and nephritic patients' diet is possible depending of the state of the renal damage; it's necessary to evaluate the risk: benefit of fish consumption in an individual way, even so, is considered that the following species would benefit the general health state if they are included in the diet in a regular way, at least thrice a week: (BR), (CF), (ST), (VS), (DF) and caitipa mojarra.

P043. HO1, BAX AND BCL-2 GENE EXPRESSION IN CELLS INCUBATED AT DIFFERENT IRON CONCENTRATIONS.
Marcela Fuentes and Miguel Arredondo
Laboratory of Micronutrients, Institute of Nutrition and Food Technology (INTA), University of Chile
marcelafuentes@gmail.com

Introduction: Bax and Bcl-2 are proteins that regulate the apoptotic process. When Bax is over-expressed, it is transported to the mitochondria where induces the release of cytochrome c. Bcl-2 binds to Bax and form a heterodimer, inhibiting Bax activity. The ratio of Bcl2/Bax is correlated with apoptosis in many types of cells. Iron (Fe) acts catalyzing reactions that increase oxidative damage. Heme oxygenase-1 (HO-1), a key enzyme in heme catabolism, also functions as an antioxidant enzyme providing cellular protection against iron-mediated (heme and non-heme) oxidant injury. Objective: The aim of this study was to determine changes in HO-1, Bax and Bcl-2 expression in cells exposed to Fe, Fe-transferrin (FeTf) and Desferioxamine (DFO, a Fe chelating agent) in two cellular models.
Methods: 3t3-l1 (a model of rat preadipocytes) and H2.35 (a mouse liver cell line) cells were cultivated in different Fe and FeTf concentrations (0.5; 5 and 20 µM Fe). 3t3-l1 cells were cultured during 3 steps of differentiation: i) differentiation (dDi) alone or differentiation by adding Rosiglitazone (a drug that accelerates growth) (dDi+R), ii) in differentiated cells (D), and iii) in the preadypocitic stage, before differentiation (BDi). H2.35 was cultured in 2 ways: acutely (2 days of treatment) and chronically (7 days of treatment). RNA was isolated using a commercial kit, quantified at 260/280 nm and retro-transcripted. mRNA expression levels of Bax, Bcl-2 and HO-1 were quantified by real-time PCR. Data are presented as mean±SD. Comparisons were performed by t- test and ANOVA. $P<0.05$ was considered significant.
Results: 1) In 3t3-l1: Bax expression increased in BDi and dDi+R stages. HO-1 increased in dDi+R. Bcl-2 was increased in BDi and D stages. There were no significant differences between different concentrations of Fe+DFO. The lowest expression of Bax was at FeTf 20 µM. The ratio Bcl2/Bax was not significantly different in any of the stages. 2) In H2.35: Bax and HO-1 increased their expression after the chronic treatment. Maximum expression of Bax was at 5 µM Fe. Fe+DFO decreased HO-1 expression after the acute treatment compared to Fe without DFO. Moreover Fe+DFO decreased Bax expression after the chronic treatment compared to Fe without DFO. HO-1 decreased at 20 µM FeTf. The highest expression of Bax was at 5 µM Fe after chronic treatment. Expression of Bax, Bcl2 and HO1 showed not differences in all treatments with FeTf. Conclusions: These results show that the ratio Bcl2/Bax is not indicative of apoptosis. DFO decreased expression of Bax and HO-1 in H2.35 cells compared to cells cultured with Fe, an indication that DFO protected cells from deleterious effects of iron, such as oxidation and apoptosis. DFO did not change gene expression in 3t3-l1 cells, but the levels of Bax and HO-1 increased after chronic treatment with iron. FeTf had a similar effect in the expression of all 3 genes at all concentrations studied. These increases could be related to the oxidative stress produced by iron.
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P044. AN IRON-CALCIUM CONNECTION IN NMDA RECEPTOR SIGNALING AND HIPPOCAMPAL SYNAPTIC PLASTICITY. Pablo Muñoz-Carvajal, Alexis Humeres, Cecilia Hidalgo and Marco T. Núñez
Centro de Neurociencias, Universidad de Valparaíso; Programa de Doctorado en Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile; Centro FONDAP de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile; Departamento de Biología, Facultad de Ciencias and Institute for Cell Dynamics and Biotechnology, Universidad de Chile.
mnunez@uchile.cl

Iron deficiency during early life is associated with significantly lower cognitive and behavioral development in infants, indicating that iron is essential for neural development in humans. In particular, nutritional iron deficiency interferes with hippocampus-depending learning and affects synaptic plasticity in animal models. Yet, current understanding of the relationship between neuronal function and brain iron status is sparse. Sustained hippocampal CA1 long-term potentiation (LTP) involves calcium-dependent stimulation of the extracellular regulated kinase (ERK) pathway. In this work, we investigated the participation of iron on a) calcium release, b) ERK1/2 stimulation induced by the glutamate agonist N-methyl D-aspartate (NMDA), and c) hippocampal synaptic plasticity. Incubation of PC12 cells or hippocampal neurons with NMDA or iron promoted reactive oxygen species generation, enhanced ryanodine receptor-mediated calcium release and activated the ERK1/2 pathway, as determined by increased ERK1/2 phosphorylation and nuclear translocation of the phosphorylated proteins. Selective iron chelation with desferrioxamine, intracellular calcium chelation with BAPTA-AM or specific RyR inhibition with ryanodine inhibited the ERK1/2 activation induced by either NMDA or iron. Pre-incubation of hippocampal slices with desferrioxamine decreased basal synaptic transmission; this inhibitory effect required NMDA receptor activation. Pre-incubation with desferrioxamine also prevented sustained (1 h) LTP induction in CA1 neurons produced by 4 cycles of theta burst stimulation. The present results suggest that hippocampal neurons require iron-derived reactive oxygen species for NMDA receptor-dependent stimulation of the ERK1/2 pathway, a requisite step of sustained LTP. Financed by ICM grant P-05-001 and FONDAP grant 15010006.

P045. PARTICIPATION OF ALDOSTERONE IN HYPOTENSION SECONDARY TO IRON DEFICIENCY ANEMIA.
Aki Konomi[1] and Katsuhiko Yokoi[2].
[1]Department of Public Health and Environmental Medicine, The Jikei University School of Medicine, Tokyo, Japan
[2]Department of Human Nutrition, Seitoku University Graduate School, Chiba, Japan
konomi_aki@jikei.ac.jp

World health organization reported that 2 billion people in the world are suffering from iron (Fe) deficiency anemia. Fe deficiency often co-occurs with zinc (Zn) deficiency. Patients with Fe and/or Zn deficiency suffer from lethargy, decreased working performance, cognitive performance and thermogenesis. Hypotension is frequently associates with Fe deficiency anemia, but the mechanism of hypotension is unknown. It is necessary to know the pathophysiology of blood pressure regulation in Fe and/or Zn deficiency. To investigate the pathogenesis of secondary hypotension in Fe deficiency anemia, we examined the effect of Fe deprivation and/or Zn deprivation on plasma aldosterone concentration and blood pressures in rats. Forty 4-week old male Sprague-Dawley rats were assigned into 4 dietary treatment groups of 10 animals each, for the 4-week study: Fe-deficient group (30 mg Zn/kg, no supplemental Fe; FD), Zn-deficient group (4.5 mg Zn and 35 mg Fe/kg; ZD), Fe/Zn-deficient group (no supplemental Fe, 4.5 mg Zn/kg; FZD), and control group (AIN-93G; Cont). At days 26 and 27, blood pressures (systolic, diastolic and mean) were measured by the tail cuff method with a body warmer. Plasma aldosterone concentration was determined by ELISA. Data were analyzed by Tukey's simultaneous multiple comparison test. Relative heart weight was significantly increased in FD and FZD groups, and was decreased in ZD compared to Cont. Compared to Cont, hematocrit and hemoglobin were similarly decreased in FD and FZD. These groups were not distinguished by common hematological parameters. Blood pressures were significantly decreased in FZD compared to Cont. Plasma aldosterone concentration was significantly decreased in FD (30% of Cont) and FZD (15% of Cont). These results suggest that secondary hypotension in Fe deficiency anemia was rather evoked by simultaneous deficiency of Fe and Zn. The mechanism of secondary hypotension in Fe deficiency anemia sensu lato or anemia with combined deficiency of Fe and Zn was partly due to decreased circulating aldosterone.

P046. IRON AND ZINC DIALYZABILITY IN QUINOA CROPS: PRELIMINARY STUDY.
Binaghi Maria Julieta[1], Dyner Luis[1], Zuleta Angela[1], Bertero Hector[3] and Pallaro Anabel[2].
[1]Cátedra de Bromatología, Universidad de Buenos Aires.
[2]Cátedra de Nutrición. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.
[3]Cátedra de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires.
Junín 956. Buenos Aires. Argentina. Tel/ Fax: 01149648243
jbinaghi@ffyb.uba.ar

The aim of this study was to assess iron and zinc dialyzability and the fiber content in different populations of quinoa seeds from Argentinean northwest. Samples subjected to analysis comprised 12 quinoa crops coming from different places of the Provinces of Salta and Jujuy. Potential iron and zinc availability was assessed using an in vitro modified method, which measures iron (FeD%) or zinc (ZnD%) dialyzability under controlled pH conditions, after a digestion simulating physiological processes. The total mineral content was determined by atomic absorption spectrophotometry after mineralization. Analyses of Total Dietary Fiber content (TDF) were assessed by enzymatic - gravimetric method (AOAC 985.29). Determinations were triplicates and statistical analysis was performed by ANOVA and a posterior Tukey test. The results are expressed as FeD%, ZnD% and g TDF/100 g sample. The values of FeD% and ZnD% were among 9.64 - 16.33 and 7.33 - 10.46, respectively. Meanwhile, the TDF were among 8.8 - 14.7. No significant differences were found among ZnD%; however significant differences were found in FeD% and TDF ($p < 0.05$). The results are shown in the following table:

SAMPLE	FED%	TDF
1	16.33	8.8
2	15.91	9.8
3	15.97	9.8
4	15.3	10.5
5	15.68	10.8
6	14.87	10.8
7	11.03	12.1
8	10.98	12.2
9	11.69	12.3
10	11.51	12.9
11	10.19	14.1
12	9.64	14.7

Conclusions: It was observed that samples with high fiber content showed lower Fe dialyzability. This preliminary study allows a first characterization of fiber and mineral content of quinoa crops collected in the Argentinean northwest. Nowadays, the study will be completed with the analyses of the rest samples obtained, in order to know if the data obtained is related to geographic zone where the samples come from.
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P047. CALCIUM INCREASES IRON UPTAKE BY CACO 2 CELL LINE. Diego Gaitán, Manuel Olivares, Miguel Arredondo and Fernando Pizarro.
Laboratory of Micronutrient, Insitute of Nutritiona nd Food Technology (INTA), University of Chile, Santiago, Chile
dgaitan@inta.cl

The evidence suggests that calcium (Ca) has a dose-dependent inhibitory effect on human iron (Fe) bioavailability from a single meal or from solutions; effect which is evident at Ca doses between 40 and 300 mg. Nevertheless, studies conducted using a complete diet in both women and children have not found that Ca affects adversely Fe nutritional status. So, it is necessary to further assess the interactions between these two minerals. The cellular model Caco 2 is a useful tool to understand interactions between up takes of nutrients at the gastrointestinal tract. Objective: to assess the effect of Ca on Fe absorption using Caco 2 cell cultures. Methods: Caco 2 cells were cultured in ISCOVE's medium (no Fe added) and 10% FBS. After confluence, up take experiments were carried out incubating the cells for 90 minutes in a transport buffer supplemented with 10 µM FeCl3 (Fe:NTA=1:2,2) and Ca was administered as CaCl2 at Ca:Fe molar ratios=0:1, 1:1, 10:1; 100:1; 250:1 and 500:1; solutions were marked with a trace of 55Fe isotope. Afterwards, cell dishes were washed with PBS 1X, cells were separated from wells using Tris Salino EDTA. Membrane lysis was conducted to obtain a cellular extract. Up take was determined as percent of the original dose administered recovered in the extract. Fe up take from solution without Ca was considered as a control and results are reported as times of increment respect to it. Results: The increments on Fe uptake ±SD of the different solutions were: 1 (control); 1.07±0.15 (1:1), 3.72±1.39 (10:1); 6.74±2.64; 7.13±2.99 (250:1) and 10.14±4.13 (500:1). Thus, Fe uptake was not changed at a Ca:Fe molar ratio of 1:1, but increased at higher molar ratios ($p < 0.0001$, $F=17.84$). Conclusion: Our result shows that Ca increases Fe uptake by Caco 2 cells. This suggests that Ca inhibitory effect on Fe bioavailability may be explained for interaction between these minerals at luminal duodenum or because Ca interferes the Fe basolateral transport at enterocytes.
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P048. IMPACT OF IRON-DEFICIENCY ON ^{210}Pb-ABSORPTION ALONG THE MURINE INTESTINE. Klaus Schümann[1], Noel Solomons[2], Monica Orozco[2], Bernd Elsenhans[3].

[1]Zentralinstitut für Ernährung und Lebensmittelforschung, Technische Universität München, Germany
[2]Cessiam, Guatemala City, Guatemala
[3]Walther Straub Institut für Pharmakologie und Toxikologie, Ludwig-Maximilians-Universität, München, Germany

KSchuemann@schuemann-muc.de

Objectives: Lead (Pb) seems to share the pathways of intestinal iron absorption at least in part, as Pb absorption increases in iron-deficient rodents. In human studies, however, findings on the impact of iron stores on Pb absorption are unequivocal. Therefore, this trial investigates 1.) if Pb-absorption rates in the distal small intestine may be substantial enough to dominate over-all Pb absorption and 2.) whether iron status was of any influence on ileal Pb absorption. **Material and Methods:** C57BL6 mice were made Fe deficient by feeding an iron-deficient diet for 5 weeks during growth. Mice were taken for the experiments at 8 and ~20 weeks of age. Intestinal 210Pb- and 59Fe-uptake into the segments and transfer of both metals into the carcass were determined in ligated small intestinal loops in situ (duodenum ~2 cm, ileum ~4 cm) after exposure to 210Pb-labelled PbSO4 and 59Fe-labelled Fe-NTA(1:2) complexes for 10 min. Iron status was analysed by determination of Hb, Hk, and hepatic Fe content. After removing the loop, Pb-absorption rates (pmol Pb/cm2 intestinal surface/min) were calculated from 210Pb-activity determined in the carcass, liver, kidney, and blood. **Results:** 210Pb values correlated well with the uptake of 210Pb into liver, kidneys, and blood (linear regression coefficient ~0.7-0.8). Duodenal absorption rates were significantly higher in Fe-deficient than in Fe-adequate adolescent and adult mice, though increments were much less marked than found for 59Fe. Ileal 210Pb-absorption rates showed no difference in response to Fe-deficiency. However, they were in the same order of magnitude as in the iron-adequate duodenum, while ileal 59Fe-absorption rates were substantially lower in the ileum. **Conclusions:** The data suggest, that the impact of Fe deficiency on duodenal Pb absorption may be compensated by almost equal Pb-absorption rates in the distal small intestine. If these murine data are also valid for man, differences in regional intestinal transit time may explain the differences seen in clinical and epidemiological studies in humans. Factors that influence this variable, such as age, dietary ligands, or pathological influences due to intestinal diseases, need to be closely monitored and controlled in human studies on Pb absorption.
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P049. EFFECTS OF ZINC SUPPLEMENTATION ON GENE EXPRESSION OF ZINC TRANSPORTERS AND METALLOTHIONEIN IN HEMODIALYSIS POPULATIONS.

Amanda Amorim[1], Rubens Santana[1], Maraysa Carvalho[1], Rafael Brandão[1], Hélida Andrade[2], José Adail Castro[1], Adalberto Silva[1], José Tiburcio Neto[1,3], Carmem Barros[3], Erasmo Oliveira[4], Waldemar Bartchewsky[4], Marcelo Ribeiro[5], Semiramis Monte[1], Nadir Nogueira1
[1]Universidade Federal do Piauí , Teresina-Brazil,
[2]Universidade Federal de Minas Gerais, Belo Horizonte-Brazil
[3]CLINEFRO, Teresina-Brazil,
[4]Med-Imagem,Teresina-Brazil
[5]Universidade São Francisco,Bragança Paulista-Brazil
libufpi@gmail.com

Introduction: Zinc is an essential micronutrient for health. Its homeostasis is maintained by absorption and excretion processes, and by the action of ZIP, ZnT and metallothionein type proteins. The main alteration of zinc metabolism is hypozincemia, which is highly prevalent in Chronic Kidney Disease patients. Aim: The aim of this work was to assess the concentrations of corporeal zinc in hemodialysis patients, its association with risk factors, and the genetic expression of ZnT1, ZIP1, ZIP3, ZIP4, ZIP14 and metallothionein in baseline conditions and after the supplementation of 30 mg of zinc gluconate. Methods: Forty three patients on maintenance hemodialysis (CLINEFRO, Teresina-Piauí, Brazil) were studied. Blood samples were taken to determine the zinc levels in serum and in erythrocytes using atomic absorption spectophotometry. Hemoglobin levels, transferrin saturation, ferritin concentration, ultra-sensitive reactive C protein (PCRu) and RNA extraction for gene expression were also assessed. Data are expressed as means+SEM and were compared using one-way ANOVA followed by the Student–Newman–Keuls multiple comparison test or Student's t test. The relationship between zinc and the studied variables was assessed using Pearson's correlation coefficient and a univariate regression model. The level of significance adopted was $p<0.05$. Results: Hypozincemia was diagnosed in 49% of the patients, whose zinc concentration was individually associated with the hemodialysis time and transferrin saturation. There was a trend of negative association with PCRu. In the group with normal plasmatic zinc levels, there was no significant association between zinc concentration and the studied variables. Using a multivariate analysis adjusted for hemodialysis time, PCRu, transferrin saturation, hemoglobin and ferritin concentration variables explained the plasmatic zinc concentration of 55%, 31%, 26% and 24% , respectively. The most abundant transcripts in patients with normal zinc levels and hypozincemia were MT- ZnT1 - ZIP14 and ZnT1- ZIP14 - ZIP4, respectively. After 60 days of zinc supplementation and maintenance hemodialysis there was a negative regulation on the transcripts abundance of the studied genes, for both groups. Conclusion: Our findings confirm the existence of Zn dyshomeostasis in hemodialysis patients and that zinc supplementation has a partial negative regulatory effect on the genetic expression of zinc transporter proteins, which suggests an important inflammatory involvement in this zinc dishomeostasis.

P050. IN-SILICO IDENTIFICATION AND CHARACTERIZATION OF EFFLUX HEAVY METALS RESISTANCE PROTEINS IN *ACIDITHIOBACILLUS FERROOXIDANS*. Mauricio Latorre[1], Pablo Moreno[2], Andrés Aravena[2], Verónica Cambiazo[1], Alejandro Maass[2] and Mauricio González[1]
[1]Laboratorio de Bioinformática y Expresión Génica, INTA – Universidad de Chile.
[2]Laboratorio de Bioinformática y Matemática del Genoma, CMM – Universidad de Chile.
mlatorre@inta.cl

Prokaryotic cells have developed different systems of resistance to heavy metals. This resistance is the results of multiples layers of mechanisms with overlapping substrate specificities. In this context, proteins able to lead the efflux of metals play an essential roll in cellular detoxification. Such components are denominated heavy metals efflux resistance proteins, which have been classified in 3 families: 1) Efflux pumps driven by proteins of the resistance/nodulation/cell division superfamily (RND), 2) P-type ATPases and 3) facilitator action diffusion (CDF). The present work has for objective find these metal transporters in the extremophiles bacteria Acidothiobacillus ferrooxidans (ATCC 23270) found associated to bioleaching environment with high heavy metal concentrations. As a first step, a list of representative members of these protein families was used like template to search them by using the algorithm BlastP. Through global alignments, analysis of genomic structure, and search of functional specific motifs we identified and classified proteins of the RND family able to transport Cu/Ag, proteins of CDF family responsible for the efflux of Zn/Fe and the P-type ATPases CopA and CopB involved in Cu transport, distributed as follow: 3 proteins RND, 5 proteins of family CDF, 1 CopA and 2 CopB. Unlike mesophiles organisms like E.coli or B.subtillus, the A. ferroxidans have a greater amount of components associated to heavy metal resistance mediated by efflux proteins, particularly to transport Cu and Fe.
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P051. CIND, A COPPER-INDUCED NITROREDUCTASE INVOLVED IN PROTEIN DENITROSYLATION IN *LACTOCOCCUS LACTIS*. Frédéric Mourlane and Marc Solioz.
University of Berne, Switzerland.
marc.solioz@ikp.unibe.ch

Lactococcus lactis IL1403 is a lactic acid bacterium widely used for the manufacture of food and dairy products. Among the different stresses this microorganism can be exposed to during industrial processes, copper challenge is common when fermentation is performed in copper vats. Although copper is an essential trace element, it is toxic when present in excess. To tightly control cytoplasmic copper levels, all living organisms have evolved homeostatic mechanisms. In *L. lactis,* fourteen genes are under the control of the copper-inducible CopR repressor. The CopR regulon encompasses the CopR regulator, two copper ATPases, a copper chaperone, and seven additional genes of unknown function. We here addressed the function of one of these genes, ytjD, which we renamed cinD (copper-induced nitroreductase). Copper, cadmium and silver induced cinD in vivo, as shown by real time quantitative PCR. CinD was overexpressed, purified and was shown to encode a flavoprotein with nitroreductase activity. It catalyzed the reduction of nitrosylated glutathione and tyrosine in vitro, using NADH or NADPH as a reductant. When the cinD gene was inactivated, L. lactis became sensitive to S-nitrosoglutathione-induced nitrosative stress. Inactivation of cinD also increased intracellular levels of S-nitrosylated proteins. These findings suggest that the induction of CinD by copper represents a novel defense mechanism against nitrosative stress and indicate a connection between metal stress and nitrosative stress.

P052. ROLE OF SELENOPROTEINS IN BRAIN FUNCTION AND DEVELOPMENT. Ulrich Schweizer.
Charité-Universitätsmedizin Verlin, Berlin, Germany.
ulrich.schweizer@charite.de

Experiments with animals have demonstrated that selenium (Se) is an essential trace element, but feeding rodent Se-deficient diets did not produce neurological phenotypes. When we inactivated the gene encoding selenoprotein P (Sepp), we showed a massive reduction of brain Se levels and selenoprotein expression in Sepp-deficient mice. These mice suffered from a movement disorder and epileptic seizures. We then asked whether hepatic or brain Sepp was required for normal brain function. We engineered transgenic mice that either lacked specifically hepatic Sepp expression or expressed Sepp exclusively in the liver. We thus found that brain, and not hepatic, SePP is required to maintain brain Se levels. However, upon dietary Se shortage, brain Sepp-deficient mice cannot maintain their brain Se levels. Recently, members of the lipoprotein-related receptor family have been identified as SePP receptors. For example, mice deficient in ApoER2 (Lrp8) develop a Sepp-KO-like phenotype when fed a Se-deficient diet. We and others showed that megalin (Lrp2) serves as a SePP receptor in the kidney in vivo. Thus, eventually Se trafficking in the body can be studied both from the ligand and the receptor sides. In order to better define the roles of Se and selenoproteins in the brain, we undertook a systematic expression analysis in the mouse brain for all selenoproteins. We showed that several brain regions are particularly rich in selenoprotein expression. In addition, expression patterns of selenoproteins are complex in the brain and suggest specific, not housekeeping, roles in brain function. We started to systematically delete selenoprotein genes in the brain and study the resulting neurological defects in mice. As a proof-of-principle approach, we inactivated the gene encoding tRNA(Sec) specifically in neurons. These mice developed a neurological phenotype one week after birth with massive neurodegeneration in the cerebral cortex and hippocampus. These mice also suffered from cerebellar hypoplasia linked to Purkinje cell death and reduced granule cell proliferation. In an attempt to define which selenoproteins are essential for neuronal cells, we deleted thioredoxin reductase 1, an essential selenoprotein, in neurons. To our surprise, the mice developed normally. In contrast, mice with neuron-specific inactivation of glutathione peroxidase 4 resembled largely tRNA(Sec)-deficient mice, with a slightly milder phenotype. Again, cortical and hippocampal neurons degenerated and cerebellar hypoplasia ensued. Using primary neuronal cultures from mouse mutants, we showed that selenoprotein-deficient neurons are more susceptiple to chemical stressors. We will now analyse the phenotypes of selenoprotein-deficient mouse mutants and try to dissect the molecular mechanisms in neurons in which selenoproteins are involved.

P053. CANCER CHEMOPROTECTION THROUGH SELENIUM: A NUTRIPROTEOMICS APPROACH TO IDENTIFY SELENIUM BIOMARKERS. Andrea Mahn[1], Héctor Toledo[2], Manuel Ruz[3] and Ricardo Vega[1]
[1] Chemical Engineering Department, University of Santiago of Chile
[2] Institute for Biomedical Science, Medicine Faculty, University of Chile
[3] Department of Nutrition, Medicine Faculty, University of Chile
amahn_2000@yahoo.es

Selenium offers important health benefits. Some chemical forms of selenium, especially the organic ones, are considered as chemoprotective, because they help in the prevention of some types of cancer. The traditional selenium indexes, such as glutathione peroxidase activity and total selenium concentration, do no account for the metabolic status of this element regarding its chemoprotective effect. In this study we evaluated the relationship between the chemoprotective status of mammalian organisms against some types of cancer and its association with a characteristic protein pattern in blood plasma. Within this pattern, some specific proteins would be identified, which could be proposed as biomarkers of chemoprotective forms of selenium. With the aim to gather information on the proteomic profiles that characterize the chemoprotective status, it was investigated if the dietary supplementation of rats with chemoprotective forms of selenium (sodium selenate or selenomethyl-selenocysteine) is reflected as differences in the abundance of some proteins in blood plasma. Additionally, the effect of the selenium dose and the length of the dietary supplementation period were investigated, in order to determine the main factors affecting the response of rats to selenium supplementation. Ten experimental groups were used, consisting in six Wistar rats each. A proteomic approach was used to quantify protein expression differences in blood plasma, consisting in two-dimensional gel electrophoresis and mass spectrometry. The differences in the abundance of plasma proteins was assessed by a Student`s t test at 95% confidence. The statistical significance of the factors and their interactions was determined by ANOVA at 95% confidence. Some proteins were significantly affected by the selenium dose; other proteins were significantly affected by the length of the supplementation period. No protein was significantly affected by both factors in the same direction, and no protein was detected to be reduced due to supranutritional selenium supplementation. In conclusion, selenium dose would be the main factor affecting the response of rats to selenium supplementation [1]. The proteomic response of rats to dietary supplementation with chemoprotective forms of selenium was characterized, resulting in some proteins whose abundance in blood plasma was increased selectively depending on the chemical form of selenium present in the diet. Apolipoprotein E, haptoglobin and transthyretin significantly increased their abundance in plasma regardless the chemical form of selenium that supplemented the diet. HNF6 responded only to SMSeC supplementation, and not to sodium selenate. Fibrinogen and alpha-1-antitrypsin increased their abundance only when sodium selenate was used. These protein patterns could probably be proposed as new selenium indexes to assess the metabolic status of this element in mammals, and would constitute a new biotechnological tool which could be used in the context of cancer prevention [2].

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[2] A. Mahn, R. Vega, M. Ruz (2008). Effect of organic selenium supplementation on rat blood plasma proteome. J. Trace Elem. Med. Biol. (submitted).

P054. EFFECT OF INORGANIC AND ORGANIC SELENIUM SUPPLEMENTATION ON GROWTH RATE, BLOOD METABOLITES, ANTIOXIDANT STATUS AND IMMUNE RESPONSE IN LAMBS. Anil K. Garg, Neeraj Kumar, Vinod K. Chaturvedi and Vijai P. Varshney
Mineral and Vitamin Nutrition Laboratory, CAS in Animal Nutrition, Indian Veterinary Research Institute, Izatnagar-243122; India.
garg@ivri.up.nic.in

Present study was aimed to investigate and compare the effect of inorganic and organic selenium (Se) supplementation on growth rate, blood metabolic profile, humoral immune response and antioxidant status in lambs. For this purpose, eighteen male lambs of about 8-9 months of age and 24.68±2.89 kg mean body weight were divided in to three equal groups of six animals each in a randomized block design. All animals were fed a common standard total mixed ration (TMR) containing concentrate mixture (CM) and wheat straw (WS) in 65: 35 ratio to meet their nutrient requirements. Animals in group T1 and T2 were additionally supplemented with 0.15 ppm Se either through sodium selenite (inorganic Se) or Jevsel-101 (organic Se). Concentrate mixture consisted of 30% crushed maize grain, 27% soybean meal, 40% wheat bran, 2% mineral mixture (without Se) and 1% common salt. Experimental feeding was administered for 90 days during which body weights of all the animals were recorded regularly at 15 days interval. To assess the humoral immune response, all the lambs were intramuscularly inoculated with a single dose (2ml) of Haemorrhagic septicaemia oil adjuvant vaccine on 0 day; and blood samples were collected at baseline and on days, 30, 60 and 90 post experimental feeding. Supplementation of Se had no effect on serum total cholesterol, total protein, albumin, globulin, albumin: globulin ratio, tri-iodothyronine (T3), thyroxine (T4), T4: T3 ratio; plasma calcium and phosphorus levels and serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase activity, as values of these parameters were found to be comparable ($P>0.05$) among the three groups throughout the experimental period and all the values were within the normal range. There was a significant increase in the plasma Se level and RBC glutathione peroxidase activity humoral immune response in both the Se supplemented groups (T1 and T2) as compared to the control (T0) group. Humoral immune response measured as antibody titre against Pasturella multocida P52 was also significantly ($P<0.05$) higher in both the Se supplemented groups (T1 and T2) as compared to the control (T0) group on 30, 60 and 90 day post vaccination. Average daily gain (ADG) was significantly higher ($P<0.05$) in both the Se supplemented groups. The highest ADG was observed in group T2 (110g) followed by group T1 (98.2g) and lowest (89.1g) in the control (T0) group. Feed required per kg gain was also less by 11.2 and 17.1% in group T1 (8.70kg) and T2 (8.12kg), respectively, as compared to control group (9.80kg). Thus, results suggest that supplementation of Se (organic as well as inorganic) at 0.15 ppm level in the diet of lambs improves their growth rate, humoral immune response and antioxidant status. Of the two sources, organic Se was more effective than inorganic Se.

P055. EFFECT OF SELENIUM SUPPLEMENTATION ON MILK YIELD IN GRAZING DAIRY COWS. Javier Neumann[1,3], Alejandro Ceballos[2], Helga Böhmwald[1] and Fernando Wittwer[1]
[1]Inst. Cs. Clínicas Veterinarias, Univ. Austral de Chile. Valdivia, Chile.
[2]Dept. Health Management, Univ. Prince Edward Island. Charlottetown, PE, Canada.
[3]Escuela de Medicina Veterinaria, Univ. Catolica de Temuco. Temuco, Chile.
javierneumann@uach.cl

Introduction Selenium (Se) is found in animal tissues as part of selenoproteins, some with a structure similar to sulphur amino acids. The blood activity of the antioxidant selenoenzyme glutathione peroxidase (GPx; EC 1.11.1.9) has been used as a biomarker of Se nutritional status in mammals. Many studies have demonstrated a positive effect of Se supplementation on milk production; however, results are still controversial. An increase in milk production has been observed associated to the supplementation with organic Se, but this response has been inconsistent. A wide range of factors can affect the response to Se supplementation, such as differences in oxidant challenges or dietary factors that interact with Se requirements. Consequently, it is necessary to evaluate the effect of different sources and routes of administration of Se on milk yield in pasture-based dairy cows. The objective of this study was to evaluate the effect of prepartum supplementation with two sources of Se on the blood activity of GPx, and on milk yield in pasture-based dairy cows. Matherial and Methods Forty healthy, multiparous, ±7 months of gestation, Chilean-Friesian cows were selected for the trial. Cows were systematically randomized to one out of three treatments. Group 1: n=13, received a single s.c. injection of barium selenate±1 month before calving (1 mL/50 kg body weight. Deposel®, Young's Animal Health, Auckland, New Zealand), which was sufficient to provide 1 mg Se/kg BW. Group 2: n=12, received 3 g/day of Se yeast since±1 month before calving (Sel-Plex®, Nicholasville, KY, USA) sufficient to provide 3 mg of Se per day; and after calving until 60 days in milk the dose was increased to 6 g/d. Group 3: n= 15, remained unsupplemented. Blood samples were collected by coccygeal vein puncture prior to supplementation and at 1, 15, 60, 105 and 150 days postpartum to measure blood GPx activity using a commercial kit (Ransel®, Randox Lab., Crumlin, UK). Milk yield was recorded from each cow at the same times. Data were analyzed using a liner mixed model in Stata release 10.0 (Stata Corp., College Station, TX, USA). Results The mean activity of GPx was 240U/g Hb, 238U/g Hb, and 190 U/g Hb for Group 1, Group 2, and Group 3, respectively at beginning the study. The activity significantly increased after the beginning of supplementation ($P<0.05$) (Group 1: 497 UI/gr/Hb, Group 2: 515 UI/gr/Hb and Group 3: 486 UI/gr/Hb), were not observed differences between treated groups. The interaction between treatment and time was not significant. Milk production was higher in barium selenate-treated cows at 60 days in milk (34 kg/d Group 1; 33 kg/d Group 2; 32 kg/d Group 3) ($P<0.05$), while the Se-yeast-treated group showed a lower production at 150 DIM ($P=0.05$). Conclusion Selenium supplementation using either organic or inorganic forms effectively increased the blood activity of GPx in grazing dairy cows. Even though barium selenate is a long-acting Se form, an effect on milk yield was evident in barium selenate-treated cows only at 60 days in milk.

P056. THE HEALTH PROMOTING QUALITY OF FOOD OBTAINED FROM CROPS FOLIARY ENRICHED WITH SELENIUM. Ivana S. Djujic
University of Belgrade, IHTM -Department of Chemistry, Serbia
ankivana@eunet.rs ankivana@EUnet.yu

Selenium (Se) is implicated in the protection of body tissues against oxidative stress, maintenance of defenses against infection, and modulation of growth and development. The natural environment has a profound influence on its contents in the food chain and the development and distribution of Se responsive diseases. To overcome the Se deficiency problem in low Se areas we developed own procedure for foliar enrichment of crops with Se. Conducted field trials showed that by the foliar application, the liquid formulations with Se salts at physiologically suitable phase of plant's development, can be obtained crops with optimal content and distribution of Se and improved values of many other parameters important for its quality, nutritive value, tolerance to environmental stressors and yield. Investigations showed that Se added in proper, for each crops specific, amounts exerts beneficial effects through multiple mechanisms. Investigations of essential elements, proteins, amino and fatty acids, vitamins, tolerance to oxidative damage and yield conducted on soybean and wheat, showed that seeds with the Se concentration in the optimal interval have improved quality and yield of crops that grew under unfavourable external conditions. Researches conducted on Japanese quails that consumed mix feed diet prepared with crops biofortified with Se foliar showed that such feed laying quails have higher egg production, mass, protein content as well as significantly increased n-3 fatty acids content and tolerance to oxidative damage. Such produced quail eggs have over 50% lower cholesterol content, much less toxic elements in eggshell and inside part of egg and are richer in many in nutrition often deficient nutrient as are choline, vitamin A, riboflavin, vitamin B12, pantothenic acid, minerals - J, Cu, Cr, Si, Ca, Fe, Zn and essential amino acids than eggs obtained by ordinary farming procedure. Optimization of raising conditions (daylight and feeding in meals) contributed that in quail eggs ratio of n-6 /n-3 omega acids rich value of 1.5. Analyses of quail muscle tissue (thigh, wing, breast) confirmed that natural Se form in feed mix contributes that undesirable changes in a number of quality parameters, including loss of water-holding capacity, texture and flavour became lower, while oxidative stability, sensory quality, shelf life and acceptability of quail meat is improved. Examinations of the health benefits that offer consumption of mentioned plant and animal products showed that its contribution to daily Se intake was enough high that Se deficient population of Serbia can assure even more Se in natural form then is RDA, as well as that, due to higher intake of many interrelated nutrients the benefit that foliar biofortification with Se can offer Se the deficient human population overcomes the benefit of any known Se supplement.

P057. SELENIUM DETERMINATION BY NEUTRON ACTIVATION ANALYSIS (NAA) APPLIED TO POULTRY PRODUCTION AND HEALTH. María Dallorso[1], Sara Resnizky[2], Rodrigo Invernizzi[2] and Ernesto Benavídez[1]
[1]Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora. Buenos Aires. Argentina.
[2]Grupo Técnicas Analíticas Nucleares, Comisión Nacional de Energía Atómica. Buenos Aires. Argentina
resnizky@cae.cnea.gov.ar

Broilers meat consumption is increasing, as its meat is rich in nutrients. Meat quality and concentration of several nutrients depend largely on the diet fed to the birds. Selenium has important physiologic effects in humans and animals. As selenium is a scarce mineral on our planet and the average concentration in igneous bedrocks is only 0.05 ppm, the way to increase selenium concentration in human diet has to be considered. Poultry meat is quite sensitive to oxidative deterioration. Many studies have indicated that lipid oxidation in meat products can be effectively controlled using antioxidants. Feeding poultry a higher level of dietary antioxidants provides the poultry industry with a simple method for improving oxidative stability and shelf life of poultry meat. Selenium is one of the key dietary factors in the nutritional modulation of oxidant protection There are then dual benefits from the Se supplementation of broilers; improved health and performance of the animal and improved product quality for human consumption. A concentration of 0.15 mg of Se/kg of diet is recommended for broiler chickens throughout the growth period. The typical broiler diet today is cereal based being a common practice to supplement broiler diets with Se. The maximum allowable level of Se supplementation is 0.3 ppm. In the present work selenium was determined in feed, premix, plasma and meat by NAA in order to provide a sensitive technique to be applied to animal nutrition studies. Feed and premix samples were obtained at different times during a complete growth period from a broiler chicken farm located in Bs.As. province (Argentina); blood and breast samples were taken from a slaughter house located in Bs.As. province. Feed and premix was grounded using freeze mill. Blood samples were taken by complete bleeding during slaughter, heparinized and plasma kept frozen until 4 milliliters plasma aliquots were lyophilized. Breasts (pectoralis major) samples were taken, weighed and homogenized with bi-distilled water; 4 milliliters of the homogenate were lyophilized for analysis. A radiochemical separation based on Se co-precipitation with HgS was applied to samples irradiated six hours in RA-3 reactor, Ezeiza Atomic Center (3× 1013cm-1s-2, 8 Mw power proximately). After 30 days the total selenium was determined through its long-lived nuclide 75Se by gamma-ray spectrometry, using appropriate standard. Certified Reference Materials were used for analytical quality control. The results show selenium levels (mean±SD) of 0.267±0.084 mg/g; 34.74±14.23 mg/g; 0.121±0. 017 mg/ml y 0.135±0.016 mg/g for “as fed” feed (n=14), “as fed” premix (n= 2), plasma (n=4) and fresh breast (n=4), respectively. The analytical procedures described in the present work could be used in selenium determination of both feed and biological samples. This advantage allows us to assay selenium levels in feed and selenium content of animal tissues during feeding trials in experimental farms. Additionally, the procedure described here could be applied to quality control assays of Se content of feed and premix employed in poultry breeding cycles in productive farms.

P058. CONGRUENCY AND DIVERGENCY OF CALCIUM IN THE BIOLOGICAL MATRICE OF THE HUMAN HAIR WITH THE OTHER OSTEOTROPHIC AND NON-OSTEOTROPHIC MEMBERS OF THE MULTIELEMENT PROFILE.
[1]Juraj Prejac, [2]Asja Čelebić, [2]Jasmina Stipetić-Ovčarićek, [2]Renata Poljak-Guberina, [2]Petra Nola-Fuchs, [3]Anatoly Viktorovich Skalny, [4]Margarita Germadievna Skalnaya, [5]BerislavMomčilović.
[1]University Hospital Center, Zagreb (Zg), Croacia
[2]School of Dental Medicine, University of Zagreb, Croacia
[3]Institute of Bioelements, Orenbrg State University, Ornburg, RUSSIA,
[4]Center for Biotic Medicine, Moscow, RUSSIA,
[5]Institute for the Research and Development of the Sustainable Eco Systems, Zg, Croacia
berislav.momcilovic@gmail.com

Multielement profile (MP) of some biological matrice, like hair, offers an non-invasive insight into their mutual context behavior relevant to the metabolism of the mineralized tissue. Indeed, identification of the elements with or without strong affinity for the bone (osteotrophic) is of considerable importance for the maintenance of the bone health, especially in the women who are more prone to the osteoporosis than men. The aim of this study was to compare the similarity in the pattern of the calcium hair deposition with the other members of the hair MP. The study was conducted by following the ethical principles of the Declaration of Helsinki for the Human Calcium congruent trend pattern Ca: \ Element: Low Normal High High ■ Normal ■ Low ■ Calcium divergent trend pattern Ca: \ Element: Low Normal High High ■ Normal ■ Low ■ Calcium like (congruent) elements are: Ca, Sr, Ba, Mg, Ag, Ni, Co, Ga, Au, La, Ti, W, Mo, P, Sb and Cu, whereas calcium inverse (divergent) elements are As, Al, Rb, B, K, Fe, and V. The other studied elements couldn't be subsumed to such a simple pattern (Be, Bi, Cd, Cr, Ge, Hg, I,Li, Mn, Na, Pb, Pt, Se, Si, Sn, Tl, Zn, Zr). This results show that the major oxygen carrying element (Fe), and the major intracellular electrolyte (K) exhibited a calcium divergent trend pattern. Indicating an inverse relationship between the mineralisation and cellular oxygenation. The study confirmed the eminent calcium congruent osteotrophic elements (Sr, Ba, Mg, Ti), and identified some new ones (Ag, Ni, Co, Ga, Au, La, W, Mo, Sb, Cu) which were not generally considered to have osteotrophic qualities. Evidently, bone health may include quite a number of trace elements beyond the most prominent trio of Ca, Mg, and vitamin D. Their precise metabolic role and requirements need to be elucidated. Acknowledgments. This work was supported by the RCI, Isle of Man, UK; McDonald's, Zagreb, Croacia; and MZOS, Zagreb, Croacia, Grant 292-0222412-2405.

P059. GENDER DEPENDENT HOMOLOGY OF THE HUMAN HAIR AND WHOLE BLOOD MULTIELEMENT PROFILE.
[1]Berislav Momčilović, [2]Jadran Morović, [3]Juraj Prejac, [4]Sandra Morović, [5]Vesna Sitar-Srebočan, [6]Anatoly Viktorovich Skalny, 7Margarita Germadievna Skalnaya.
[1]Institute for the Research and Development of the Sustainable Eco Systems, Zagreb (Zg), CROATIA (CRO),
[2]Psychiatric Ambulance, Mental Health Center, Community Health Services – Center, Zg, CRO,
[3]Unibersity Hospital Center, Zg, CRO,
[4]Clinical Hospital "Sestara milosrdnica", Zg, CRO,
[5]Croatia osiguranje, Zg, CRO,
[6]Institute for Bioelements, Orenburg State University, Orenburg, RUSSIA,
[7]Center for Biotic Medicine, Moscow, RUSSIA
berislav.momcilovic@gmail.com

Multielement profile analysis of a biological matrice allows for the study of the element metabolism in their mutual context. The aim of this study was to explore the role of gender on the multielement profile pattern of 58 women and 38 men of adult age. The study was conducted by following the ethical principles of the Declaration of Helsinki for the Human Subject Research. Forty one element were analyzed in the occipital scalp hair (H) and whole blood (WB) by the ICP MS (Elan 9000, Perkin Elmer, USA) (Momčilović et al. TEM (Moscow), 2006;7(4):35-42). Median difference of an element of 10% or more between men and women was considered to be significant. The results allows for element grouping into four homologous patterns: Men>Women (H, WB): As, B, Mo, Rb, V, Zr Women>Men (H, WB): Ca, Co, Cu Men>Women (H), Women>Men (WB): Cd, Na Women>Men (H), Men>Women (WB): Au, Ba, Ga, Ge, Sn, Sr Other studied elements (Ag, Al, Be, Bi, Cr, Fe, Hg, I, K, La, Li, Mg, Mn, Ni, P, Pb, Pt, Sb, Se, Si, Ti, Tl, W, Zn) did not exhibit such a simple gender dependent homologous pattern between the hair and the whole blood, albeit more complex associations can't be excluded at this time. The higher values of Ca, Co, and Cu in both the H and WB of women than man appears to be especially intriguing. This is the first evidence to our knowledge of such evident gender dependent differences in the metabolism of several elements between men and women. Acknowledgments. This work was supported by the RCI, Isle of Man, UK; McDonald's, Zg, CRO; and MZOS, Zg, CRO, Grant 292-0222412-2405.

P060. DEPRESSION DEPENDENT HOMOLOGY OF THE HUMAN HAIR AND WHOLE BLOOD MULTIELEMENT PROFILE.
[1]Berislav Momčilović, [2]Jadran Morović, [3]Juraj Prejac, [4]Sandra Morović, [5]Ninoslav Mimica, [2]Irena Bezić, [2]Tanja Radionov, [2]Ankica Svirč, 6 Anatoly Viktorovich Skalny, 7 Margarita Germadievna Skalnaya.
[1]Institute for the Research and Development of the Sustainable Eco Systems, Zagreb (Zg), CROATIA (CRO),
[2]Psychiatric Ambulance, Mental Health Center, Community Health Services-Center, Zg, CRO,
[3]University Hospital Center, Zg, CRO,
[4]Clinical Hospital “Sestara milosrdnica”, Zg, CRO,
[5]Psychiatric Hospital “Vrapce, Zg, CRO,
[6]Institute of Bioelements, Orenburg State University, Orenburg, RUSSIA ,
[7]Center for Biotic Medicine, Moscow, RUSSIA
berislav.momcilovic@gmail.com

Multielement profile analysis of the hair (H) and whole blood (WB) biological matrice allows for the study of the element metabolism in their mutual context. The aim of this study was to explore the role of depression on the multielement profile pattern of depressed (33 women and 15 men) and control subjects (25 women and 23 men) of adult age. Depression was diagnosed according to the DSM-IV criteria. The study was conducted by following the ethical principles of the Declaration of Helsinki on Human Subject Research. Forty one element were analyzed in the occipital scalp H and WB by the ICP MS (Elan 9000, Perkin Elmer, USA),(Momčilović et al. TEM (Moscow), 2006; 7(4): 35-42). Median difference of an element of 10% or more between control and depressed subjects was considered to be significant. The results allows for element grouping into four homologous patterns: Depressed>Control (H,WB): Ag, As, B, Ba, Cd, Ga, Ge, La, Li, Mn, Rb, Sb, V, W Control>Depressed (H, WB): Bi, Se Depressed (H>WB), Control (WB>H): K, Na Control (H>WB),Depressed (WB>H): Au, Cu, Hg, Mg, Sr, Zr Other studied elements did not show such a simple depression dependent homologous pattern between the hair and the whole blood (Al, Be,Ca, Co, Cr, Fe, I, Mo, Ni, P, Pb, Pt, Si, Sn, Ti, Tl, Zn), albeit more complex associations can’t be excluded at this time. Evidently, there is a long list of predominantly non-essential elements that are higher in both H and WB of depressed than in the healthy control subjects. It’s not surprising that the essential Se is decreased in depression, but the same hold for Bi considered to be non-essential. The fact about the high K and Na in the hair of depressed as opposed to the blood of control subjects is intriguing since it indicates some basic discrepancy in the control of the water and electrolytes balance to be at the core of the depression. Acknowledgements. This work was supported by the RCI, Isle of Man, UK; the McDonald’s, Zg, CRO; and the MZOS, Zg, CRO, Grant 292-0222412-2405.

P061. MUCIN DOES NOT IMPROVE HEME IRON BIOAVAILABILITY. Gustavo Cediel, Manuel Olivares, Fernando Pizarro. Institute of Nutrition and Food Technology (INTA), University of Chile,
gcediel@inta.cl

Background: Previous studies have shown that heme iron absorption is saturable, and it is mediated by a receptor in the apical membrane of the enterocyte. It has been established that meat present in the diet increases heme iron bioavailability. There is a lack of information regarding the role that some of the proteins found intraluminally may have. Recent studies using the Caco-2 cell model have shown that mucin increases heme iron absorption; however, there is no evidence that this also occurs in humans. Objective: To establish the role of the mucin in the absorption of heme iron in humans. Design: Fourteen apparently healthy women between 35-45 years of age were selected to participate in one iron absorption study. Informed consent was obtained from all the volunteers prior to the absorption studies. The women received 5 mg of iron as heme intrinsically labeled with either 3 uCi 55Fe or 1 uCi 59Fe plus 1.7 g of mucin. On days 1, 2, 14 and 15 the subjects intake heme alone, heme + mucin, partially digested hemoglobin, partially digested hemoglobin + mucin, respectively. On days 14 and 28 blood samples were collected to assess iron status and to determine the amount of circulating radioactivity using the Eakins and Brown double isotope technique. Repeated measures ANOVA was used to compare the mean percent bioavailability of iron from the test meals. Results: Geometric mean (range±1SD) of heme iron absorption was 16.4% (10.5-25.7), 13.1% (9.0-18.9), 13.7% (9.0-20.7) and 11.8% (7.6-18.3) for heme alone, heme + mucin, partially digested hemoglobin, partially digested hemoglobin + mucin, respectively (ANOVA, F=2.89, N.S.). Conclusion: Mucin does not improve heme iron bioavailability.
Financial Support: Fondecyt Grant 1061060.

P062. ANEMIA IN PRE-SCHOOL DAY CARE CENTERS IN SAO PAULO: EVALUATION AFTER 2 YEARS OF INTERVENTION IN CONTROL OF IRON DEFICIENCY WITH THE FORTIFICATION OF WHEAT AND CORN FLOUR.

Edna Machado, Cleber Costa, Celia Colli, William Latorre and Sophia Szarfarc.
Post-Graduating Program in Aplied Human Nutrition Faculty of Pharmacy/Faculty of Economy and Administration/ Faculty of Public Health. University of S.Paulo, Brazil
ehe_cap@ig.com.br

SUMMARY Introduction: Preschool children are one of the most vulnerable groups to iron deficiency anemia. Objective: The objective of this study was to evaluate the prevalence of anemia of children from 24 months to 5 years old who attended day-care centers in the city of S.Paulo/Brasil. Methodology: 465 pre-school children, of six day-care centers of the ButantÃ£ regional, from municipality of Sao Paulo, had their peripheral hemoglobin concentration determined (HEMOCUE) and their nutritional status evaluated through the Z-scores of weight / age, height / age and weight / height. Results: The prevalence of anemia (Hb< 11g/dL) was 20.9% significantly smaller than that (69.7%) found in the same region before the implementation of the National Program for the Iron Fortification of Wheat and Corn Flours (4mgFe/100g) in 2004. A prevalence of 2.4% of malnutrition, 0.9% of current malnutrition and 5.1% overweight were also found. There was no association of anemia with the anthropometric parameters nor with the socio-economic and domestics conditions of the children, what is possibly due the homogeneity of the population assisted by the day-care centers. Conclusion: There was significant reduction of anemia in the group studied compared to the previous values obtained in other studies in the same region, which may have occurred due to an increase of iron intake through the iron fortified flours once bread and / or biscuits and / or cake are present in their daily menus.
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P063. CORRELATES OF FASTING CIRCULATING 25-HEPCIDIN LEVELS AND 25-HEPCIDIN RESPONSES TO ORAL IRON SUPPLEMENTATION IN HEALTHY, GUATEMALAN MEN. Noel W. Solomons[1], Klaus Schümann[2], María-Eugenia Romero-Abal[1], Guenter Weiss[3], Dorine Swinkels[4].
[1]Cessiam, Guatemala City, Guatemala
[2]Center Institute for Nutrition and Food Research, Technische Universität Muenchen, Germany,
[3]Dep. General Internal Medicine, Clinical Immunology and Infectious Diseases, Medical University Innsbruck, Austria
[4]Dep. Clinical Chemistry Radboud University, Nijmegen, The Netherlands.
KSchuemann@schuemann-muc.de

Background: Hepcidin is a peptide of hepatic origin, discovered in 2001. The active hormonal (25-hepcidin) form down-regulates the egress of iron from cells via the ferroportin mechanism. Only recently has valid assay techniques for serum levels been developed (2008) within our collaboration. **Objectives:** To apply a newly developed quantitative assay for 25-hepcidin in serum to human diagnostic and metabolic research. **Design and Methods:** Fasting and 1-, 2- and 3-h post-challenge serum samples were collected after oral dosing of 8 Guatemalan male volunteers with 0, 15, 30, 60, 120 and 240 mg of iron as ferrous sulfate in August-September 2005. Ferritin, serum iron, and % transferrin saturation and prohepcidin (84-hepcidin precursor peptide) were measured within 3 months of collection in collaborating European laboratories by extant assays. It was only until 2008, however, that development of an assay for 25-hepcidin in serum allowed its measurement in samples stored at -20oC. **Results:** No significant correlation between 25-hepcidin and prohepcidin within serum samples was found. There were, however, significant and inverse correlations between baseline (fasting) 25-hepcidin status and circulating ferritin and % transferring saturation. Baseline 25-hepcidin, moreover, was inversely related to the area-under-the-curve of oral iron at the 240 mg dosage. None of the doses of iron produced a post-dose circulating 25-hepcidin response. **Conclusions:** Despite attenuation of analytic fidelity from sample storage, biological bases for an expected relationship of 25-hepcidin levels iron status is demonstrated, without any time-dependent response to oral iron supplementation.
This study was supported by the Hildegard-Grunow-Foundation, Munich, Germany

P064. ZINC NUTRITIONAL STATUS OF UNIVERSITY STUDENTS IN SÃO PAULO, BRAZIL. Vanessa Cristiane Miyazato and Silvia Maria Franciscato Cozzolino.
Laboratory of Nutrition-Minerals, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil
smfcozzo@usp.br

One of the essential trace elements found in the human body in zinc (Zn), a mineral that aid the catalytic activity of many enzymes, besides taking part in the metabolism of proteins, carbohydrates, lipids, and nucleic acids. The aim of this research was to evaluate the diet of 30 academic students from 19 up to 30 years old, for three days, through the record of the consumed food, and to correlate the results with the plasmatic and erythrocyte zinc levels. Moreover, the centesimal composition of the most consumed foods registered in the software of evaluation was determined, and afterwards the new obtained data by the food record list was recalculated. The results showed that at least 30.0 % of the students presented deficient intake of zinc (<6.8mg/d) and 26.7% from 6.8 to 8.0mg/d. In relation to the biochemical parameters, the zinc nutritional status of the subjects was deficient in the most part of them; the plasma Zn mean concentration was 63.3±10.5µg/dL (83.3%<75 µg/dL) and erythrocytes Zn mean concentration was 40.2±8.9 µgZn/gHb (43.3% <40 µgZn/gHb). According to BMI levels, 80% of them were eutrophic.

P065. EFFICACY OF AN IRON-BASED INTERVENTION ON ZINC STATUS OF YOUNG ADULT NEW ZEALAND WOMEN WITH MILD IRON DEFICIENCY.
Nicolas Prosser[1], Anne-Louise Heath[1], Sheila Williams[2] and Rosalind S Gibson[1]
[1]Department of Human Nutrition, University of Otago, Dunedin, New Zealand
[2]Department of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand
Rosalind.Gibson@Stonebow.Otago.AC.NZ

Interventions to combat mild iron deficiency in women of child-bearing age may affect zinc nutriture. Supplemental iron may impact adversely on zinc absorption and metabolism, especially when high-dose iron supplements are taken without food. In contrast, iron-based dietary interventions may enhance zinc as well as iron status because zinc and iron share many food sources and factors affecting bioavailability. We used a combination of dietary, biochemical, and functional indices to assess change in zinc status during a four-month partially blinded placebo-controlled iron-based intervention in women with low iron stores (serum ferritin<20 ug/L and haemoglobin>120g/L) from Dunedin, New Zealand. Exclusion criteria were anaemia, pregnancy, lactation, or health problems and/or medications likely to affect iron or zinc status. Subjects aged 18 – 40 years were randomly assigned to a dietary advice group (DG; n=29), a daily iron supplement group (SG; 50 mg Fe as amino acid chelate) with meals (n=23), and a placebo group (PG; malto-dextrin; n=26). A dietitian provided individual dietary advice to the DG at baseline, and at weeks 2 and 6, with telephone check-ups at weeks 10 and 14. Advice included strategies to increase intakes of iron-containing foods (especially flesh foods) and factors considered to enhance non-haem iron absorption, and to decrease absorption-inhibitors, particularly with meals. A validated semiquantative food frequency questionnaire (SFFQ) was administered at 0, 4, 8, 15 weeks; fasting morning blood samples assayed for serum zinc and alkaline phosphatase (ALP) at baseline and 4, 8, 12, and 16 weeks; hair zinc and taste detection thresholds by electrogustometry were measured at baseline and 16 weeks. Data were also collected for variables that could possibly confound these indices, including: serum C-reactive protein, oral contraceptive use, and season. Based on the average of 3 SFFQs, intakes of meat, fish, poultry, and vitamin C increased, whereas calcium, phytate, and phytate-to-zinc molar ratios decreased ($p<0.001$) in DG compared to SG+PG, with no significant increases in zinc or total iron intakes. Changes in zinc indices, after accounting for likely confounders, were compared between the two intervention groups and placebo. Serum zinc values increased in DG (adjusted, $p=0.001$); however the same rise occurred in PG, and the difference between the two groups was not significant. In contrast, a small decrease in serum zinc concentrations in SG was significant when compared to placebo ($p=0.02$). A similar, though non-significant trend was apparent for ALP activity. Changes in hair zinc concentrations were not significantly different between groups. Taste acuity was not consistently affected by the intervention: changes in taste threshold values from baseline to post-intervention showed no association with changes in serum zinc, ALP activity, or the dietary measures, despite being weakly associated with serum and hair zinc concentrations at baseline and with change in hair zinc concentrations ($p=.01$). In conclusion zinc status was not improved compared to placebo by a dietary intervention designed to improve iron levels. However, a moderate-dose iron supplement taken daily with meals appeared to impair zinc status in these young adult women. Funded by HRC, New Zealand.

P066. EFFECT OF ZINC SUPPLEMENTATION ON THE ANTIOXIDANT STATUS OF PHYSICALLY ACTIVE ADOLESCENTS. Josely C Koury, Karla JF de Oliveira, Astrgildo V Oliveira-Junior and Carmen M Donangelo
Instituto de Nutrição UERJ, Instituto de Educação Fisica e do Desporto UERJ, Instituto de Química UFRJ- Rio de Janeiro Brasil.
jckoury@gmail.com

Puberty associated with intense physical activity results in oxidation stress. Zinc supplementation may benefit antioxidant capacity. The aim of this study was to compare the effect of nutritional counseling and zinc supplementation with nutritional counseling alone on antioxidant status in physically active male football players (13±0.4 y). The subjects were divided in two groups and studied during 12 wks under nutritional counseling and Zn-supplementation (Zn-SUP, Zn: 22 mg/d as zinc gluconate, n=21), or nutritional counseling alone (NC, n=26). Nutrition counseling was based on the dietary health guide for the Brazilian population that encourages the intake of foods groups according to the food pyramid. Each adolescent received a list of food replacements considering food groups and food portions. The adolescents were stimulated to consume at least 4 to 5 meals per day. Zinc in plasma, erythrocyte and urine, was measured by inductively coupled plasma–optical emission spectrometry. Ferric-reducing ability (FRAP), ceruloplasmin and conjugated dienes were measured in plasma. Erythrocyte osmotic fragility was measured in vitro in saline-washed erythrocytes. The effect of treatment on variables within each group (Zn-SUP or NC) was evaluated by comparing results after-treatment with those at baseline, using paired t-test. Differences between groups were determined by ANOVA. At baseline, there were no significant differences in biochemical indices between the two groups. After treatment, plasma zinc increased in both groups ($p<0.001$); urinary zinc increased only in Zn-SUP ($p<0.001$), and erythrocyte zinc decreased only in NC ($p=0.002$). Plasma ferric-reducing ability and plasma conjugated dienes increased in both groups ($p<0.01$), although after the 12 wk-treatment the latter two were significantly lower in Zn-SUP compared to NC ($p<0.01$). Erythrocyte osmotic fragility decreased in both groups after 12wk-treatment were significantly higher in Zn-SUP compared to NC ($p=0.006$). Plasma ceruloplasmin was significantly higher in Zn-SUP ($p<0.05$). In conclusion, this study indicates that the use of 22 mg/d of supplemental zinc during 12 wks in adolescent athletes who also received nutritional orientation, improved markers of antioxidant status, although only slightly better than nutritional counseling alone. Financial Support: FAPERJ and CNPq (Brazil)

P067. METABOLIC ALTERATIONS OF ZINC RELATED TO AGING. Anna Cecília Queiroz de Medeiros, Maria das Graças Almeida, Vanessa Teixeira de Lima Oliveira, Débora Azevedo Nascimento, Kênio Costa Lima, Lorena dos Santos Tinoco and Lucia de Fátima Campos Pedrosa.
UFRN - Federal University of Rio Grande do Norte
annacqm@yahoo.com.br

Although many studies point to alterations in the organic concentrations of zinc in elderly patients, the mechanisms by which aging might cause changes in the metabolism of this nutrient remain unclear. Thus, the aim of this study was to assess plasma zinc and ZnBCPP in adults and elderly individuals, to determine zinc-related changes in nutritional status caused by the aging process. This cross-sectional study was conducted with a group of elderly subjects older than 60 years of age, of both sexes (n=14); and the other was composed of university undergraduates (n=57) from the city of Natal, Brazil. The subjects were chosen randomly from those who declared themselves healthy and who had no history of chronic diseases and/or health problems in the previous year. The elderly underwent the Mini-Mental State Examination. The following exclusion criteria were established: the presence of gastrointestinal disorders, obesity or current malnutrition, chronic and/or acute diseases and the use of vitamin-mineral supplementation or other medication that might interfere in normal zinc metabolism. The study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (UFRN). The zinc analyses were performed by atomic absorption spectrophotometry and the Zinc Binding Capacity to Plasma Protein (ZnBCPP) was determined using methodology standardized by Argemi et al. (1988) and Cunningham et al. (1994), modified by Pedrosa et al. (2000). The saturation index was calculated according to Arcasoy et al. (2001), using the %SI formula (plasma Zn/ZnBCPP x 100). A statistically significant difference ($p<0.001$) was found between the two groups, in relation to plasma zinc and SI, but the ZnBCPP did not differ between the younger and older subjects. In agreement with this result, it was shown in the young group that 76% ($R^2=0.760$) of the ZnBCPP variations are explained by the variations in plasma zinc and SI. In the elderly group this measure decreased to 30.5% ($R^2=0.305$). The dietary ingestion of zinc/day was 9.73±0.91 mg in the Younger group and 9.41±1.0 in the Older group, without statistically significant differences between them ($p=0.8642$). Thus, we can deduce that the amount of dietary zinc has no effect on the differences found between the groups, in terms of plasma zinc and saturation index. We can infer, therefore, that the Older group had less “demand” and/or “competence” in binding zinc to the plasma protein pool, suggesting changes in zinc homeostasis. This could represent a greater difficulty in organic supply owing to a shortage and/or need to increase the continuous offer of zinc to reestablish this equilibrium. We conclude, therefore, that aging may be a factor associated to changes in control mechanisms and in zinc homeostasis, and could even alter ZnBCPP response patterns and other zinc-related indicators of nutritional status.

P068. EFFECT OF ZINC SUPPLEMENTATION ON HEMATOLOGICAL INDICES IN MARATHON RUNNERS. Josely Koury; Carla Bogéa; Aderval Luna and Carmen Donangelo.
Instituto de Nutrição UERJ, Instituto de Quimica UERJ, Instituto de Quimica UFRJ
jckoury@gmail.com

Hematological indices may be influenced by exercise and zinc intake. The hematological effect of oral supplementation of zinc was investigated in 14 male marathon runners (30±6 yr). Subjects took zinc gluconate (providing 24 mg Zn /d) for three months (T1) followed by placebo for another three months (T2). Dietary intake of zinc was approximately 11 mg/d during the study. Fasting blood samples were taken in the resting state. Hematological indices were assessed by an automatic counter (Cell Dyn COBAS) and plasma zinc was measured by atomic absorption spectrometry. Differences between treatments were determined by one-way-repeated-measures ANOVA. Plasma zinc was higher at T1 and T2 (11±1 µmol/L) compared to baseline (9±1 µmol/L) ($p=0.05$). Leukocyte count and Red Cell Distribution Width (RDW) were higher at T2 (6±2 x 103/mm3, and 15±1%, respectively) compared to baseline (5± 1 x 103/mm3, 13±1%, respectively) ($p<0.05$). These results suggest that zinc supplementation has a positive effect on cell immunity and on erythrocyte cell homogeneity in marathon runners. Financial Support: FAPERJ and CNPq (Brazil)

P069. ZINC SUPLEMENTATION IN INFANTS WITH DOWN SYNDROME. Guillermo Venegas, Paulina Escobar and Aldo Rodriguez
Biomedical Center. University of Concepcion,Chile
gvenegas@udec.cl

The purpose was to evaluate the effect in a long term Zinc supplementation in the plasma levels of this element and copper. A group of 20 infants with Down Syndrome were randomized getting 10 of them 5 miligrams/ day of elemental Zn for 1 year and the others placebo. The plasma level of Zn and Cu were determined in both groups at the beginning and every 3 months by atomic spectrophotometry. Both groups were similar in gender and age.The basic zinc levels were comparables from statisistical point of view. The zinc plasma after 1 year of supplementation was significantly higher in the supplemented group that placebo group (99±31ug/dl vs 63±9ug/dl, respectively).There was not significant difference in the copper levels between the placebo control group and the experimental group (supplemented). The Zn supplementation with 5 miligrams day for 1 year did not affect the Cu levels whereas the Zn levels were normal in infants with Down Syndrome.

P070. EFFECT OF COPPER ON IRON BIOAVAILABILITY. Manuel Olivares[1], Fernando Pizarro[1], Alejandra Martin[1] and Manuel Ruz[2].
[1]Laboratorio de Micronutrientes, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, Chile.
[2] Departamento de Nutrición, Facultad de Medicina, Universidad de Chile, Santiago, Chile.
molivare@inta.cl

Iron deficiency is the single most common nutritional disorder and the main cause of anemia worldwide. It is prevalent in most of the developing world where it coexists with other micronutrient deficiencies. Combined supplementation with both micronutrients is one strategy that can be used to improve the iron and copper status of a population. However, there is concern about the negative interactions between these two minerals. Copper uptake by the enterocyte is mediated by the specific copper transporter hCTR1 and the divalent metal transporter 1 (DMT1) a proton-coupled transporter, which is the main Fe2+ transporter and participates actively in Cu1+ transport. The possible negative interaction between iron and copper could be due to a competitive binding to DMT1. The aim of the study was to determine the dose-response effect of copper on iron bioavailability. Twenty-nine healthy adult women were selected to participate in two iron absorption studies. Iron (as ferrous sulfate) with or without copper (as copper sulfate) was given in an aqueous solution on days 1, 2, 14, and 15 of the study. Iron bioavailability was measured on the basis of erythrocyte incorporation of 55Fe or 59Fe 14 days after administration. The first group received 0.5 mg of iron given alone and with copper in molar ratios Cu:Fe of 0.5:1, 1:1 and 2:1 (0.28, 0.57 and 1.14 mg of Cu, respectively); the other group received 0.5 mg of iron given alone and with copper in molar ratios Cu:Fe of 4:1, 6:1 and 8:1 (2.28, 3.41 and 4.55 mg of Cu, respectively). No significant effect of copper on iron absorption was found at Cu:Fe molar ratios up to 8:1. (ANOVA for repeated measures NS). In conclusion, copper administration combined with iron in an aqueous solution did not inhibit iron bioavailability. The lack of negative interaction between iron and copper suggests that copper uptake by the enterocyte is mediated largely by the high affinity copper transporter hCTR1. It is possible that a negative interaction could occur at higher Cu:Fe molar ratios.
Supported by Fondecyt Grant 1070665.

P071. CHANGES IN SERUM FE, CU, ZN, LDH AND LIVER AMINO TRANSFERASES AFTER A UNIQUE ORAL COPPER DOSE IN HEALTHY WOMEN CHRONICALLY SUPPLEMENTED WITH COPPER. Jorge Botero-López, Fernando Pizarro and Magdalena Araya. INTA, University of Chile, Santiago, Chile.
jbotero@inta.cl

Previous studies showed that responses to chronic copper administration are associated with sex and that 8 mg oral Cu/d for 6mo does not induce significant changes on liver aminotransferases, in healthy adults. Objective: To assess the acute changes of serum Fe, Cu, Zn, LDH and liver aminotransferases in healthy women, before and after 6mo Cu supplementation. Methods: 37 healthy adult women received 10 mg oral copper (as copper sulfate), before and after 6mo supplementation with 8 mg Cu/day. Serum concentrations of Fe, Cu and Zn were measured by AAS and LDH, GOT, GPT and GGT by commercial kits, at times 0, 0.5, 2, 4, 6, 24, 48 and 96 hrs. Results were analyzed by ANOVA repeated measures. Results: Liver aminotransferases remained below the cut offs during the whole study period. Comparison of the 96hr curves (acute responses) before and after copper supplementation showed that GOT and GPT curves were different by treatment ($p<0.0001$ and 0.04 respectively), by time ($p<0.0001$) and interaction treatment*time ($p<0.0001$ and <0.04). GGT and LDH curves were significant by treatment ($p<0.004$ and <0.0001) and by time ($p<0.0001$ and <0.001). Fe and Zn curves differed by time ($p<0.001$ and <0.0001) and that of copper by time and interaction treatment*time (both $p<0.0001$). Discussion: After 6mo copper supplementation, both the liver aminotransferases and LDH curves tended to flatten after the acute copper dosing, suggesting adaptation. Serum curves of minerals suggest that intraindividual variability and variables associated with seasonal changes (temperature, humidity, light/dark cycles, others) influence mineral concentration in serum.

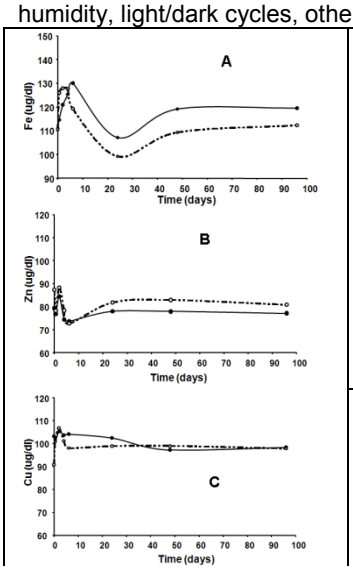

Figure 1. Fe (A), Cu (B) and Zn (C) serum concentrations after 10 mg Cu challenge before (●) and after (○) 6mo Cu supplementation.

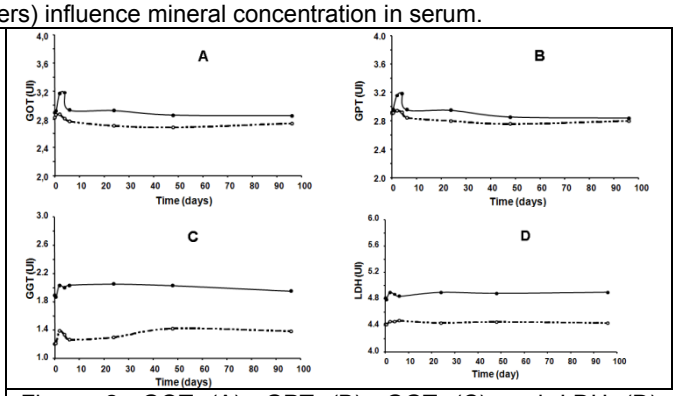

Figure 2. GOT (A), GPT (B), GGT (C) and LDH (D) activities after 10 mg Cu challenge before (●) and after (○) 6mo Cu supplementation.

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P072. ASSESSMENT OF SELENIUM NUTRITIONAL STATUS OF RIVERINE CHILDREN FROM RONDÔNIA STATE, WESTERN AMAZON. Ariana Vieira Rocha[1], Rafael Barofaldi Bueno[1], Cristiane Cominetti[1], Maritsa Carla Bortoli[1], Liliane Viana Pires[1] Milena Barcza Pinto[1], Luís Marcelo Aranha Camargo[2] and Silvia Maria Franciscato Cozzolino[1].
[1]Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental,Universidade de São Paulo – USP, Brazil
[2]Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo
avr@usp.br

There are only a few studies that assess nutritional state in Rondônia, especially isolated riparian settlement, as communities like Demarcação, and Preto River. People living in theses communities are exposed to many factors that may interfere with nutritional status, among then, lack of basic sanitation conditions, high levels of malaria, limited food availability and medical care due to access difficulty. Besides, these communities may be at risk from mercury intoxication since their main food item consists of fish. All these factors contribute to low nutritional status, specially children, who are more vulnerable. Selenium is a mineral with many organic functions, like antioxidant, immune system repairer and heavy metal, like mercury, detoxifier. Hence, the aim of this study was to assess selenium and the nutritional status of riparian children, aged between 3 and 9 years, living in two different communities, Demarcação and Preto River, both in the State of Rondônia, Western Amazon. Anthropometric parameters, as weight and height, were measured and analyzed according to WHO 1995 indexes. To assess selenium status, in plasma and erythrocyte, 5mL blood sample was collected, and analyzed by hydride generation atomic absorption spectrometry. Forty two children formed the group of study, 74% of them were from Demarcação and 26% were from Preto River. According to established anthropometric cutoffs, in Demarcação, 10% of the children presented under nutrition, 87% of them were eutrophic and 3% obese by the weight/age index; for the weight/height index the percentages were respectively 3%, 87% and 10%. In the Preto River community, the results were similar to Demarcaçao, with 91% of the children adequate and 9% of them obese, for both indexes. For the last index, height/age, Demarcação had 6% of under nutrition and 94% of eutrophic children, and in Preto River, they were all eutrophic. According to selenium status assessment, 84% of the children from Demarcaço had normal selenium plasma levels, and only 16% showed low levels. In Preto River, however, all children had high selenium plasma concentrations, above the reference parameters (60-120µg/L). For selenium erythrocyte concentration, in Demarcação, 45% of the children presented low levels, and 55% of them were adequate; in Gleba, 45% showed adequate levels and 55% had higher levels than the reference ones (90-190µg/L). Thus, most part of the children from both communities were eutrophic according to anthropometric parameters, although in relation to selenium status Demarcaçao children presented high prevalence of selenium deficiency, while in Gleba they were in risk of toxicity. These differences may be attributed to the geographic localization of the communities, that even close, are settled in different Rivers.

P073. EFFECT OF BRAZILIAN NUTS SUPPLEMENTATION ON THE LEVELS OF ERYTHROCYTE SELENIUM AND GLUTATHIONE PEROXIDASE IN HEMODIALYSED PATIENTS.
MB Stockler-Pinto[1]; NE Farage[1], GT Boaventura[2], D Mafra[2], SMF Cozzolino[3].
[1]Renalcor, NutriRim, Rio do Janeiro; Brazil
[2]Universidade Federal Fluminense-UFF, Niterói, Rio do Janeiro,Brazil
[3]Universidade de São Paulo-USP, São Paulo, Brazil.
milbarcza@usp.br

Introduction: Patients with Chronic Kidney Disease (CKD) in hemodialysis (HD) may undergo oxidative damage which causes reactive oxygen species (ROS) formation. Uremic toxins, hemodialysis treatment and the decrease in antioxidant system are known to result in the ROS generation. Antioxidant includes the selenium (Se) and the Se-dependent enzyme glutathione peroxidase (GSHPx) and the studies report Se deficiency in these patients. By the way, the Brazilian nuts are the main food source of this element. Objective: To evaluate the effect of Brazilian nuts supplementation on the erythrocyte levels of Se and GSHPx activity in hemodialysed patients. Subjects and Methods: A total of 77 HD patients (49.7±12.9 years old, average time HD of 85.4±106.0 months and BMI of 24.7±4.9 kg/m2) from RenalCor and RenalVida Clinics in Rio de Janeiro, Brazil were studied. The patients received one nut (average of 58.1 µg/g of Se) each day during three months. The Se concentration was determined through atomic absorption spectrophotometry with hydride generation (HITACHI®, Z-500). The levels of GSHPx were measured using RANDOX® commercial kits. Results: The assessment of the erythrocyte Se concentration before supplementation showed that 62% of patients had levels below the normal range (90-190 µg/L) with average values found in 84.3 µg±31.2 µg/L and after supplementation the levels increased to 240.4±122.0 µg/L ($p<0.0001$). Thirteen % of patients had activity of GSHPx below the normal range (27.5 – 73.6 U/gHb) before supplementation (mean of 46.9±15.5 U/gHb) and after supplementation the levels increased significantly to 61.4±21.9 U/gHb ($p<0.0001$), so that all patients showed activity of GSHPx within the normal range. Conclusion: The data revealed that the investigated patients had erythrocyte Se levels below the normal values before supplementation, whereas the activity of GSHPx was normal. Thus, the consumption of just one Brazil nut each day (5g) was effective to increase on Se concentration and GSHPx activity in the erythrocyte of patients with HD, thus contributing to improve the antioxidant condition of these patients. Suport: Capes, Cnpq.

P074. COMPARATIVE ANALYSIS OF DETERMINING THE CONCENTRATION OF SELENIUM IN DIETS OF PATIENTS WITH HYPOTHYROIDISM AND HYPERTHYROIDISM IN THE STATE OF SAO PAULO – BRAZIL. Luciana Sigueta Nishimura, Liliane Viana Pires, Carla Soraya Costa Maia, José Alexandre Coelho Pimentel, Silvia Maria Franciscato Cozzolino
PRONUT – USP (Pos graduate program in Applied Human Nutrition at the University of São Paulo, Brazil)
lucianasn@usp.br

Selenium is a trace mineral found in different concentrations in the soil, depending on geochemical factors. The amount ingested by an individual will depend on his eating habits. More recently was verified the importance of micronutrients in the metabolism of the thyroid, namely in the conversion of T4 to T3. The purpose of our study was to compare the amount of selenium in prepared diets of patients with hypothyroidism and hyperthyroidism using two methods. For both groups, were prepared diets with the most food consumed by patients with hypothyroidism, hyperthyroidism and the control group, from data collected by food recall of three days method. It was conducted a comparison of the concentration of selenium present in these diets produced by chemical analysis and the analysis made by the software NutWin. In the chemical analysis of the three diets were evaluated the concentration of selenium by the hydride generation-atomic absorption spectrophotometry technique (HGQTAAS) and proximate food composition according to AOAC. The results of the comparison of the methods showed a statistically significant difference ($p<0.05$) between the averages found for the concentration of selenium of the prepared diets of patients with hypothyroidism and hyperthyroidism. The concentration of selenium analyzed in prepared diets of groups with hypothyroidism and hyperthyroidism, was lower (10.33µg and 21.24µg, respectively) than that found by means of software NutWin that utilize other composition tables (47.88µg and 45.83µg, respectively). Moreover, these values were also below those recommended for daily intake of selenium. It was observed statistically significant difference in the energy (kcal) and percentage of lipids of these groups. In the case of carbohydrates and proteins, there was no statistically significant difference. It was observed that the prepared diet of the control group showed no statistically significant difference for the nutrients evaluated, including the concentration of selenium. This fact demonstrates the difficulty of assessing the nutrients consumption in diets based only in tables of food composition, which it developed for different regions with foods that suffer regional interference.

P075. NUTRITIONAL STATUS OF SELENIUM ON HEALTHY ADULT SUBJECTS OF THE STATE OF SÃO PAULO – BRAZIL. Luciana Sigueta Nishimura, Carla Soraya Costa Maia, Liliane Viana Pires, José Alexandre Coelho Pimentel, Clara Satsuki Mori, Rafael Barofaldi Bueno and Silvia Maria Franciscato Cozzolino
PRONUT – USP (Pos graduate program in Applied Human Nutrition at the University of São Paulo, Brazil)
lucianasn@usp.br

Selenium (Se) has an antioxidant role because its participation in selenoproteins, including the enzyme glutathione peroxidase, that acts protecting the cells against the effects of free radicals that are produced during metabolism. Se food content is influenced by geographical location, seasonal changes, protein content and food processing. People who live in areas where the soil is poor in micronutrients, such as the southeastern region of Brazil, usually have low selenium intake, since the levels of this nutrient in soil generally reflect its presence in food. Thus, the aim of this study was to evaluate the nutritional status of selenium on healthy adult subjects of the State of Sao Paulo – Brazil. The study included 30 healthy individuals aged 18 to 59 years, resident of the State of Sao Paulo, Brazil. The evaluation of body composition were assessed by weight and height measures, and then calculated the body mass index (kg/(m)2). Selenium concentration in plasma and erythrocyte was determined by the hydride generation-atomic absorption spectrophotometry technique, and the erythrocyte enzymatic activity of glutathione peroxidase was measured by the method proposed by Paglia and Valentine (1976), using a kit commercially available (RANSEL-RANDOX®). We observed that 30% of the subjects were eutrophic, 40% were overweight and 30% were obese. The assessment of selenium concentration in plasma showed that only 13% was below of the cutoff point established (53-110mcg/L). Regarding concentration of this mineral in the erythrocytes was observed that 37% found itself with lower amounts to the value of reference used. The mean of the glutathione peroxidase activity in the group was 52.23±20.43 IU/gHb, and we observed that everybody showed adequate activity of this enzyme. The results allowed conclude that selenium nutritional status is adequate in the most of the individuals evaluated, as the parameters used.

P076. AGE-RELATED ANTIOXIDANT DEFENCE WEAKENING IS ALTERED BY LOW SELENIUM INTAKES. I Margaritis[1], I Hininger[2], A Botta[3], L Farout[3].
[1] French Food Safety Agency – France
[2] Université de Grenoble – France
[3] Université de Nice Sophia-Antipolis – France
i.margaritis@dg.afssa.fr

Selenium is a trace element playing an important role in the machinery that protects cell components from oxidative damages. Numerous studies that deal with long and/or high selenium depletion, report deleterious effect at the cellular level, associated with an increase of pathologies occurrence. The aim of the present study was to determine whether a both mild and short depletion of selenium intakes (-30% during 3 weeks) could alter skeletal muscle and heart cell physiology in young (6 months) and old (24 months) adult rats. Results exhibit an alteration of total selenium distribution among tissues.
Selenoproteins GPx, MsrB1 activities are highly inhibited, and level of oxidized protein increased in both skeletal muscle and heart. These changes are exacerbated during ageing. Using a 2D electrophoretic method, changes of protein expression patterns have also been observed in these tissues after 30% of depletion of food selenium content for 3 weeks. Skeletal muscle and heart selenium status seem to be greatly dependent on food selenium contents, and even a mild short depletions can induce these alterations. In theory, a food contribution lower by 30% compared to nutritional recommendations intakes is supposed to cover national needs. According to our results, the question of whether French recommendations -based on nutritional needs- are underestimated with concern to ageing, can be raised. These results have to be completed by further research, clinical experiments, epidemiological studies, which could help and contribute to a higher accuracy of selenium intake recommendations.
The research has been supported by *Merck Medication Familiale*, Dijon, France

P077. CONSUMPTION OF ANIMAL FOODS, RESTRAINED EATING BEHAVIOUR AND BIOMARKERS OF NUTRITIONAL STATUS IN YOUNG WOMEN.
Flavia Fayet1, Victoria Flood[1], Peter Petocz[2], Peter Stewart[3], Ian Caterson[1], Samir Samman[1]
[1]Discipline of Nutrition and Metabolism, School of Molecular and Microbial Biosciences G08, University of Sydney, NSW, Australia
[2]Department of Statistics, Macquarie University, NSW, Australia,
[3]Department of Clinical Biochemistry, Royal Prince Alfred Hospital, NSW, Australia
s.samman@mmb.usyd.edu.au

Background – Meat and poultry are good dietary sources of bioavailable nutrients such as iron and zinc. Exclusion of animal products from the diet may have adverse consequences on nutritional status. Objective – To investigate the effects of eating behaviour on nutrient intake and biochemical markers of nutritional status in young women. Design – Female tertiary students (n=308; age 22.4±3.8 y; BMI 21.4±2.6 kg/m2; mean±SD) participated in a cross-sectional study. Participants were categorized as avoiders or non-avoiders of 'meat and poultry' based on their responses to a Food Frequency Questionnaire. Eating behaviour was assessed by the Three Factor Questionnaire. Blood samples were analysed for biomarkers of iron and vitamin B12 status, and concentrations of plasma zinc, copper, selenium, and fatty acids. Outcomes – Serum ferritin concentrations <15 ug/L were observed in 32% of subjects and 16% had serum transferrin saturation <15%. Mean concentrations of plasma zinc (13.8±2.5 µmol/L), copper (16.2±5.9) and selenium (1.1±0.2) were within the reference ranges. Consumption of zinc was positively correlated with serum vitamin B12 concentrations and plasma n-3 fatty acids. Serum ferritin and serum vitamin B12 concentrations were positively correlated with consumption of red meat (P<0.001) and chicken (P<0.001). Intakes of zinc, selenium and vitamin B12 were significantly lower in 'meat and poultry' avoiders than in non-avoiders. Restraint eating scores were significantly higher in red meat avoiders (11.0±5.6; arbitrary units) than in non-avoiders (8.0±5.0). Individuals who consumed <2 serves of red meat per week had significantly lower concentrations of ferritin and vitamin B12 in serum, and higher restraint scores than those who consumed>2 serves per week. Conclusion – Restrained eating behaviour and avoidance of 'meat and poultry' contribute to an increased risk of nutrient deficiencies in young women. (Funded by a Human Nutrition Research and Development grant from Meat & Livestock Australia).

P078. EFFECT OF COTHERAPY OF CHELATING AGENTS AND ANTIOXIDANTS AGAINST ALUMINIUM INDUCED NEPHROTOXICITY.
Sadhana Shrivastava[1], Abhilasha Sharma[1], Varsha Singh[1], Deepmala Joshi[1], Sangeeta Shukla[1] and Mohammed Abdullah[2]
[1]Reproductive Biology and Toxicology Laboratory, School of Studies in Zoology, Jiwaji University, Gwalior, India
[2]Trace Element- Institute for UNESCO, Lyon, France.
dr_sadhana59@rediffmail.com

Aluminum (Al) is an ubiquitous mineral in our environment; it is the most prevalent element in the earth's crust. The toxicity of Al can be traced to increased deposition in bone and the central nervous system, particularly in the presence of reduced renal function. Because aluminium competes with calcium for absorption, increased amounts of dietary aluminium may contribute to the reduced skeletal mineralization (osteopenia) observed in preterm infants and infants with growth retardation. Excessive consumption of antacids containing Al compounds and excessive use of aluminium-containing antiperspirants are more likely causes of toxicity. Maximum use of Al containing deodorant which caused kidney disease due to removal of certain minerals and metal from bloodstream. It is primarily directed at Al nutrition and toxicity in infants and children. Cautioning against the use of old Al utensil or subjecting it to high heat and acidic food. Tea and acidic food material, according to experts, is best kept away from its utensils. It has been linked with diseases of the brain, bone and blood. Normal adults on an average ingest 2-5 mg Al per day through food and significant amounts from drinking water. Kidneys do filter excessive Al but those with faculty kidneys, however don't have the advantage and are thus at more pronounced risk, DNA damage and inhibition of its repair and apoptosis in human peripheral blood lymphocytes. This study aimed at evaluating the protective effects of combination therapy on histopathological and biochemical parameters in kidney of rats. Al $(NO_3)_3$ (5mg/Kg/day, *i.p.*) was administered to rats for 90 days, followed by therapy with NAC/HEDTA+Ca+Fe for 7 days. Induction of CYP 2E1 by Al is one of the central pathway by which Al generates oxidative stress in kidney which caused significantly rise were observed ($P<0.05$) in the activities of creatinine, urea, serum alkaline phosphatase, uric acid and BUN whereas serum protein was found to be declined after toxicant exposure. Secondary products of lipid peroxidation that is MDA levels were estimated by measuring the (TBARS) levels. Our results indicate that TBARS levels, acid phosphatase were significantly higher and total glutathione content were significantly lower in renal toxicity. The decrease in activity of various enzymes viz. succinic dehydrogenase, adenosine triphosphatase, glutathione reductase and glutathione -6-phosphatase dehydrogenase in kidney were noted but marginal inhibition was noted in glutathione peroxidase. The tissue retention of Al varied among the organs. Animals showed significantly higher levels of Al in kidney ($P<0.001$) when compared to control animals. Co therapy was significantly effective in removing Al from the organs and showed over all improvement in all the biochemical changes. TEM of kidney after Al exposure represents degeneration in mesangial cell, distorted filtration membrane, reduced no of foot processes, disruption of endoplasmic reticulum, vacuolization and degenerated mitochondria of necrotic cells. Proximal tubules revealed damaged microvilli. Conjoint treatment showed rounded nuclei, elongated mitochondria with clear seen crests. Epithelial cell of tubule exhibits abundant microvilli. Treatment with HEDTA+Ca+Fe was more effective when compared to NAC+Ca+Fe.

P079. THE EFFECT OF DAILY IODINE SUPPLEMENTATION ON COGNITION IN MILDLY IODINE DEFICIENT SCHOOL CHILDREN: A RANDOMISED, CONTROLLED, DOUBLE BLIND STUDY. Rosie Gordon[1], Meredith Rose[1], Sheila Skeaff[1], Kirstie Morgan[2], Ted Ruffman[2] and Andrew Gray[3]
[1]Department of Human Nutrition; University of Otago, Dunedin, New Zealand
[2]Department of Psychology; University of Otago, Dunedin, New Zealand
[3]Department of Preventive and Social Medicine; University of Otago, Dunedin, New Zealand
sheila.skeaff@stonebow.otago.ac.nz

I Iodine deficiency is the most common nutrient deficiency in New Zealand children. Research studies carried out in parts of the world with severe and moderate iodine deficiency show that an inadequate intake of iodine during childhood adversely affects cognition, which can be improved with additional iodine. The consequences of mild iodine deficiency on cognition have been reported in one cross-sectional Spanish study; to our knowledge there have been no intervention studies published in this area. The aim of this research was to conduct a randomised, controlled, double blind, clinical intervention trial investigating the effect of a daily iodine supplement or placebo for 26 weeks on cognition in New Zealand children aged 10-13 years. Children living in Dunedin, New Zealand, were recruited primarily through their school and randomly allocated to either the iodine or placebo group. At baseline, children were asked to provide a casual urine sample for determination of urinary iodine concentration (UIC), a 1 mL finger prick blood sample for serum free T4 (fT4) and thyroglobulin analysis (Tg), complete an iodine-specific food frequency questionnaire, and have their heights and weights measured. Children also underwent cognitive testing involving four sub-components (i.e. letter number sequencing, symbol search, matrix reasoning, and picture concepts) of the Wechsler Intelligence Scale for Children (WISC-IV) modified for use in Australia. Children were asked to consume a tablet each day for 26 weeks containing either 0 (i.e. placebo) or 150 µg of iodine; at the end of each month, unconsumed tablets were returned as a check for compliance. All measurements and tests will be repeated at the end of the intervention, in October 2008. At baseline, 184 children (mean age=11.1 years) participated in the study; 93 children (51 boys, 42 girls) in one group and 91 children (50 boys, 41 girls) in the other group. The mean (±SD) height of the study population was 149.9 ±8.0 cm and the mean weight was 45.4 ±11.5 kg. The median UIC of the sample was 63 µg/L (Interquartile Range, 46 to 84). The mean fT4 and Tg concentration was 101±16.1 nmol/L and 18.7 ±13.6 µg/L, respectively. The mean iodine intake was 55±23.1 µg /day. There were no significant differences between the two groups with respect to biochemical measurements, dietary intakes or cognitive test scores. The effect of daily iodine supplementation on all indices will be reported at the conference. An improvement in cognitive scores in children taking the iodine supplement will provide strong evidence that even mild iodine deficiency can have detrimental effects on brain development in children.
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P080. PECULIARITIES OF MINERALS IN IRANIAN DAILY DIETS. AGGharib[1], M Gharib[2], SG Mohseni[3]
[1]Physic Faculty, Amir-Kabir University of Technology, Hafes Avenue, Tehran – Iran, Tafresh Univ. and Ashtian Azad Univ.
[2]Biology Dept. of Azahra University, Tehran-Iran
[3]Faculty of Engineering, Tehran University, Tehran -Iran
agharib@aeoi.org.ir

In this article, some of the main aspects of a comprehensive study on trace elemental content of a few typical daily diets of Iranians, along with the same study in a few more countries under conduction of International Atomic Energy (IAEA) are going to be presented. The actual purpose was to work out the intakes of important nutritionally trace elements via daily diets by certain study groups and /or by a reference man. This work was completed internationally till 1999, but it could be accomplished locally very recently. The objective of this recent study was not only a review over the detailed work for 24 nutritionally important minerals measurement, but to look at the peculiarities such as interrelation, bioavailability, interaction between and among trace elements (essential, toxic, radioactive ones) plus carrying out some sort of crosschecking. Also energy intakes in mg(s) of trace elements per MJ per head are presented. In this recent work a few further study groups from Iranian were added to previous groups with the same objectives and to increase/verify the reliability of applied methodologies. The diets prepared by recording dietary regimes (RD), duplicate diets(DD) and market basketing (MB). Methods for elemental measurement were mostly NNA, ICP, AAS and for the measurement of some other parameters such as phytate, fiber and energy, the appropriate proven chemical methods were applied. Since the results are very plenty, therefore apart from briefing them in a few tables, the attempt is presenting them in forms of various and appropriate figures individually and all together where it is needed to do so. The results in compare with other countries and Recommended Daily Allowances (RDA) generally are fair but no doubt there are some deficiencies in I, Se, Fe, Zn in a few Iranian study groups and also being high or in rather borderline exposure of a few toxic trace elements in other study groups. There should be very much discussion about the bioavailability, interrelation among the most of trace elements such as Zn, Fe in presence excess amount of phytate and Ca, meantime from other hand some interaction and imbalances among essential, toxic and counterpart radio elements are observed which all are taken into consideration.

P081. TISSUE AND BLOOD LEVELS OF CADMIUN AND SELECTED TRACE ELEMENTS IN WOMEN WITH UTERINE MYOMAS AND ENDOMETRIAL CANCER.
Marzenna Nasiadek[1], Tomasz Krawczyk[2], Jadwiga Szymanska[1] and Andrzej Sapota[1]
[1] Medical University of Lodz, Poland
[2]Polish Mother's Health Center-Research Institute, Lodz, Poland
asapota@pharm.am.lodz.pl

Uterine myomas (UM) form the group of the most frequently reported benign neoplasms of the reproductive organs. Uterine myomas and endometrial cancer (EC) are determined among others by an excessive stimulation of estrogen. Cadmium (Cd), classified as one of the major carcinogens, has recently been found to possess the estrogen receptors (ER)-binding ability. Cadmium is one of the most toxic transition metals associated with air and water pollution, as well as with cigarette smoking. A heavy smoker can easily be exposed to 1.5-60 µg of Cd everyday. The aims of the present study were to investigate the relationship between cigarette smoking and Cd levels in women with UM or EC, and to determine the effect of Cd on the homeostasis of metals essential for human metabolism. The study group consisted of 58 women after total abdominal hysterectomy because of malignant (15 patients with EC) or benign diseases (43 patients with UM) of female reproductive organs. The control group was composed of 28 healthy volunteers subjected to routine gynecological and pelvic ultrasound examinations, which revealed no pathology. Tissue samples were collected only from women with EC or UM, immediately after surgical removal of uterus or myoma. All the tissues were subjected to histopathological examination. Normal myometrium (NM) from “healthy” parts of uterine wall served as the tissue control for EC and UM. In the study group, including EM and UM cases, blood samples were collected from the antecubital vein in the morning on the day of surgery. At the same time the control group underwent a routine gynecological examination, during which blood samples were collected. In tissue and blood samples, concentrations of Cd, Cu, Fe, Mg, Zn, and Ca were assessed using AAS. The study revealed significant differences in the concentration of metals between women with EC and women with UM. Cu, Fe, Ca and Mg concentrations were significantly higher in tissues of women with EC than in UM and NM tissues. Cd concentrations in tissues (EC, UM, NM) of smoking women was higher than in those of non-smokers. The highest Cd concentration was found in NM tissues collected from smoking women with EC, whereas the effect of smoking on the concentration of other metals was insignificant in all tissues under study. Contrary to tissues, pathological changes (in EC and UM) had no significant effect on the blood levels of essential metals as compared with healthy women. It is known that smoking contributes to an evident increase in blood Cd concentration. In our study, smoking significantly increased the blood level of Ca and Cu in the study and control groups. To sum up, the reported study performed in a group of women with uterine myomas, regarded as a preliminary one, may suggest that smoking and related high Cd concentration in tissues of the uterus and blood may interfere in steroidogenesis through interacting with essential elements, especially with copper and calcium. The study was supported by the Joint Project (No 502-13 -483) of the Medical University, Lodz.

P082. TYPE 2 DIABETES MELLITUS PATIENTS HAVE ELEVATED IRON STORES AND LIPOPEROXIDATION INDEX.
Valeria Candia and Miguel Arredondo.
Micronutrient Laboratory, Nutrition Institute and Food Technology (INTA), University of Chile, Santiago, Chile
vcandia@inta.cl

Introduction: Type 2 diabetes mellitus (DM2) is a disease that in the last years it has become in an authentic pandemia. DM2 is frequently associated with obesity and abnormal carbohydrate, lipid and protein metabolism. These alterations produce an important metabolic unbalance.
Objective: To determine hematologic parameters and lipoperoxidation index in non-diabetic (controls) subjects, non-diabetic, but with metabolic syndrome individuals (MS) and type 2 diabetes mellitus patients (DM2). Methods: Arterial blood samples were obtained from 120 controls, 120 MS individuals and 120 DM2 patients. Hematologic variables included: Serum iron, transferrin receptors (TfR) and serum ferritin (SF) concentrations. Using TfR and SF values we calculated total body iron (TBI). We also measured HO1 activity in mononuclear leukocytes (MNCs) stimulated with H2O2. TBARS were measured by the thiobarbituric acid method. Results were expressed as mean±SD. One way ANOVA was used to compare the groups. Results: Serum iron did not differ significantly between the 3 groups. SF was significantly higher in both DM2 and MS than controls (64.7±30.4; 62.9±31.5 and 43.3±26.4 μg/L, respectively; $p<0.001$). Despite of this difference, no iron overload (SF>200 μg/L) was detected in either of the groups. TfR was significantly lower in both DM2 and MS than controls (5.5±2.0; 6.2±1.6 and 6.7±2.7 μg/ml; respectively; $p<0.001$). Both DM2 and MS patients had significantly higher TBI than controls (6.9±2.9; 5.6±2.5 and 4.8±3.9 mg/kg, respectively; $p<0.001$). HO enzymatic activity was also significantly higher in both DM2 and MS than controls (0.7±0.09; 0.6±0.09 and 0.2±0.1 nmole bilirrubin/mg protein/hr, respectively; $p<0.0001$). Finally, TBARS values were significantly higher in both DM2 and MS than controls (1.7±0.5; 1.2±0.4 and 0.9±0.4 nmole/ml, respectively; $p<0.0001$).
Conclusion: Type 2 diabetes mellitus patients and subjects with metabolic syndrome have elevated levels of iron stores and HO enzymatic activity; this is translated in an increased possibility of develop oxidative stress, which is reflected in high values of lipoperoxidation index.
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P083. MINOR AND TRACE ELEMENTS IN BRAZILIAN COFFEE SAMPLES. Maria do Carmo Freitas and Ho Manh Dung.
Reactor, Technological and Nuclear Institute, E.N. 10, 2686-953 Sacavém, Portugal
cfreitas@itn.pt

Coffee is a national and frequent drink in Portugal. It is used as an “expresso”, which means little amount of water and a lot of coffee powder, turning it a strong drink. Coffee is drunk at the end of the two main meals (lunch and dinner) as well as in the morning and middle of afternoon. Some Portuguese even drink in-between. In general, at least 4 coffee cups are drunk per day. Portugal cultivates coffee beans in S. Jorge island, in Azores archipelago, however the amount is too small to cover the needs, mostly it is imported from Angola and Brazil. Powder of coffee beans cultivated in Brazil were analysed by instrumental neutron activation analysis in order to get the composition in trace elements. The coffee powder was from 3 different packages to test its homogeneity. Analytical control was made by analysing it similar conditions the reference material from National Institute for Standards and Technology (NIST) Citrus leaves, SRM 1572. Short and long irradiations were performed to extract the maximum number of chemical element concentrations from the technique: aluminium, bromine, calcium, chlorine, cobalt, chromium, caesium, copper, iron, potassium, magnesium, manganese, sodium, rubidium, scandium, titanium, vanadium, and zinc. Calcium, magnesium and potassium concentrations accounted for 2.1% of the total composition; the other concentrations are at mg kg-1 level except cobalt, chromium, caesium, scandium and vanadium which were at µg kg-1 level. As pollutants copper and vanadium might be pointed as such, as soil contaminants scandium and titanium might be referred. The discussion is made in terms of the needs in nutrients of the human body and the contribution of coffee for that purpose. The amount of potassium and magnesium may justify the known contribution of coffee drink in decreasing the fatigue. Other favourable characteristics concern the relatively large amount of iron and the reduced amount of sodium.

P084. REDOX BALANCE, ELEMENTAL LEVELS AND CARDIOVASCULAR RISK FACTORS IN AZOREAN PATIENTS WITH CONFIRMED CORONARY ARTERY DISEASE. Rita Ferin[1], Patrícia Napoleão[2], Carla Gomes[1], Ana R Castro[1], Paula Lopes[3], Dinis Martins[4], M. Cristina Santos[3], José Baptista[1], Ana M. Viegas – Crespo[3], Teresa Pinheiro[2] and M Leonor Pavão[1]
[1]CIRN, Universidade dos Açores, Ponta Delgada, Portugal
[2]Laboratório de Feixes de Iões, ITN, Lisboa, Portugal
[3]CBA, Universidade de Lisboa, Lisboa, Portugal
[4]Hospital do Divino Espírito Santo, Ponta Delgada, Portugal
lpavao@uac.pt

Oxidative stress involves any situation in which oxidant metabolites, namely reactive oxygen species (ROS) can exert their deleterious effects as a result of increased production or altered cellular mechanisms of protection. The balance between ROS generation and antioxidant activity is critical to the pathogenesis and progression of stress-related disorders. Atherosclerosis and consequent cardiovascular diseases represent a state of heightened oxidative stress characterized by lipid and protein oxidation in the vascular wall. Trace elements such as Cu, Fe, Se and Zn can play key roles in both the generation of oxidant species and in endogenous antioxidant systems as cofactors of antioxidant enzymes. This work aimed at evaluating the association of lipid peroxidation (serum thiobarbituric acid reactive substances concentration-TBARS), protein oxidation (plasma protein carbonyls contents-C) and antioxidant markers, such as whole blood glutathione peroxidase and erythrocyte superoxide dismutase activities (Se-GPx and Cu,Zn-SOD, respectively) and serum Vitamin E concentration in blood from 20 patients living in the island of S. Miguel (The Azores Archipelago, Portugal), aged 40 to 65 years, with coronary artery disease previously submitted to percutaneous revascularization (PCI). A similar group of apparently healthy subjects was taken as control. Plasma and blood cell Cu, Fe, Se and Zn concentrations were determined by PIXE. All subjects gave informed consent to participate in this study. Results were analysed by taking into consideration some cardiovascular risk factors, such as gender, dislipidemia, obesity and hyperhomocysteinemia. Control women, albeit with overweight, were normolipidemic and exhibited plasma total homocysteine concentrations (tHcy) within normal range. Both control and PCI men had overweight and were dislipidemic and moderately hyperhomocisteynemic. However, the degree of dislipidemia (particularly serum cholesterol levels) was less severe in patients since they were medicated with statins. TBARS increased significantly by 38% in PCI women (not in men) relative to controls but no significant differences in C were found between PCI and control groups in both genders. TBARS had a positive correlation (R=0.701, p=0.035) with C in male controls but not in PCI men or women. In PCI women, who were all obese and dislipidemic, TBARS correlated well (R=0.806, p=0.009) with tHcy, which mean was also above reference values and about 70%higher than in the respective control group. Se-GPx and serum vitamin E levels were significantly lower (24% and 15%, respectively) in patients (regardless gender) than in controls. Zn concentration in blood cells of PCI subjects (namely women) was increased as compared to the respective counterparts. In both genders, no variations were observed concerning Cu,Zn-SOD, as well as the other trace element concentrations either in blood cells or in serum. Results suggest the presence of oxidative

stress and a decreased antioxidant status in patients, namely in women, who are therefore less protected than men. Besides hyperlipidemia, hyperhomocysteinemia can aggravate oxidative vascular injury in PCI women and potentiate a risk of recurrent vascular events.

P085. ASSESSMENT OF TRACE ELEMENT INTAKES IN CHILDREN WITH CEREBRAL PALSY. Ujang Tinggi[1], Niikee Schoendorfer[2], Peter Davies[2], Roslyn Boyd[3], Pieter Scheelings[1], Henry Olszowy[1]
[1]Centre for Public Health Sciences, Queensland Health Forensic and Scientific Services, 39 Kessels Road, Coopers Plains, Queensland 4108, Australia.
[2]Children's Nutrition Research Centre, University of Queensland, Royal Children's Hospital, Herston, Queensland 4029, Australia.
[3]Queensland Paediatric Rehabilitation Service, University of Queensland, Royal Children's Hospital, Herston, Queensland 4029, Australia
ujang_tinggi@health.qld.gov.au

Abstract Cerebral palsy (CP) is a non-progressive brain disorder which is characterised by inadequate muscle tone, abnormal body posture, and disordered body movements. These disabilities in CP children can cause malnourishment and underweight when compare with healthy children of similar age. There has been a limited study on the assessment of trace element status in these CP children, and there is a concern that they may not receive sufficient trace element intakes in their diet. It is estimated that the prevalence of CP is about two per 1000 live births. The aims of this study are to assess and compare intakes of trace elements between CP children and the healthy controls, and to determine for signs or symptoms of trace element deficiencies in these CP children. In this study, children with CP (20 subjects), 4-12 years of age, and a control group (20 subjects) of healthy, non-disabled children of matching age are selected. The study also includes CP children (20 subjects) with tube feeding via percutaneous endoscopic gastrostomy (PEG). Food and drink intakes for 3 consecutive days are collected and weighed for each subject for the study. The foods are to be analysed as a composite sample and drinks are to be analysed individually. Special dietary formulas used by the CP children with PEG are also collected. Other biochemical parameters such as blood glutathione peroxidase activity, plasma zinc and ferritin, and thyroglobulin levels will also be determined. The analyses of trace elements (copper, zinc, iron, selenium, chromium, molybdenum, iodine, manganese) are carried out using ICP-MS and ICP-AES with appropriate standard reference materials for quality control. Other major minerals (calcium, sodium, magnesium, potassium, phosphorus) in the diet of these children are also determined. The preliminary results showed a wide range of estimated trace element intakes (mg) per day for CP children (4.5-10.9 for iron, 0.7-0.9 for copper and 3.2-7.1 for zinc). For the major mineral intakes (mg) per day, these ranged from 135-570 for calcium, 1310-1630 for potassium, 136-194 for magnesium, 650-1780 for sodium, and 530-790 for phosphorus. The preliminary findings of this study will be presented and discussed.

P086. MULTIVARIATE OPTIMIZATION PROCEDURE USED TO DEVELOP A METHOD FOR ANTIMONY DETERMINATION IN HUMAN SERUM SAMPLES BY GF AAS. Henrique José Ferraz Fabrino, Waldomiro Borges Neto, Alfredo de Miranda Goes andJosé Bento Borba da Silva
Federal University of Minas Gerais, Belo Horizonte, Brazil
hfabrino@yahoo.com.br

Sb background level in bodily fluids is measurable at ppt levels. Some organs contain amounts of 0.1-0.7 ppm. Patients who had been given antimony-based drugs against parasitic infections were found to retain the element for longer than expected. The level in urine was 1 ppm and 0.25 ppm after 6 months and one year, respectively. A few hours after ingestion, Sb enters the bloodstream, but less than 10% are absorbed. It first accumulates in the liver, moves to other parts of the body and is slowly excreted by the kidneys. The average daily intake is 0.5 mg and the total body burden in the average person is 2 mg. Sb may block enzymes that are necessary for liver, kidneys and heart muscle function. Trace analysis in complex matrices may require sample preparation involving several steps. In-situ digestion is an advantage of GF AAS. In the present work a method was developed to determine Sb in human serum using GF AAS. A simple 1:4 dilution of the serum with 1% HNO3 and 0.1% triton X-114 was performed. Pyrolysis and atomization temperatures as well as the use of permanent chemical modifiers were studied by multivariate approach. First, modifiers were evaluated (Ir, Rh, Ru, Ta, Nb, Zr, W and no modifier) using the furnace program recommended by the manufacturer, in order to select two of them to be used in the factorial design. Zr and Ta (500 µg) were found to be the best modifiers. The 23 factorial design showed that all variables studied had a significant effect on the answer (integrated absorbance) at a 95% confidence level. A CCD was constructed to obtain the answer surface using the tube treated with the modifier selected in the factorial design (Zr) and studying variables pyrolysis and atomization temperatures. The equation describing the answer surface resulted in values of 800 and 2100 ^{o}C for pyrolysis and atomization temperatures, respectively. Matrix effect was verified by comparing the slopes of aqueous and matrix-matching calibration curves and results showed no significant difference at a 95% confidence level (n=3). Figures of merit were determined using aqueous calibration between 0-30 ppb, which presented a coefficient of linear correlation of 0.9977±0.0005 (n=3). Recovery studies and intra- and inter-assay precision tests (in three days) were made at three concentration levels (12.5, 17.5 and 22.5 ppb). Recovery was 101±1% (n=21) and coefficients of variation of 2.7±0.1 (n=21) and 5.0±0.6 (n=63) were found for intra- and inter-assay precision, respectively. Equations LD=3S and LQ=10S were used to calculate the detection and quantification limits, respectively, where S is the standard deviation of 10 independent readings of the blank. LD and LQ were, respectively, 0.3 and 1.0 ppb and the characteristic mass was 13±1 pg (n=6). Sb serum levels in 60 volunteers ranged between (truncated summary)

P087. TRACE AND MINOR ELEMENTAL CONTENT OF VARIOUS CALCIUM SUPPLEMENTS: DOES THE CHOICE OF CALCIUM SUPPLEMENT INDUCE RISK OF METAL INTOXICATION TO RENAL INSUFFICIENT PATIENTS? Raphael Jakubovic[1], Eric Da Silva[2] and Ana Pejovic-Milic[1]
[1]Department of Physics, Ryerson University, Toronto, Ontario, Canada, M5B 2K3
[2]Department of Chemistry & Biology, Ryerson University, Toronto, Ontario, Canada, M5B 2K3
e2dasilv@ryerson.ca

Objectives. Haemodialysis treatment for patients with end-stage renal failure induces significant stress on bone health due to imbalances in the elemental absorption of phosphorous, calcium and various trace elements. Various trace elements including most notably aluminum and strontium have been found in dialysate fluids at concentrations approaching up to fifty-time normal dietary intake levels (Daelemans *et al.*, 2001; Padovese *et al.*, 1992; D'Haese & De Broe, 1999). It is now believed that aluminum and strontium may act in synergy to decrease bone formation and create a mineralization defect in dialysis patients (Cohen-Solal, 2002). Hyperphosphatemia is also a concern for haemodialysis patients as is the associated hypocalcaemia. Both disorders may require parathyroidectomy. Not surprisingly, the management of haemodialysis patients involves a significant amount of calcium supplementation. Post-parathyroidectomy patients are typically prescribed 2-6 g of elemental calcium per day (Cozzolino *et al.*, 2004). Similar amounts of calcium supplementation are required for patients with intact parathyroids to bind phosphates after meals. Therefore, there is a significant obligation to quantify the differences in the strontium content in various calcium supplement forms (including antacids, synthetic calcium carbonate, natural (*i.e.* oyster shell) calcium carbonate, calcium citrate and calcium glutonate) to determine if the choice of supplement can influence the overall strontium (and aluminum) burden to patients with renal insufficiency. In particular, given that strontium is most abundant in sea water, it is of interest if the oyster shell-based supplements have a sufficiently larger concentration of strontium per gram of elemental calcium. **Method.** Thirteen calcium supplements were purchased that included antacids, synthetic calcium carbonate, natural source calcium carbonate (oyster shell, limestone and coral), calcium citrate and calcium glutonate. Various forms were purchased that were further enriched with other elements such as magnesium. The samples were ashed sequentially from 450-1100°C to remove any organic constituents and to calcinate the products to their oxides. The analysis was performed by wavelength-dispersive X-ray fluorescence spectrometry (S4 Explorer, Bruker-AXS) using the associated standardless software package (SpectraPlus®) on pressed pellets. **Results and discussion.** Our findings indicated increased concentrations of strontium in calcium supplements composed of oyster shell carbonate with strontium contents in the range of 1.98-2.26 mg Sr/g Ca. Limestone and coral calcium sources contained intermediate levels of strontium up to 0.71 mg Sr/g Ca, while synthetically produced calcium carbonate and calcium citrate contained the lowest concentrations at levels <0.40 mg Sr/g Ca. No aluminum was observed in all cases indicating that if present, it is at a concentration below the estimated detection limit of approximately 100 mg/g Ca (as Al). **Conclusions.** The data indicates that the choice of calcium supplement source may have considerate bearing on the amount of strontium ingested daily. The increased levels of

strontium may influence the bone health in not only dialysis patients consuming considerable amounts of calcium supplements, but also osteoporosis patients, who may be supplementing their diet with large doses of strontium ranelate.

P088. EFFECT OF SODIUM SELENITE SUPPLEMENTATION ON BLOOD ACTIVITY OF GPx IN CHILEAN CRIOLLO HORSES KEPT IN PASTURE. Mirela Noro, Ricardo Chihuailaf, Macarena Rioseco, Bruno Menarim and Fernando Wittwer
Instituto de Ciencias Clínicas Veterinarias. Universidad Austral de Chile, Valdivia, Chile.
mirelanoro@gmail.com

Introduction: Selenium (Se) is an essential microelement for animals therefore a nutritional and metabolic balance is necessary for maintenance and performance. Clinical cases of Se deficiency in horses kept at pastures with low content of this element have been reported. The aim of this study is to evaluate the effect of sodium selenite supplementation on the blood activity of the selenium enzyme glutathione peroxidase (GPx, EC: 1.11.1.9), in Chilean Criollo grazing horses. Materials and Methods: The study was performed with 23 Chilean Criollo horses grazing on pasture with a low selenium content. The horses were randomly distributed in three homogeneous groups: G1, n=7, supplemented with a single dose of selenium (Se= 0.05mg/kg) as sodium selenite 1.67% solution, im; G2: n=8, supplemented two doses of selenium (Se= 0.05mg/kg each), the first as G1 and repeated on day 15; G3: n=8 not supplemented. Blood heparin samples were obtained from jugular vein, on days 0, 30, 60, 90 and 120, from September to January. Blood activity of GPx was determined by a NADPH-dependent technique using a commercial kit (Ransel®, Randox Lab). Normality and homoscedasticity of data were analyzed and the mean and standard deviation determined. Differences between groups were established by ANOVA and contrasted by Tukey test, considering significance of $P< 0.05$. Results: GPx activity previous to supplementation was similar in all groups (X: 42±21 U/g Hb; $P>0.05$), and lower than the reference value (>130 U/g Hb). At day 30 of treatment, the supplemented groups demonstrated increased GPx activity, being superior in G2 (110±21 U/g Hb), intermediate in G1 (84±12 U/g Hb) and lower in G3 (55±22 U/g Hb). The higher activity of blood GPx was observed at day 90 on both supplemented groups ($P<0.05$) (G1= 121±52 U/g Hb; G2= 124±69 U/g Hb). The repeated dose was able to increase GPx activity faster than a single supplementation, but both treatments were unable to reach the reference values of GPx in horses. Conclusion: Single or twice supplementations with 0.05mg/kg of selenium as sodium selenite increases blood activity of GPx, in a insufficient magnitude to get an adequate balance of this element in selenium deficient horses.

P089. SELENIUM METABOLIC BALANCE IN GRAZING CHILEAN PUREBRED HORSES IN SOUTHERN CHILE. Ricardo Chihuailaf, Mirela Noro and Fernando Wittwer
Instituto de Ciencias Clínicas Veterinarias, Universidad Austral de Chile, Valdivia, Chile.
rchihuailaf@uach.cl

Introduction: Selenium (Se) is an essential nutrient for equines and its deficiency is associated with nutritional muscular dystrophy and steatosis. Pastures in the south of Chile are known to have a low Se content. The aim of this study was to determine Se metabolic balance, in spring and autumn period, in grazing Chilean purebred horses. Material and methods: Horses from 10 stud farms located in the province of Valdivia, Chile (39º52`S 72º34`W) were used. Horses were selected regardless of their age, sex and physiological status. During autumn 39 animals kept on pastures (G1) and 47 animals kept on pastures and supplemented with grain oats (Avena sativa) (G2) were analyzed. For the spring period 41 grazing horses were studied (G3). Se metabolic balance was determined by the blood activity of selenium dependant enzyme gluthatione peroxidase (GPx, E.C. 1.11.1.9). A blood heparinized sample of each horse was obtained to measure the haemoglobin concentration (Hb) and GPx activity, expressed as U/g Hb, by a NADPH-dependent kinetic method using a commercial kit (Ransel®, Randox Lab). Normality and homocedasticity were determined and the medians (Me), first (Q1) and third (Q3) quartiles and percentages are presented. Comparison between groups by Kruskall-Wallis test was performed. The level of significance was set at 5%. Results and discussion: The GPx activity showed that Se metabolic balance was considered deficient for 67% of the horses from G1 (< 60 U/g Hb) and no horse reached an adequate status (>130 U g/Hb). In G2 only 32% of the animals were deficient and also no horse reached an adequate status. In G3, the values indicated that 34% of the equines had a deficient status and only 17% had an adequate Se metabolic balance. GPx activity was higher in G3 (Me=85, Q1=44 and 318 U/g Hb) and G2 (Me=92, Q1=13 and Q3=251 U/g Hb) compared to G1 (Me=36, Q1=12 and Q3=85 U/g Hb) ($P<0.05$) indicating a better Se metabolic balance in spring and a deficient status in autumn. Autumn deficiency can be improved through supplementation with grain oats. Conclusion: In grazing Chilean purebred horses, a low Se metabolic balance is registered; this is influenced by the period of the year.

P090. MALONALDEHYDE AND TOTAL GLUTATHIONE PLASMA CONCENTRATIONS AFTER A RODEO EXERCISE IN CHILEAN PUREBREED HORSES WITH DIFFERENT SELENIUM NUTRITIONAL STATUS. Juan S. Galecio, Ricardo Chihuailaf and Fernando Wittwer.
Instituto de Ciencias Clínicas Veterinarias. Facultad de Ciencias Veterinarias. Universidad Austral de Chile.
juangalecio@uach.cl

Introduction. Exercise increases cellular oxygen consumption and free radicals production, inducing oxidative stress with damage of lipids, proteins and DNA molecules. The selenoenzyme glutathione peroxidase (GPx) and other antioxidants prevent or inhibit oxidative damage from free radicals generated during the exercise. A high prevalence of horses with a deficient status of selenium (Se) according to low blood GPx values has been described in the South of Chile. Objective. This study evaluated the effect of the rodeo exercise on the concentrations of malonaldehyde (MDA) and total glutathione (TGSH) in Chilean Purebreed horses with low and adequate nutritional status of Selenium. Material and methods. Two groups of horses with different status of Se, measured by the blood activity of GPx, Group A (n=15; GPx <70 U/g Hb) and Group B (n=15; GPx >130 U/g Hb) were exercised in a sand covered arena with 28 meters arch, where performed three typical Chilean Rodeo runs at a speed of 6.5±0.9 m/s. Blood jugular heparinized samples were obtained in two occasions; the first 5 minutes before exercise and the second 10 minutes after the last run. Plasma concentrations of MDA (TBARS test) and TGSH (Gluthatione reductase kinetic method) were determined. Mean and standard deviation of the data were determined and the comparison between groups were performed by a “t” test and Pearson correlation analysis considering a significance of $P<0.05$. Results. Plasma concentrations of TGSH before exercise was lower ($P<0.05$) in Group A (4.5±0.7 µM). than Group B (5.7±1.4 µM). After exercise TGSH increased in both groups ($P<0.05$); the magnitude of this increment (Δ) was higher ($P<0.05$) in Group B (Δ=1.3±1.0 µM) than in Group A (Δ=0.2±0.5 µM). Plasma TGSH concentration was correlated to blood GPx activity (r= -0.61), according with this result animals with a low GPx activity presents higher plasma TGSH concentrations. Plasma concentrations of MDA was similar ($P>0.05$) for both groups during the pre-exercise period (Group A=347±78 nmol/mL; Group B=333±81 nmol/mL). The exercise produces a significant ($P<0.05$) increase in the mean concentrations of MDA of the same magnitude in both groups (Group A Δ=440±102 nmol/mL; Grupo B Δ=621±371 nmol/mL). The increment of MDA suggests that lipoperoxidative damage induced by the exercise was not related to the Se status before the race. Conclusion. A deficient nutritional status of Se in Chilean Purebred horses, expressed by a low blood activity of GPx, was associated to increased plasmatic concentrations of TGSH, but did not induced differences in the magnitude of MDA increase after the rodeo exercise.

P091. ANTAGONISTIC EFFECT OF ORGANIC SELENIUM AGAINST THE TOXICITY OF LEAD. Xiaowei Li and Junquan Gao*
National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, 29 Nan Wei Road, Beijing100050, China
jqgao@vip.sina.com

Experiment design: In this study, the effect of three species of organic selenium that were soybean selenoproteins, tea seleno-amylose, and selenic acid ester-amylose on lead excretion had been compared. The antagonistic effect of the three forms of selenium supplements in three dosages to against the toxicity of lead was further studied. We also conducted the effect of three forms of selenium supplements: soybean selenoproteins, selenic acid ester-amylose and Na2SeO3 on selenium content and anti-oxidation system. Materials and methods: Wistar rats were randomly divided into twelve groups. All of them drink the water containing 300mg/L lead-acetate except the blank control group. In the meantime, the rats were treated with the intervention factors: blank and mode control groups received double distilled water; positive drug group received DMSA solution; Na2SeO3 group, soybean selenoproteins group, selenic acid ester-amylose group, respectively orally through gastric intubation daily. After 30 days, blood was drawn and brain, spleen, liver, kidneys, tibias were collected, the content of lead & selenium were analyzed. In addition, we also determined the activity and content of SOD, GSH, MDA in serum and GSH-Px in whole blood. The analytical quality assurance was severely executed in this study. Results: It was indicated that all of the three selenium supplements could drive lead from blood and organs. The effect of selenic acid ester-amylose on lead excretion was the best, and high dosage was better than low and middle dosages. There were negative correlations between selenium supplement and the lead levels in blood and organs of rats. In addition, it was found that all selenium supplements could increase hemoglobin level efficiently ($P<0.05$). We also founded that selenium increase the activities or contents of GSH-Px in whole blood as well as SOD, GSH in serum, and reduce the content of MDA in serum. There were positive correlations between selenium supplement and the contents of GSH-Px, SOD, and GSH in whole blood or serum, and there was negative correlation between selenium supplement measure and the contents of MDA in serum. Conclusion: Three selenium supplements in three dosages could clearly decrease the lead content and evidently increase the selenium content in lead exposure rats. And the content was enhanced along with the dosages of selenium increasing. It was consider that selenium and lead in rats could combine into a selenium-lead-protein compound that promoted lead excretion and reduced lead aggradation. It was showed that selenium enhancing the activity of anti-oxidation system in rats' body was one of the mechanisms of antagonistic action of selenium on lead poisoning. Keywords: Soybean selenoproteins; Tea seleno-amylose; Selenic acid ester-amylose; Organic selenium; Lead; Lead exposure; Anti-oxidation system; Antagonistic action. * Author to whom correspondence should be addressed.
Prof. Junquan Gao Phone: 8610-67776790; Fax: 8610-67776790

P092. TOXIC TRACE ELEMENTS-NUTRIENTS INTERACTION: LONG-CHAIN POLYUNSATURATED FATTY ACIDS N-3 AND N-6 CONCENTRATIONS IN THE PLACENTA OF RATS EXPOSED TO CADMIUM DURING PREGNANCY. Marcela Araya, Arnaldo Gatica, Ricardo Uauy and Ana M. Ronco.
Laboratory of Nutrition and Metabolic Regulation, Institute of Nutrition and Food Technology, INTA, University of Chile
amronco@inta.cl

Cadmium (Cd2+), a transition metal with an extremely long biological half-life, has become a ubiquitous environmental pollutant during the past several decades. Cadmium is accumulated in smoking women's placentas inducing intrauterine growth retardation (IUGR) through unknown mechanisms. Docosahexaenoic acid (DHA), a n-3 polyunsturated fatty acid (PUFA) is essential for intrauterine growth and development. Our objective was to study, in a rat model, if IUGR induced by Cd2+ exposure during pregnancy (50 ppm) is, at least in part, mediated by mechanisms involving alteration of placental fatty acid metabolism. Virgin female Wistar rats (n=9) were exposed to 10 ppm of Cd2+ in drinking water at 24 days of age until mating. Immediately after detecting pregnancy, rats were exposed to 50 ppm during the whole pregnancy period (20 days). Both groups (experimental and control) were fed with a standard diet containing 4.5 % lipids (Champion). At day 20 of gestation, fetuses, placentas and organs were collected. Fetuses were weighted and organs were frozen at -80 ° C. Total lipids were extracted, trans-esterified to form fatty acid methyl esters and analyzed by gas chromatography mass spectrometry (GC-MS). Statistical analyses were performed using the Kruskal-Wallis test. Anthropometric measurements: in the Cd2+-treated group, a significant decrease in birth weight (3.75 g±0.22 vs 3.12 g±0.34, p <0001) and size (3.6 cm±0.14 vs 3.4 cm±0.12, p <0001) of offspring was observed. This effect was higher in males than in females. There was no difference in the placental weight. The maternal food intake (21.1±0.8 vs 19.9±1.4, p <0001) and maternal weight at term (371±9.4 vs. 320±9.7 p <0001) were decreased in the Cd2+-treated group. When adjusting birth weight to maternal food intake, birth weigh differences between groups were maintained (p <0001). No significant differences in n-6 fatty acids content (arachidonic acid: ARA) between control and Cd2+-treated group was found (120±5 vs 140±20 mg/ 100 g placental tissue). However, a significant change in n-3 fatty acids (eicosapentaenoic acid: EPA and DHA) placental concentrations between both groups was observed; EPA concentration was reduced (120±5 vs 6±1 mg/100 g, p <0.05) and DHA was slightly increased (76±6 vs 93±13 mg/100 g, p<0.05) by Cd2+ treatment. EPA / DHA ratio was reduced from 0.16 to 0.07 (p <0.05) in Cd2+- treated rats. ARA / EPA ratio was significantly augmented in placentas treated with Cd2+ (12.7 to 20.2, p <0.05). There was no difference in the ARA / DHA ratio between both groups. Conclusions: our results suggest an alteration in placental PUFA metabolism as a result of Cd2+ exposure during pregnancy that could be related with Cd2+ effects on IUGR. This work was supported by Fondecyt Nº 1071110

P093. MANAGING MINERAL BALANCE WITHIN BEEF-CATTLE SYSTEMS. Isabel Blanco-Penedo[1], Richard F. Shore[2], Marta Miranda[3], José Luis Benedito[1] and Marta López-Alonso[1]
[1]Universidade de Santiago de Compostela, Departamento de Patoloxía Animal, Facultade de Veterinaria, Lugo, Spain
[2] Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK; [3]Universidade de Santiago de Compostela, Departamento de Ciencias Clínicas Veterinarias, Facultade de Veterinaria, Lugo, Spain.
marta.lopez.alonso@usc.es

Provision of adequate levels of trace metals in cattle diet is essential to promote growth and maintain animals in good health. The increasing intensification of animal production on intensive systems, designed to achieve high productivity, involve widely practiced mineral supplementation incorporated into purchased concentrates. However, concentrate rations are often formulated with large “safety margins” so nutrient intakes largely exceed requirements and trace metals included as mineral supplements may have toxic effects at supra-optimal concentrations. In contrast, farm husbandry practices on extensive systems using a high proportion of local or in-farm produced forage and low/no mineral supplementation can be associated with mineral deficiencies. While mineral deficiencies can be corrected by including concentrates in the diet, organic farms can only give cattle organic feed and 60% of the food ration must consist of on-farm roughage, and the use of mineral supplements is restricted. The aim of the present study was to determine how trace metal concentrations in beef-cattle varies between farms across NW Spain (including farms that have intensive, conventional and organic management practices) and what the likely major causes of such variation are. In terms of overall trace metal nutrition, our results indicate that farm husbandry practices that involve use of a high proportion of in-farm produced forage and low or no mineral supplementation can lead to mineral deficiencies; in fact, a high proportion of calves from organic farms showed Co, Cu and Se concentrations within the deficient-marginal levels. No such deficiencies were found in calves from intensive or conventional animals from the same areas, which indicate that the widely practiced mineral supplementation of concentrates guarantees that the physiological trace element requirements of calves are met, even when concentrates comprise a relatively low proportion of the diet. On the contrary, a high proportion of calves from intensive systems and, to a lesser extent, from conventional farms showed hepatic Cu concentrations above the adequate levels. Cu is the micronutrient that best exemplifies the conflict between short-term animal welfare, because of the great susceptibility of cattle to Cu deficiency worldwide, and the long term sustainability of soil fertility, associated with run-off of Cu-enriched slurries from highly Cu-supplemented animals. Furthermore, the husbandry practices related to grazing in organic systems may have a positive or beneficial effect on the status or other elements, such as Fe. Fe tissue concentrations of calves from organic farms were the highest amongst those measured in our study, even though dietary Fe concentrations were not exceptionally high. This most likely reflects ingestion and assimilation of Fe from soil. In conclusion, organic farms require a strict management of the feed ration to meet the physiological requirement of the animals and avoid mineral imbalances including sub-clinical or marginal deficiencies which are difficult to diagnose clinically but can cause physiological stress and decreased production. Particular care must be taken when concentrates are

supplemented above physiological requirements because of the particular susceptibility of ruminants to chronic Cu toxicity.

P094. MODERATE IRON OVERLOAD IN RATS: INTERACTION WITH FRUCTANS AND/OR PHYTATE IN THE HEPATIC AND BONE METABOLISMS. Maria Lucia Cocato[1], Alexandre Rodrigues Lobo[1], Primavera Borelli[2], Anna Karenina Azevedo Martins[3], Lílian Rosa Marques de Sá[4], Célia Colli[1]
[1]Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, SP, Brazil
[2]Departamento de Análises Clínicas e Toxicológicas da Faculdade de Ciências Farmacêuticas, ; Universidade de São Paulo, SP, Brazil
[3]Departamento de Fisiologia e Biofísica do Instituto de Ciências Biomédicas; Universidade de São Paulo, SP, Brazil
[4]Departamento de Patologia da Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brazil
cecolli@usp.br

Excess Fe in the organism generates potentially toxic reactive oxygen species (ROS). However, the magnitude of the effects of a moderate Fe overload and its interaction with factors which inhibit or facilitate mineral absorption is not known. The aim of the present work was to evaluate such effects and their interaction with fructans and/or phytate (compounds which facilitate and inhibit Fe absorption, respectively) on serum iron status indices, on the profile of serum lipids and on hepatic and bone metabolism parameters. In the experiment, thirty-four male Wistar rats initially weighing 49.3±3.9g were used. The rats were housed in individual stainless-steel wire-mesh cages for 92 days. An AIN-93G diet (Diet 1: Control Group) and four modified AIN-93G diets were used in the study. The modified diets presented the following formulations: Diet 2: Moderate Fe overload with 550mgFe/kg diet (IO Group); Diet 3: Moderate Fe overload + 18% yacon flour (IO-YF Group); Diet 4: Moderate Fe overload + 0.6% phytate (IO-Phy Group); Diet 5: Moderate Fe overload + 18% yacon flour + 0.6 % phytate (IO-YF-Phy Group). The results demonstrated that a moderate Fe overload or its interaction with yacon flour and/or phytate did not alter the serum iron status indices. An increase in the serum AST activity was observed only in the IO group ($p=0.055$). In the IO and IO-YF groups, there was a reduction in the serum cholesterol concentration ($p=0.002$) and a reduction in the serum VLDL concentration was observed only in the IO-YF-Phy group. In the liver, there was a significant increase ($p=0.002$) in non-heme Fe concentration in the IO (+83%) and IO-Phy (+117%) groups. Also, GPx activity was significantly increased ($p=0.000$) in all IO groups. CAT activity was lower ($p=0.036$) only in the IO-YF-Phy group. A significant increase in hemosiderin deposition around Kupffer cells was observed in all IO groups ($p=0.000$). Apoptosis was increased in all IO groups, whereas the IO-YF and IO-YF-Phy groups showed the largest number of apoptotic bodies/area (+405% and +342%, respectively) ($p=0.000$). There was no alteration in the parameters related to bone metabolism. In the IO-YF group, there was a significant increase in Ca apparent absorption ($p<0.05$).In conclusion, the moderate Fe overload did not alter the serum iron status indices, but led to alterations in the hepatic tissue and in the profile of serum lipids. Except for the profile of serum lipids where only phytate seemed to have a protective effect, in the other evaluated parameters the interaction with yacon flour rich in fructans and/or phytate partially or totally reversed the alterations induced by the moderate Fe overload.

P095. EFFECT OF MOLYBDENUM AND SULPHUR ON COPPER STATUS AND MOHAIR QUALITY IN MERGHOZE GOAT. Mohammad Mehdi Moeini, Ebrahim Nooriyan and Manochehr Souri.
College of Agriculture, Razi University, Kermanshah, Iran.
mmoeini2008@yahoo.com

This study assessed the effects of a normal diet containing 12.8 mg Cu/kg DM (dry matter of feed), to which molybdenum and sulphur were added gradually, on the copper status and fibre quality in eight 1- year Merghoze goats. Group 1 (n=4 mean weight 31±2.0 kg) was treated with Mo and S supplements for 20 weeks, group 2 (n=4 mean weight 32± 2.1 kg) served as controls. Blood samples were obtained to measure copper status in plasma and copper content and quality of fleeces were measured every 6 weeks. Mohair measurements were carried out by taking patch samples (10 * 10 cm 2) from the mid-side area of the animals. Determinations included plasma copper concentrations (Pl Cu), Trichloroacetic acid soluble copper concentrations (TCA-Cu) and fleece copper content. Results indicate that the addition of 25- 30 mg Mo and 2-2.5g S /kg DM to the normal diet resulted in sub clinical copper deficiency. There was loss of fleece pigmentation and poorer crimp. Also, there was a significant decrease in Pl Cu ($P<0.05$) along with a significant increase in thiomolybdate (MoS) production after 4 months. The Pl Cu minus TCA-Cu plasma became more than 2 uM in the blood of treated goats, indicating that there was a significant thiomolybdate formation in the body. The sub clinical signs of copper deficiency and mohair quality are likely derived from the high molybdenum intake and thiomolybdate formation in the body.
Key word: Copper deficiency, Molybdenum, Sulphur, Fiber, Goat

P096. EFFECT OF SELENIUM AND VITAMIN E SUPPLEMENTATION DURING LATE PREGNANCY ON SERUM IGG CONCENTRATION IN HEIFERS AND SERUM IGG CONCENTRATION AND PASSIVE IMMUNITY IN THEIR CALVES. Mohammad Mehdi Moeini, Elham Mikaeili, Hamed Karami and Amir Kiani.
College of Agriculture, Razi University, Kermanshah, Iran.
mmoeini2008@yahoo.com

This study was conducted to determine the effect of injecting selenium and vitamin E to pregnant heifers at the last stage of gestation on Se status, serum IgG concentrations of heifers and passive immunity and growth rate of their calves. Fifty Holstein heifers were randomly assigned to one of five treatments. Four and two weeks before expected calving, heifers were injected intramuscularly 10 ml (T1), 20 ml (T2), 30 ml (T3), 40 ml (T4) selenium and vitamin E respectively. The control group (C) received no supplement. Each ml of the supplement containing of 0.5 mg Se as sodium selenite and 50 IU of D-L alpha-tocopheryl acetate. Blood samples were collected from heifers four weeks before expected calving and on calving day. Blood samples in calves were drawn from the jugular vein at birth and at 7 days of age. White blood cell and differential leukocyte counts were measured. Serum concentrations of Se were determined using hydride generation atomic absorption spectrophotometry. Serum IgG concentrations were measured by sandwich ELISA method. Results indicate that before administration of Se and vitamin E serum Se concentration of heifers was not different among the 5 groups ($p>0.05$). After calving, Se concentration of heifers was significantly higher in animals receiving T3 and T4 ($p<0.05$). Similarly, calves of heifers that received the supplements, had higher serum Se and this was highest in T4. Colostral concentration of Se was increased by maternal Se supplementation. Calves born to mothers that received Se supplement had higher Se status at birth and 7 days of age. Changes in serum IgG concentrations before and after calving did not differ among heifers. Treatments did not modify colostral IgG concentration ($p>0.05$) or the mean values of serum IgG among calves. The white blood cell count was higher in calves receiving T4 treatment as compared to the control group ($P<0.05$) but the percent of neutrophils was not different in calves. Treatments did not affect the birth weight and weaning weight of calves ($p>0.05$).
Keyword: Selenium, Vitamin E, IgG, Immunity, Heifers, calves

P097. A STATISTICAL APPRAISAL OF THE RESULTS OF THE BLOOD MICROMINERALS METABOLIC PROFILE TEST ON CHILEAN DAIRY HERDS. Mirela Noro, Ricardo Chihuailaf, Marcela Cabrera, Helga Böhmwald and Fernando Wittwer
Instituto de Ciencias Clinicas Veterinarias, Universidad Austral de Chile, Valdivia, Chile
mirelanoro@gmail.com

Introduction. Mineral metabolic unbalances are frequently diagnosed in grazing cows according to local conditions of the forages. The blood metabolic profile test (MPT) has been used for the diagnosis of the metabolic disorder in the dairy herds of the South of Chile. The aim of this study is to describe the prevalence of micro-minerals disorders determined by MPT realized to local dairy herds in the Clinical Pathology Laboratory of the Universidad Austral de Chile during the last 5 years. Material & Methods. The results from 611 MPT from dairy herds of the South of Chile, realized in the Clinical Pathology Laboratory of the Universidad Austral de Chile in the period January 2004 – May 2008 were used. Each MPT was formed by 1 to 3 groups of 5 or more blood samples obtained of grazing dairy cows and analyzed to obtain the mean concentrations of Cooper (Cu), Zinc (Zn) and glutathione peroxidase activity (GPx, EC:1.11.1.9) as marker of selenium metabolic balance. Information of physiological condition of the cows (pre-partum; early lactation; middle and end of lactation) and season of the year (summer, autumn, winter, spring) were also registered. Cu and Zn concentrations were determined by Atomic Absorption Spectrophotometer and GPx by a kinetic technique using commercial kit (Ransel®, Randox Lab). The means (X), standard deviations (SD) coefficient of variation (CV) and physiological and seasonal differences using ANOVA or Kruskal-Wallis Test were calculated using the program Statistic 8.0. A difference was considered significant when $p < 0.05$. Also the percentage of values below or above the reference values established for the Laboratory (Cu = 10 – 22 µmol/L; Zn = 8 – 24 µmol/L; GPx >130 U/g Hb) were determined. Results. The blood concentration of Cu was lower in pre-partum cows (11.4 ± 3.0 □mol/L) than in lactating cows (12.3 ± 2.2 □mol/L) (p□0.05), and also lower during winter compared to other seasons of the year (p□0.05). Blood activity of GPx was also lower in pre-partum cows (230 ± 140 U/g Hb) than in lactating cows (278 ± 151U/g Hb); but not seasonal differences were observed. Blood concentration of Zn was lower in early lactation cows (13.9 ± 3.5 □mol/L) compared to pre-partum (15.5 ± 4.3 □mol/L) and middle-end of lactation cows (15.4 ± 4.3 □mol/L), with higher values during the autumn. The highest prevalence of micro mineral metabolic unbalances were Cu and Se deficiencies. Hypocupremia was observed in 18.3 % of the groups, especially during pre partum period (31.5%). Se deficiency, diagnosed by a low blood activity of GPx, was done in 21.7% of the groups, especially in pre-partum (28.8%) and early lactation cows (21.0%). Zn deficiency was observed only in 1.2% of the herds. Conclusion. Se deficiency and hypocupremia, presumed to a Cu metabolic deficiency, presented elevated prevalence in grazing dairy herds of the South of Chile during the period January 2004 – May 2008.

P098. HCP1 AND FLVCR EXPRESSION IN RESPONSE TO HEME AND NON-HEME IRON. Solange Le Blanc[1], Marco T. Nuñez[2] and Miguel Arredondo[1].
[1]Laboratorio de Micronutrientes, INTA, Universidad de Chile, Santiago, Chile.
[2]Facultad de Ciencias, Universidad de Chile, Santiago, Chile.
solange.leblanc@gmail.com

Introduction: Iron is an essential element for almost all living organisms. Iron can be found in the diet either as heme Fe or inorganic iron. Heme Carrier Protein 1(HCP1) has been characterized as an apical transporter that is responsible for the uptake of heme into duodenal cells. Feline Leukemia Virus subgroup C Cellular Receptor 1(FLVCR) has been shown to export heme out of erythrocytes and probably plays a role in intestinal heme transport since FLVCR is highly expressed in Caco-2 cells.
Objective: To determine HCP1 and FLVCR transporter expression in intestinal epithelium-like Caco-2 cells incubated at different heme Fe or non-heme Fe concentrations and incubation times. Methodology: Caco-2 cells were incubated with 50 μM heme Fe for 0-72 hr or incubated with 0–80 μM of heme or non Fe for 12 hr. Total intracellular Fe content was measured by EAA. We purified total RNA, determined the relative expression of HCP1 and FLVCR by Real-Time RT-PCR and correlated these expressions with both intracellular heme content and the expression of heme oxygenase enzyme. Results: Intracellular heme Fe or no heme Fe increased ($p<0.01$) when Caco-2 cells were incubated with increased heme or no heme Fe concentrations, respectively. Caco-2 cells incubated for different period of time with 50 μM heme induced a decrease of HCP1 and FLVCR expressions. When Caco-2 cells were incubated with increasing concentrations of heme, HCP1 showed a decrease in its expression while FLVCR showed a biphasic response; first it decreased and then it increases. However, this response was specific only to heme, since an increase in extracellular non-heme Fe concentration did not produce a significant variation in HCP1 or FLVCR expression. Conclusion: The expression of HCP1 and FLVCR is down-regulated by elevated intracellular heme concentration.
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P099. THE OXIDATIVE STRESS GENERATES A SIMILAR GENETIC RESPONSE TO IRON DEFICIT IN ENTEROBACTERIAS. Guadalupe López-Rodríguez[2], Angélica Reyes-Jara[1], Patricia Águila[1] and Mauricio González[1].
[1] Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile.
[2] Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, México.
ljhons22@yahoo.es

Iron is an essential micronutrient for all eukaryotes and the majority of prokaryotes, but in excess it can induce oxidative stress. The cellular metabolism of iron involves proteins participating in different cells processes such as uptake, iron handling, users and metal efflux. In general, under iron deficit, the genes that encode iron-uptake transporters increase their expression to ensure iron requirements. However, this response does not seem to be exclusively associated to metal deficiency, and it has been detected under oxidative stress conditions. Here, our aim was evaluate whether oxidative stress produced by iron excess induces the expression of genes associated to iron deficit. In cells of Enterococcus faecalis OGIRF exposed to iron excess, we evaluated the transcriptional response of iron metabolism genes, the iron content and the GHS intracellular content (indicator of redox status). The abundance of genes was determined by qPCR and included the following elements: a) genes involved in iron uptake (three operon of ABC-type system of siderophore and 1 operon of ferrous uptake), b) transcriptional factors (TFs) that respond to iron (Fur) and H2O2 (Per), c) catalase, SODMn, thioredoxines, glutathione reductase and one bifunctional enzyme for GSH synthesis used as reporter of stress response. Glyceraldehyde 3-phosphate dehydrogenases gene was used as a housekeeping marker. The intracellular iron content was determined by atomic absorption spectrophotometry (AAS). The results shown that E. faecalis growing 6 h in a medium supplemented with 500 μM of FeCl3:NTA (1:2) have a significantly increment of iron respect to cells not exposed to iron (12.5 ± 2.2 vs 27.9 ± 4.9 mmoles Fe/mg protein, $p<0.05$). After iron exposure, the GSH content (determined by Griffith OW method) decreased from 10.1 ± 1.6 to 3.8 ± 0.9 nmoles GSH/mg protein ($p<0.05$), which was correlated with an increment of transcripts associated to stress response: catalase (3-fold change), SODMn (10-fold change), thioredoxine (4-fold change), glutathione reductase (3-fold change) and GSH synthetase (2-fold change). In addition, an increment of TF Per was observed. Thus, our results indicated that iron excess induce a redox stress in E. faecalis cells. By other side, cells exposed to iron showed an significant increment in the relative abundance of transcripts associated to iron uptake: the system of transport of ferri-enterobactin (3-fold change) and the ferrichrome ABC transporter (2.5-fold change), as is expected for bacteria cultured in a deficit of iron. In contrast, the expression of Fur appears to respond to excess of iron. In conclusion, the results suggest that genes encoding for components of iron-uptake system are target of transcriptional regulatory network activated by redox stress.

P100. SUPEROXIDE-DEPENDENT REDUCTION OF FREE Fe^{3+} AND RELEASE OF Fe^{2+} FROM FERRITIN BY THE PHYSIOLOGICALLY-OCCURRING $Cu(I)[GSH]_2$ COMPLEX.

Francesca Burgos-Bravo[1], Margarita Aliaga[1], Edgar Pastene[1], Catalina Carrasco-Pozo[1] and Hernán Speisky[1,2]

[1]Micronutrients Unit, Nutrition and Food Technology Institute, University of Chile, Santiago, Chile
[2]Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical & Pharmaceutical Sciences; University of Chile, Santiago, Chile
francesca.burgos@gmail.com

The interaction between Cu^{2+} ions and reduced glutathione (GSH), at molar a ratio equal or greater than 1:3, leads to the swift formation of $Cu(I)-[GSH]_2$, a complex reported to occur in copper-overloaded cells. Under aerobic conditions, the $Cu(I)-[GSH]_2$ complex reacts with oxygen to generate superoxide radicals ($O_2^{\cdot-}$), in a continuous and reversible reaction. As a $O_2^{\cdot-}$-generating species, the complex could induce either the oxidation or the reduction of certain redox-susceptible target molecules. In the present study, we addressed Fe^{3+} as a possible target, since its interaction with $O_2^{\cdot-}$ anions would lead to the formation of the redox-active Fe^{2+} species. As known, in the presence of hydrogen peroxide, Fe^{2+} ions catalyze the formation of hydroxy radicals. Under physiological conditions, most iron is bound to Ferritin. This protein stores iron under a redox-inactive form (an Fe^{3+}-oxide-phosphate mineral) which prevents the metal from catalyzing free radical formation. Previous in vitro evidence has revealed that superoxide anions are capable of inducing the reduction of iron in ferritin and its subsequent release as Fe^{2+} into the media. Considering the latter, we evaluated the ability of the $Cu(I)-[GSH]_2$ complex to induce in vitro the reduction of Fe^{3+}, both, as free and bound to ferritin. Results from our study indicate that: (i) the superoxide anions generated by the $Cu(I)-[GSH]_2$ complex are capable of reducing free Fe^{3+} ions (assessed through the batophenantroline-OD_{530nm} and triazine-OD_{593nm} colorimetric chelation assays), (ii) when H_2O_2 is present, the Fe^{2+} ions generated in (i) are capable of catalyzing the generation of hydroxyl radicals (assessed using fluorescein as probe), and (iii) through its ability to generate superoxide anions, the $Cu(I)-[GSH]_2$ complex can induce the reduction and subsequent release of Fe^{2+} from ferritin (assessed through the batophenantroline assay).
These results indicate that during its interaction with iron (whether in its free or bound forms), the $Cu(I)-[GSH]_2$ complex generates redox-active Fe^{2+} ions which could favour the occurrence of superoxide-driven Fenton reactions. Thus, on the basis of its pro-oxidant activity, it is suggested that the complex could exacerbate iron-dependent oxidative damage in copper over-loaded cells.
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P101. THE UPTAKE AND METABOLISM OF SOYBEAN FERRITIN BY INTESTINAL CELLS. Brie K. Fuqua and Elizabeth C. Theil
CHORI: Council on BioIron, Centers for Nutrition and Metabolism and for Sickle Cell Disease; University of California-Berkeley: Department of Nutritional Sciences and Toxicology, USA.
briekat@berkeley.edu

New knowledge of dietary iron absorption is crucial for prevention and treatment of iron deficiency, which affects billions of people worldwide1. Ferritin, the primary form of iron in legumes, is well absorbed by both humans and animals (20-25%)2-5. Ferritin iron absorption differs from that of other non-heme iron sources by: 1. absorption in the presence of phytate, an inhibitor of ferrous (sulfate) absorption2,5,6; 2. the relative stability of ferritin to digestion in vitro, suggesting some ferritin reaches the intestine intact; 3. delivery of >1000-fold more Fe to cells/transport cycle. Part of the molecular mechanism of absorption of iron from ferritin involves clathrin and the μ2 subunit of the AP2 complex -dependent endocytosis7, but other molecules involved are unknown. We hypothesize that the ferritin protein nanocage structure influences ferritin iron absorption and metabolism by intestinal cells. Thus, we are studying the effects of targeted mutations in two highly conserved major surface features of plant (Soya) ferritin: 1. the eight 3-fold axis pores, found in all ferritins, and 2. an N-terminal extension peptide located near the pores and found uniquely in plant ferritins, on iron absorption by an intestinal cell model (polarized Caco-2). The L134P mutation in soybean ferritin, an extensively characterized pore-unfolding mutation in animal ferritins8, also unfolds plant ferritin pores based on the 3-fold increase in the rate of iron release in the presence of reductant and chelator. Preliminary studies suggest that this mutation inhibits iron absorption from ferritin, indicating that folded pores may contribute to ferritin recognition during iron absorption. In addition, we compared effects of phytate on absorption of 59Fe from ferrous sulfate and ferritin by Caco-2 cells and observed that preincubation of ferrous sulfate with phytate (phytate: Fe=10:1) for 30 minutes, as would occur during digestion, reduced absorption by >95%, but had a much smaller effect on ferritin iron absorption, supporting the concept that the ferritin nanocage prevents the formation of the iron-phytate complex. mRNA profiles from the duodenum of iron-deficient mice fed diets supplemented with ferrous sulfate or ferritin revealed upregulation of several novel genes, confirmed by immunoblotting. The effect of altering expression of the genes with RNAi is being analyzed to further understand the molecular properties of iron absorption from plant ferritin. Support: CHORI Foundation (ECT), NIH-DK20251 (ECT, BKF), NSF (BKF).

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P102. IRON AND GLUCOSE OVERLOAD MODIFY MITOFUSIN 1 AND MITOFUSIN 2 MRNA EXPRESSION. Alejandra Espinoza[1], Amaya Oyarzún[2], Megan Rourk[3], Francisco Pérez[2], Miguel Arredondo[1].
[1]Laboratorio de Micronutrientes, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Santiago, Chile
[2]Laboratorio de Epidemiología Nutricional y Genética, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Santiago, Chile
[3]Michigan University, USA.
aespinoza@inta.cl

Mitofusin 1 (MFN-1) and Mitofusin 2 (MFN-2) participate in mitochondrial fusion and division processes. MFN-2 has been pointed out as a key gen in the development of type 2 diabetes mellitus (DM2) and/or obesity, showing a decreased expression in both illnesses. Furthermore, MFN-2 is one of the main determinants of oxidative stress mediated apoptosis in cardiomyocytes. It has also been shown that DM2 patients have decreased expression of MFN-2 in skeletal muscle. On the other hand, a positive association between high levels of storage iron (Fe) and risks of cardiovascular disease, metabolic syndrome, gestational diabetes and DM2 has been reported. Fe participates in oxidative stress generation through the Fenton reaction and hydroxyl radicals produced by Fe participate in early insulin resistance and later on in the decrease of insulin secretion, which contributes to DM2 development. The aim of this study was to analyze the effect of Fe and glucose overload on mitofusin 1 and 2 mRNA expression. Caco-2 (a intestinal epithelium-like cell) and MIN6 (beta pancreatic cells) cells were incubated with different stress conditions: 1) control (G0)(3,5□M Fe/10mM glucose); 2) high glucose concentration (G1) (3,5□M Fe/25mM glucose); 3) high Fe concentration (Fe) (50□M Fe/10mM glucose) and 4) high Fe and glucose (Fe/G) (50 □M Fe/25 mM glucose). Total Fe concentration was measured in protein extracts by AAS. MFN-1 and MFN-2 expression was assessed by RT-PCR. Intracellular Fe content (in nmoles Fe/mg protein) in Caco-2 cells was significantly higher in cells incubated with high Fe than those without Fe (Fe: 3.4+0.4, Fe/G: 3.5+0.9, G0: 1.4+0.7 and G1: 1.8+0.7; One way ANOVA, $p<0.01$). Similar results were found in MIN6 cells (Fe: 3.5+1.1, Fe/G: 4.5+1.0, G0: 1.4+0.6 and G1: 1.3+0.1; One way ANOVA $p<0.01$). MFN-1 y MFN-2 mRNAs showed a different expression pattern in Caco-2 cells. Fe and high glucose challenge decreased MFN-2 expression (One way ANOVA, $p<0.01$). A higher MFN-1 expression was found in cells incubated with high glucose and in control condition, while Fe challenge decrease its expression (One way Anova, $p<0.01$). MTN-2 mRNA expression increased mainly in MIN6 cells incubated with high Fe (One way ANOVA $p<0.01$). In conclusion, we show that Caco-2 and MIN6 cells have different expression patterns. Despite these differences, oxidative stress generated either by glucose or Fe overload induced changes on the expression of both mitofusins.
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P103. DOUBLE EDGE REDOX-IMPLICATIONS FOR THE INTERACTION BETWEEN ENDOGENOUS THIOLS AND COPPER IONS: *IN VITRO STUDIES*. Catalina Carrasco-Pozo[1,2], Margarita Aliaga[1], Claudio Olea-Azar[3] and Hernán Speisky[1,2].

[1]Micronutrients Unit, Nutrition and Food Technology Institute, University of Chile, Santiago, Chile.
[2]Department of Pharmacological and Toxicological Chemistry, University of Chile, Santiago, Chile.
[3]Department of Analytical Chemistry[3], Faculty of Chemical & Pharmaceutical Sciences; University of Chile, Santiago, Chile.
catacapo@gmail.com

The present study investigated the redox-consequences of the interaction between various endogenous thiols (RSH) -glutathione, cysteine, homocysteine, □-glutamyl-cysteine and cysteinyl-glycine- and Cu^{2+} ions, in terms of their free radical-scavenging, ascorbate-oxidizing and $O_2^{\cdot-}$-generating properties. Upon a brief incubation (3-30 min) with Cu^{2+}, the free radical-scavenging properties (towards $ABTS^{\cdot+}$ and $DPPH^{\cdot}$) and thiol-titrateable groups of the RSH added to the mixtures decreased significantly. Remarkably, both effects were only partial even in the presence of a large molar Cu^{2+}-excess, and were unaffected despite increasing the incubation time. At equimolar concentrations, the RSH/Cu^{2+} mixtures led to the formation of (EPR paramagnetic) Cu(II)-complexes that were time-stable and ascorbate-reducible, but redox-inactive towards oxygen. In turn, at a slight molar thiol-excess (3:1), the mixtures resulted in the formation of time-stable Cu(I)-complexes (EPR silent) that were unreactive towards ascorbate and oxygen. The only exception was seen for the thiol, glutathione, whose mixture with Cu^{2+} mixture which displayed a $O_2^{\cdot-}$-generating capacity (Cytochrome c- and Lucigenin-reduction). The data indicate that, depending on the molar ratio, the tested thiols would give place to mixtures containing either: (*i*) time-stable and ascorbate-reducible Cu(II)-complexes which display free radical-scavenging properties, or (*ii*) time-stable but redox-inactive towards oxygen Cu(I)-complexes. Among the latter, the only exception was that of glutathione.
Supported by Fondecyt Grant 1070613.

P104. BIOGENESIS OF CYTOCHROME C OXIDASE IN *A. THALIANA*: TWO PUTATIVE COPPER CHAPERONES SIMILAR TO YEAST SCO1 AND COX11. Talía del Pozo, Patricia Hanna, Verónica Cambiazo and Mauricio González.
Laboratorio de Bioinformática y Expresión Génica, INTA – Universidad de Chile.
tdelpozo@inta.cl

Copper is required within the mitochondria for the function of two metalloenzymes, cytochrome c oxidase (CcO) and superoxide dismutase (Sod). Copper metallation of these two enzymes occurs within the mitochondrial intermembrane space and it is mediated by metallochaperone proteins. In yeast Cox 17 is a copper donor to two accessory proteins, Sco1 and Cox11, to form the two centers in the mature CcO complex. In humans there are two Sco proteins; mutations in hSco1 cause neonatal hepatic failure, whereas mutations in hSco2 lead to neonatal encephalo-cardiomyopathy underscoring the importance of these proteins for development and cell viability. Recently, it has been shown that these mutations in humans cause significant reduction in cellular copper contents both tissue an allele specific. Up to now neither Sco1 nor Cox11 have been described in plants. A searching in the genome of Arabidopsis thaliana (a model plant) allowed us to identify two single sequences At4g39740 (AtCox11-like) and At3g08950 (AtSco1-like) using as templates yeast sequences of Cox11 and Sco1, sharing 50% and 38% of identity respectively to yeast proteins. In silico analysis predicts mostly a mitochondrial destination for both, and the topology suggests a single transmembrane domain as have been reported in human and yeast Cox11 and Sco1. The protein alignments showed a highly conserved copper binding motif CxxxC for AtSco1-like and CxC for AtCox11-like across several species and plants. Also, we performed experiments to analyze the transcript levels by real-time PCR in roots and shoots de A thaliana, finding that both genes are weakly expressed in these tissues. In plants exposed to copper (50 µM $CuSO_4$, 24 h) AtCox11-like was upregulated in shoots and roots, whereas AtSco1-like only in roots. Experiments with the specific copper chelator Bathocuproine disulphonic acid (50 µM BCS, 5 days) did not affect their transcriptional response. Yeast complementation failed to rescue the mutations for both proteins, despite the high conservation of the sequences related to yeast proteins suggesting that its Cu-chaperone function could be species specific. Further analyses expressing chimeric proteins in yeast will be necessary to better characterize these proteins and theirs possible roles in plant copper homeostasis.

P105. Cu(I)-GLUTATHIONE COMPLEX: A POTENTIAL SOURCE OF SUPEROXIDE RADICALS GENERATION. Maritza Gómez[1], Catalina Carrasco-Pozo[1], Edgar Pastene[1] and Hernán Speisky[1,2].

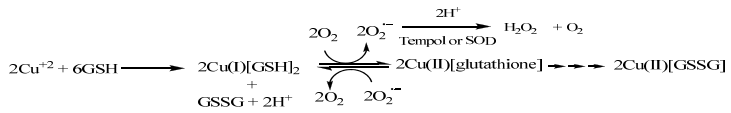
[1]Nutrition and Food Technology Institute, University of Chile;

[2]Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile.
magomez@inta.cl

Copper is an essential but also a potentially toxic trace element. Its status is controlled by efficient intracellular homeostatic mechanisms which operate not only to secure its metabolic requirements but also to prevent its occurrence as free copper ions. Upon entry of copper into the cell, glutathione (GSH), the single most abundant endogenous thiol, swiftly interacts with Cu^{2+} ions to form a Cu(I)-$[GSH]_2$ complex. We investigated the potential capacity of this complex to reduce molecular oxygen into superoxide anions ($O_2^{\cdot-}$). The redox activity of the Cu(I)-$[GSH]_2$ complex towards oxygen was assessed: polarographically, measuring the changes in oxygen concentration (Clark-electrode); through ESR, measuring the changes in paramagnetic properties of the metal and its complexes and addressing the emergence of oxygen-based radicals; and spectrofotometrically and spectrofluorometrically, using cytochrome c and dihydroethidium (DHE), respectively, as a $O_2^{\cdot-}$-sensitive probes.
While the concentration of oxygen in a solution containing the Cu(I)-glutathione remained constant along time (0-30 min), the addition of SOD to such solution led to a sustained decline of the basal oxygen level. On the other hand, the complex (8 µM) was able to induce the reduction of cytochrome c and the oxidation of dyhydroethidium into 2-hydroxyethidium. Both effects were totally blocked by SOD. The ability of the complex to generate $O_2^{\cdot-}$ radicals was confirmed by EPR spin-trapping. Cu(I)-glutathione induce no oxidation of fluorescein, a hydroxyl radical-sensitive probe. We conclude from these experiments that in solutions containing the complex, oxygen would be continually reduced into superoxide anions, and that –in absence of interceptors- the latter radicals would be quantitatively re-oxidized into molecular oxygen. We suggest that coupled to the re-oxidation of $O_2^{\cdot-}$, the Cu(I)-$[GSH]_2$ complex would be re-generated from an “oxidized form” of the former. A further characterization of the Cu(I)-$[GSH]_2$ complex revealed that its $O_2^{\cdot-}$-generating ability depends on its concentration. Addition of GSH in excess to the Cu(I)-$[GSH]_2$ complex (8 µM) exerted a biphasic effect; while GSH concentrations $\leq$ 25 µM inhibited (by around 75%) DHE oxidation, concentrations of the tripeptide >25 µM (and up to 500 µM) were found to linearly reverse such effect, increasing DHE oxidation to an extent greater than 2-fold the initial rate. The latter effect of GSH was accompanied by concomitant increases in the concentration of GSSG. We suggest that the GSH-induced higher capacity of the complex to oxidize DHE (which serves to remove superoxide) results from a GSH-mediated increased re-generation of Cu(I)-$[GSH]_2$.
We speculate that by functioning as a continuous source of superoxide anions, the Cu(I)-$[GSH]_2$ complex could potentially affect a broad range of $O_2^{\cdot-}$-susceptible biological targets.
Supported by Fondecyt Grant 1070613.

P106. FORMATION OF A Cu(II)-GSSG COMPLEX DURING THE REMOVAL OF SUPEROXIDE ANIONS GENERATED BY THE Cu(I)-$[GSH]_2$ COMPLEX. Margarita Aliaga[1], Francesca Burgos[1], Maritza Gómez[1], Claudio Olea-Azar[2], Carolina Jullian[2] and Hernán Speisky[1,3].
[1]Micronutrients Unit, Nutrition and Food Technology Institute, University of Chile, Santiago, Chile
[2]Department of Analytical Chemistry, Faculty of Chemical & Pharmaceutical Sciences; University of Chile, Santiago, Chile.
[3]Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical & Pharmaceutical Sciences; University of Chile, Santiago, Chile
mealiaga@uc.cl

The present study addressed the characterization of the Cu(II)-GSSG complex, whose formation, as shown here, results from the interaction (Scheme I) between Cu(I)-$[GSH]_2$ and oxygen in the presence of superoxide-interceptors (Tempol or SOD). The latter was studied in aqueous solutions using: spectrophotometric methods (assessing thiol density as DTNB reduction at OD_{412nm}, GSSG as NADPH oxidation at $OD_{340nm;}$ and Cu(II)-GSSG formation as appearance of a band at 625 nm), and spectroscopic methods (nuclear magnetic resonance, NMR and electron spin resonance, EPR).
Results show: (a) that the incubation of the Cu(I)-$[GSH]_2$ complex (prepared as a Cu^{2+}/GSH 10:30 □M mixture) with Tempol (8□M at 37°C) leads to a time-dependent decrease in the thiol density of the mixture and to an increase in the concentration of GSSG (when assessed in the presence but not absence of EDTA); (b) that the incubation of the Cu(I)-$[GSH]_2$ complex (prepared as a Cu^{2+}/GSH 5:15 mM mixture) with either Tempol (4mM) or SOD (2000 U/mL), 37°C, leads to emergence of a band at 625 nm which is characteristic of the Cu(II)-GSSG complex. The latter band was found to disappear, time- and concentration-dependently, upon addition of increasing concentrations of GSH (6-160 mM); (c) for the mixture in (b) NMR and EPR spectra which are coincident with those featured by a pre-formed Cu(II)-GSSG complex .
According to these results, removal of the superoxide radicals, generated during the interaction between the Cu(I)-$[GSH]_2$ complex and oxygen, result in the oxidation of Cu(I) into Cu(II) (EPR data) and that of GSH into GSSG molecules. Based on these data, on the NMR spectra, and on the appearance of a band at 625 nm data, we postulate that, as proposed in Scheme I, Cu(II)-GSSG molecules are formed as final product.
Supported by Fondecyt Grant #1070613 and #3080025 (Postdoctoral).



Scheme I

P107. MECHANISM OF SELENIUM CYTOPROTECTION AGAINST CHOLESTEROL OXIDE-INDUCED VASCULAR DAMAGE IN RATS. Kaixun Huang, Hongmei Liu, Qingzhi Wu, Rong Tang and Huibi Xu.
Department of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan, PR China
hxxzrf@mail.hust.edu.cn

Selenium (Se) is an essential trace element and functions primarily in the form of selenoproteins. The intake of Se can to be inversely correlated with the incidence of atherosclerosis and coronary heart disease. Oxidative damage to the endothelium is thought to be a primary event in the pathogenesis of atherosclerosis and oxidatively modified low-density lipoprotein (Ox-LDL) is riched in cholesterol oxidation products (Ch-Ox), which are found in atherosclerosis lesions and are markedly elevated in plasma and vascular tissues in hypercholesterolemic humans and animals.

In this paper, the mechanisms of Ch-Ox-induced cellular toxicity and the protections by Se were investigated. The main results are as follows:

1. Damages induced by triol to the vascular endothelium of long-term Se-deficiency rats and protections through injecting Se were studied by scanning electron micrography and transmission electron micrography. The results indicated that triol severely injured the vascular endothelium of long-term Se-deficient rats, and pre-injected with Na2SeO3 and ebselen exhibited significant protections against damages induced by triol.
2. The mechanisms of selenium against oxysterol-induced vascular smooth muscle cells (VSMCs) apoptosis were investigated. The supplement of selenium could keep the cellular levels of antioxidant capacity of VSMCs and protected markedly against apoptosis and damage induced by Triol through stabilizing the membranes of cell and organelles such as mitochondria and sarcoplasmic reticulum, decreasing the generation of reactive oxygen species, keeping the intracellular Ca2+ homeostasis and maintaining the levels of mRNA of protooncogene c-myc and bcl-2.
3. The cytotoxicity of triol to ECV-304 cell and protections by ebselen were investigated. Ebselen protects ECV-304 cells against damages induced by triol in a dose-dependent manner. The protections of ebselen against damages induced by triol, however, can be inhibited by the presence of GSH. The mechanism of inhibition was discussed.

P108. THIMEROSAL REDUCES THE EXPRESSION OF MT-1 MRNA IN THE CEREBELLUM MICROGLIA CELL LINE, C8-B4. Takeshi Minami and Eriko Miyata.
Laboratory of Environmental Biology, Department of Life Sciences, School of Science & Engineering, Kinki University
minamita@life.kindai.ac.jp

Thimerosal is one of the most famous preservative chemicals used in vaccines. Thimerosal is divided into ehtylmercury and thiosalicylate in the body and the relationship between thimerosal use and autism became a topic about ten years ago. Although the relationship was denied by the large-scale epidemiological survey, the effect of thimerosal in the central nervous system is still not clear. Therefore, we are studying the effect of thimerosal in cerebellum using metallothionein (MT). When C8-B4 cells, microglia cell line established from mouse cerebellum, were incubated 24 hours with thimerosal, 50 % of cytotoxic dose (CD50) of thimerosal was 2.5 μM. When mRNA levels of MTs were compared in cells 6 hours after thimerosal was added, MT-1 mRNA level decreased on a dose-dependent manner and MT-2 mRNA did not change. However, the expression of MT-3 mRNA increased dose-dependently. Then, we measured MT mRNA levels time-dependently in C8-B4 cells after the addition of 2.5μM thimerosal. MT-1 mRNA level decreased dramatically at 4 hours and gradually recovered time-dependently. The level of MT-1 mRNA in cells at 24 hours did not change the level of the control group. On the contrary, both levels of MT-2 and MT-3 mRNAs did not change from the control group until 24 hours. It is suggested that MT-1 is the target against thimerosal in C8-B4 cells. The reason why the expression of MT-1 mRNA decreases in the cells by the addition of thimerosal is still unknown.

P109. THE ROLE OF MITOCHONDRIAL FERRITIN IN CELLULAR IRON METABOLISM.
Enrique A. Armijo, Natalia Mena and Marco T. Núñez
Biology Department, Faculty of Science, University of Chile, Santiago, Chile
armijoenrique@hotmail.com

Iron is an essential element required for normal cell function, it participates in redox reactions by reversely donating or receiving an electron. Still, the reducing conditions of intracellular environment favor the reaction of iron with hydrogen peroxide, generating hydroxyl radicals (•OH) through the Fenton reaction. Therefore, considering its potentially toxic properties cells regulate tightly iron uptake, storage and mobilization. Cytosolic ferritin (Ft) is a nanobox protein that plays a critical role in intracellular iron homeostasis by storing it inside its multimeric shell and exerting important protective roles against iron-mediated free radical damage (Hintze & Theil, 2006). Therefore, although excess iron is stored primarily in cytosol, most of the metabolically active iron is processed in mitochondria. The discovery of mitochondrial proteins implicated in iron homeostasis, such as Mitochondrial Ferritin (MtFt) (Levi et al., 2001), a novel ferritin H chain-like protein, suggest a level of complexity of cellular and mitochondrial iron metabolism not previously imagined. MtFt expression correlates with cellular mitochondrial number rather than iron storage capacities, presenting higher expression in testis and neuronal cells rather than liver cells (Napier et al., 2005). Previous studies demonstrated that MtFt overexpression significantly affects intracellular iron homeostasis in mammalian cells, which show elevated Transferrin Receptor (TfR) levels, decreased Ft levels and increased cellular iron uptake. Nevertheless, MtFt function and regulation remain largely unknown (Nie et al., 2005; Nie et al., 2006). We have studied the expression of MtFt in human neuroblastoma cell SHSY-5Y exposed to different extracellular iron concentrations and to the neurotoxin Rotenone as a cellular model of Parkinson's Disease. In particular, unlike Ft, MtFt expression increased in response to low iron conditions, a reaction which may help secure an adequate iron supply in this organelle. Furthermore, MtFt were also increased in comparison to control cells, when placed in high iron concentrations, a response that may allow sequestering iron under excess conditions. These functions inside mitochondria are particularly interesting, since these organelles are the sites where heme and Fe-S complex are synthesised and therefore exposed to heavy iron traffic. Interestingly, increased levels of MtFt correlated with an increase in TfR and divalent metal transporter 1 (DMT1) levels, IRPs RNA-binding activities and an increase in cellular iron uptake in cells treated with rotenone. This increased in MtFt may lead to a redistribution of iron from various sources in the cytosol to mitochondria, causing a similar cytosolic iron starvation phenotype (elevated DMT1 and increased cellular iron uptake) observed in cells treated with a complex I inhibitor (Salazar et al., 2006). Finally, we studied the effect of a knock down MtFt on others proteins implicated in iron homeostasis at SHSY-5Y cells.

P110. APPLICATION OF THE DUAL-ISOTOPE-TRACER-RATIO TECHNIQUE TO MEASURE ZINC ABSORPTION IN PIGLETS - A PILOT STUDY. Dorthe Carlson[1], Nancy F Krebs, Sian Lei[2], Jamie E Westcott[2], Jakob Sehested[1], Hanne Damgaard Poulsen[1]
[1]Faculty of Agricultural Sciences, Dept of Animal Health Welfare and Nutrition, University of Aarhus, Tjele, Denmark.
dorthe.carlson@agrsci.dk
[2]Section of Nutrition, Department of Pediatrics, The University of Colorado Health Science Center, Denver, CO, USA
dorthe.carlson@agrsci.dk

Traditionally, the balance technique is used when studying the absorption of nutrients in pigs. However, in addition to unabsorbed Zn, feces contains Zn from absorbed and re-excreted Zn and endogenously secreted Zn (Davidsson, 1994). Thus, in a typical balance study, Zn absorption may be biased. In human studies the most common method employs stable isotopes to label dietary- and endogenously secreted Zn (Krebs and Hambidge, 2001). This pilot study aimed to apply the dual-isotope-tracer-ratio (DITR) method in pig studies and compare the results with traditional balance results. Two 4-wk old castrated piglets were fitted with a jugular vein catheter and placed in stainless steel cages. The diet consisted of fresh cow's milk collected from the cow stables (Faculty of Agricultural Sciences, Denmark) every morning. Piglets were fed three times daily (0800, 1200 and 1430) and milk intake was recorded. After a 7-d adaptation period the balance method and the DITR method were initiated (d1) and the two methods went on simultaneously for 7 days. Balance method: Feces were collected in plastic bags situated on the back of the pigs and urine ran into a tray underneath the pigs. Total urine and complete feces were collected throughout the balance period. All collection materials were acid washed in advance. Milk, feces and urine were analyzed for total Zn concentrations by AAS. Zinc absorption coefficients (ZAC) were calculated as: (intake-output)/intake. DITR method: The enriched stable isotopes 67Zn and 70Zn were given as the oral and IV dose, respectively. The doses were prepared with minor modifications of the method described previously (Hambidge et al., 2004). On d1 oral doses were administered via a tube guided through the esophagus to the stomach just before each meal. The IV doses were administered through the jugular vein catheters at1530 on d1. Blood samples were taken at 1500 on d0 and at 0900 and 1500 on d3 to d7. Zinc isotope enrichment in plasma was measured by ICP-MS and fractional absorption of Zn (FAZ) was determined as described before (Hambidge et al., 2004). The two piglets consumed 915 and 1755 g milk/day. The Zn concentration in a pooled milk sample was 2.84 mg/kg. The ZAC obtained from the balance method were 0.58 and 0.83, respectively. The corresponding FAZ obtained by the isotope technique were 0.65 and 0.67, respectively. When converting these coefficients to absorbed Zn (AZ) the balance technique resulted in 1.50 and 4.15 mg AZ/d and the corresponding values from the isotope method were 1.70 and 3.33 mg AZ/d, respectively. It is concluded that the DITR method is feasible in piglet studies; however, modifications and validation with a higher number of animals are needed to obtain reliable results.

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P111. QUANTIFYING TRACE AND TOXIC METALS IN HUMAN BONE WITH ACCELERATOR-BASED IN VIVO NEUTRON ACTIVATION ANALYSIS.

Ana Pejović-Milić[1,2], Aslam[2], Fiona E. McNeill[2] and David R. Chettle[2]
[1]Department of Physics, Ryerson University, Toronto, ON, Canada
[2]Department of Medical Physics and Applied Radiation Sciences, McMaster University, Canada
anamilic@ryerson.ca

Introduction: The development of novel techniques and diagnostic tools capable of measuring trace and typically toxic metals in animal and human tissues, both *ex vivo* and *in vivo*, has been an important area of research in recent decades. In this presentation an overview of a non-destructive and non-invasive neutron activation analysis (NAA) technique suitable for analysis of animal and human bone will be provided. **Method:** Non-invasive, delayed or prompt, neutron activation analysis is one of the techniques available at the McMaster Accelerator Laboratory (Hamilton, Canada) suitable for measurement of low concentrations of metals accumulated in living tissues. Typically the NAA technique includes our high current tandem accelerator or a ^{238}Pu/Be source as neutron sources, an irradiation/shielding cavity and a 4π NaI(Tl) detection array designed for measurement of an animal or human extremity or a small animal. **Results:** Delayed *in vivo* NAA is currently utilized for measurement of trace amounts at the level of a part per million of aluminum, manganese, magnesium, indium and fluorine in the bones. *In vivo* measurements of eighteen healthy subjects living in Southern Ontario indicated that this technique could be applied for cumulative bone aluminum estimation while delivering a low effective radiation dose of only 14.4 μSv. The mean hand bone aluminum concentration of (27.9±1.0) μgAl/gCa is comparable to the expected levels of 20 – 27 μgAl/gCa. Following feasibility studies, a first pilot study, to measure manganese in the bones of the hand of ten healthy male human subjects, who had no known history of exposure to Mn, was conducted. The inverse variance weighted mean value of Mn/Ca is (0.12±0.68) *μ*g Mn/g Ca, which is comparable within uncertainties with the estimated range of 0.16–0.78 *μ*g Mn/g Ca from cadaver data. Magnesium determination in humans using *in vivo* NAA of hands has been also demonstrated to be feasible, with effective doses as low as one-quarter of those delivered in chest x-rays. The results are found to be in the range of the *in-vitro* measurements reported for other cortical bones collected from different sites of the human skeleton, which confirms that this technique mainly provides a measure of the amount of magnesium in hand bones. The average concentration of magnesium determined in human hands was (10.96±1.25) mg Mg/g Ca. On the other hand, the ^{238}Pu/Be source based prompt NAA is investigated for determination of cadmium and gadolinium accumulated in bone. A highlight of this technique is its ability to be used on living animals and humans, and an emphasis will be given in this presentation to describe the *in vivo* NAA in the light of documented need to measure trace and toxic metals accumulated in living tissues of animals and humans reliably.

P112. A NON-LINEAR COMPARTMENTAL MODEL FOR MOLYBDENUM. Augusto Giussani[1], Federico Tavola[2], Marie Claire Cantone[2], Vera Höllriegl[1] and Uwe Oeh[1].
[1]Helmholtz Zentrum München - Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Radiation Protection, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany.
[2]Università degli Studi di Milano, Dipartimento di Fisica, via Celoria 16, 20133 Milano, Italy.
augusto.giussani@helmholtz-muenchen.de

A compartmental model for the description of molybdenum biokinetics was developed on the basis of recent studies conducted by a number of research groups using stable isotopes as tracers. In those studies intestinal absorption, blood plasma kinetics, urinary and faecal excretion were investigated under different experimental conditions, in particular to find out how these processes were affected by the mass of intake and by the chemical form in which molybdenum was administered.
The results of the studies can be roughly summarized as follows: molybdenum was rapidly and nearly completely absorbed into the systemic circulation when administered in liquid solution, it was absorbed more slowly, and to a lesser extent (40-60%) when incorporated into foodstuffs. Systemic molybdenum was predominantly excreted through the urine; excretion of the stable tracers was observed to be very fast and to increase as the amount of circulating molybdenum increased. At elevated levels of administration also the excretion of endogenous molybdenum was enhanced, thus indicating a mobilization of the material stored in organs and tissues.
The developed model structure consists of a central transfer compartment, split into two subunits, representing blood plasma and extracellular fluids, and of a series of compartments describing the gastrointestinal tract, the kidneys and the urinary path, and an unspecified pool representing organs and soft tissues. The effects observed during the tracer kinetic studies could be simulated by introducing non-linear kinetics at the levels of intestinal absorption and of passage through the renal excretion pathway. Non-linearities were described by making use of saturable kinetics and mass-dependent parameter values. The model structure was created and its parameters were evaluated using SAAMII and Adapt software packages.
The model structure and its parameters will be described and discussed with reference to the current knowledge of molybdenum metabolism and biokinetics. A linearization of the model for application in the field of radiation protection will be also presented.

P113. A COMPARATIVE STUDY OF HAIR ELEMENTS AND PANCREAS FUNCTION.
Tatjana D. Lalic[1] and Ivana S Djujic[2]
[1]IHIS - Science & Technology Park, 11080 Belgrade, Serbia
[2]University of Belgrade, ICHTM – Department of Chemistry, Njegoseva 12, 11000 Belgrade, Serbia
tatjanalali@yahoo.com

The pancreas is a large gland. It secretes insulin, enzymes are of fundamental importance in the handling of glucose, and digestive enzymes that help digest fats, proteins and carbohydrates from food. If the pancreas is not functioning properly, diabetes and/or pancreatitis may develop. Wide renge of factors may cause alterations in pancreas functions. In In the present study we have evaluated in 71 adult subjects alterations in pancreas function, chromosomeal instability and the concentrations of I, Se, Cu, Zn, Fe, Mn, Co, Cr, Mo, Ni, Al, As, Pb, Cd, Hg, Ca, Mg, K and Na in hair. For diagnostic pancreas function and chromosomal instability was used bio/resonance diagnostique. In 41 (58%) individuals were registerd low grade disturbances in pancreas functions, 16 (23%) chronic pancreatitis, 7(10%) Diabetes mellitus type 2 with while and 7 (10%) did not registered functional disturbances in pancres. Such disturbances are undectetable by. Investigations of chromosoms in subjects showed that in 41 casese (58%) exist intability on chromosome 2 where is beside other gen thet regulate expression of pancreatitis-associated protein (inhibits the inflammatory response), 41% on chromosome 4 where 5 genes regulates a family of nyzmes that catalyse oxidative conversion of various alcohol to the corresponding aldehydes, 23% on chromosome 19 where are genes responsible for diabetes mellitus type II and Alzheimer disease, hyperlipoproteinemia atherosclerosis and other, 14% on chromosome 15, 11% on the chromosome 10, and under 10%on chromosomes 17, 20, 11, 6, 12 and 8. Values measured for C-reactive protein in pancreas (early prediction of pancreatic necrosis) in investigated subjects correlated with the severity of pancreatitis. Hair analysis data showed that all subjects with chronic pancreatitis and diabetes significantly increased concentrations of Ca and Mg, and in some cases Si, K, Na, P, Fe, Cu, as well as that over 80% of subjects with altered function of pancreas have significantly decreased cocentrations of Zn, Mn, I, Se and Co. Disturbances in other elements except Cr that is as a rule decreased in hair of subjects with diabetes, were detected in only some samples. These observations indicate that these could play important role in pancreas function maintaining.

P114. SEMEN QUALITY PARAMETERS IN RELATION TO SERUM ZINC, COPPER AND SELENIUM IN MEN

Alica Pizent[1], Jasna Jurasović[1], Božo Čolak[2]
[1]Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2]University Clinic for Diabetes, Endocrinology and Metabolic Diseases "Vuk Vrhovac", Zagreb, Croatia
apizent@imi.hr

The relationship between serum zinc (SZn), copper (SCu) and selenium (SSe) and parameters of semen quality was examined in 210 men 19-52 years of age who had never been occupationally exposed to metals. The subjects were randomly selected among those reporting for examination in the andrology unit of the Vuk Vrhovac Clinic in Zagreb. The SZn and SCu measurements were performed by flame AAS method. SSe was measured by electrothermal-AAS method. Parameters of semen quality, including semen volume, immature sperm cells, sperm concentration, sperm count, motility and viability, were determined by using computer-aided sperm analysis (CASA). Oligozoospermia (sperm concentration<20 x 10^6/ml) was observed in 19 (9%) men, asthenozoospermia (sperm motility<40%) in 52 (25%), oligoasthenozoospermia (sperm concentration<20 x 10^6/ml and sperm motility<40%) in 80 (38%) and normozoospermia (sperm concentration ≥ 20 x 10^6/ml and sperm motility ≥ 40%) in 59 (28%) men. Men with oligoasthenozoospermia showed significantly lower SZn as compared to men with normozoospermia, and significantly lower SSe as compared to men with oligozoospermia. No significant difference between the groups was found for age, smoking habits, alcohol consumption and SCu levels. Results of the Spearman's rank correlation in all 210 subjects showed a significant increase in sperm concentration (p=0.0046), sperm count (p=0.0029), percentage of viable sperm (p=0.049), viable sperm count (p=0.013), motile sperm count (p=0.006) and progressively motile sperm count (p=0.010) with respect to an increase in SZn. These results confirmed beneficial role of zinc in spermatogenesis and sperm motility.

P115. A SIMPLER ANALYTICAL METHOD FOR PHYTATE. Donald Oberleas, Alemzewed Challa, Barbara Stoecker and Barbara F Harland.
Texas Tech University Emeritus, Lubbock, Texas; Hawassa University, Awassa, Ethiopia, Oklahoma State University, Stillwater, Oklahoma; and Howard University, Washington, DC; USA
Doberlea@aol.com

Phytate is an integral part of understanding zinc nutrition in monogastric animals and man. Historically, methods have ranged from a titration, through precipitation and ashing to what was considered the ultimate, HPLC. Each of these methods has advantages and disadvantages. The major problem with the HPLC method is the initial cost that makes the acquisition of instrumentation and maintenance nearly impossible for scientists in developing countries. The reagents required are similar to those used in the HPLC method. The basic methodology published by Latta and Eskin (J. Agri. Food Chem. 28:1313-1315, 1980) required some modification. The method utilizes gravity flow columns of about 0.25 inch ID x 24 inch length with some means to control flow. All reagent solutions are aqueous. The equipment required is a colorimeter or spectrophotometer that reads in the visible spectrum. An AG1 x 8, 100-200 mesh or 200-400 mesh resin is necessary. This is a major initial expense but the resins may be reused many times, and may be washed and stored in ethanol between uses. There are large dilutions and precision pipetting is required for successful results. The current method was developed with a Beckman-Coulter 800 spectrophotometer read at 500 nm. A 6-point standard curve from 25 µg through 400 µg had a correlation coefficient of –0.9998. Twelve samples of kidney beans ranged from 6.54 to 12.36 mg phytate/g with a mean of 8.88 mg/g. A corn sample contained 3.78 mg phytate/g and the phytate could be decreased with 24 hour germination. The best estimate is that results may be obtained with<5% variation. Most of the samples analyzed were grown, harvested, dried, and ground in Southern Ethiopia and the flour samples analyzed in laboratories at Oklahoma State University.

P116. CONTENT OF TRACE ELEMENTS, ANTIOXIDANTS AND OTHER NUTRIENTS IN FOUR ANDEAN VEGETABLE SPECIES OF BOLIVIA. Felipe Chuquimia[1], J Antonio Alvarado[1], J Mauricio Peñarrieta[1,2,3], Björn Bergenståhl[3], Björn Åkesson[2]
[1]Instituto de Investigaciones Químicas, Universidad Mayor de San Andrés, La Paz, Bolivia,
[2]Biomedical Nutrition, Pure and Applied Biochemistry, Lund University, Lund, Sweden,
[3]Food Technology, Lund University, Lund, Sweden
jaalvkir@gmail.com

In the present work the quantification of iron, zinc and copper of four vegetable species of the Andean region of Bolivia was carried out. The selected plants are used as foodstuffs, in particular in times of famine. The plants are: the rhizomes of chijura (Stangea rhizantha); the roots of the cactus achacana (Neowerdermannia vorwerckii), the white basal leaves in form of rose of siki (Hipochoeris meyeniana var.brachylepis) and the shaft of the flower of amañoke (Ombrophytum subterraneum Asplund), an eatable parasite Balanophoraceae that grows on roots of thola (Lepidophillum quadrangulare) and it is also used as a medicine. The quantification by AAS was performed according the AOAC method V1, Met.975.03 15th Ed., and was carried out in triplicate. Several interesting results regarding the trace element content of the studied species were obtained. All the species presented high Fe values, and the highest values were obtained for chijura (10.9 mg/100 g). Regarding Zn, achacana (2.6 mg/100 g), amañoke (1.5 mg/100 g) and chijura (1.8 mg/100 g) presented high values. Regarding the content of Cu, amañoke stands out with a content of 0.46 mg/100 g. Keywords: trace elements, antioxidants, Andean species The study was part of a collaborative project between UMSA and ULUND sponsored by the Swedish International Development Agency.

P117. THE ELEMENTOME MATRIX MAP FOR A COMPLEX CONTEXTUAL INTERACTIONS IN THE HUMAN WHOLE BLOOD.
[1]Rastko Momčilović, [2]Juraj Prejac, [3]Dalibor Veber, [4]Nikola Ivičić, [5]Jadranka Pongračić, [5]Anica Benutić, [6]Glenn Irvin Lykken, [7]Berislav Momčilović.
[1]Faculty of Electro Engineering/Computing, Zagreb (Zg), CROATIA (CRO),
[2]University Hospital Center, Zg, CRO,
[3]Occupational Health Outpatient Clinic Dr.Terzić & Dr. Hoffert, Osijek, CRO,
[4]Inst Med Res Occup Hlth, Zg, CRO,
[5]Croatian Institute for Public Health, Zg, CRO,
[6]Department f Physics, UND, Grand Forks, ND 58201-7219, USA,
[7]Institute for the Research and Development of the Sustainable Eco Systems, Zg, CRO
berislav.momcilovic@gmail.com

Interactions between the elements have a long and fruitful history in the nutritional sciences. Today, the medical importance of these interactions is only increased by the widespread shelf accessibility of the mineral/vitamin supplements. Indeed, uncritical consumption of such supplements of various bioavailability may lead to iatrogenic complications and toxicity. The aim of this work is to analyze and show the map of the possible elemental interactions by the analysis of the multielemental profile (MP) of blood in ten healthy women 20-30 years old. The study was conducted by following the ethical principles of the Declaration of Helsinki for the Human Subjects Research. Blood samples were acid digested and analyzed for Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Ge, Hg, I, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pt, Rb, Sb, Se, Si, Sn, Sr, Ti, Tl, V, W, Zn, and Zr by the ICP MS (Elan 9000, Perkin Elmer, USA),(Momčilović et al. TEM (Moscow), 2006; 7(4): 35-42). The elemental interactions within the MP were analyzed by the Pearson's multiple correlation test at $p<0.05$, $p<0.10$, and $p<0.15$; only the data for $p<0.10$ are shown in a diagonal network display. A complex contextual elementome matrix map of interactions in the normal whole blood emerged. Thus, the results showed a wide range of proportional, inverse and/or neutral interactions among elements, whereas the number of possible interactions increased with the increase of the probability [$(p<0.10)>(p<0.10)>(p<0.15)$]. Such results indicate that the interactions among the elements occur even at their physiological range level in the whole blood. Thus, homeostasis of the MP "milieu interior" in the whole blood is the resultant common vector of the dynamic equilibrium of metabolic processes in the human body. Moreover, a large number of elements from this MP were not tested for their interaction capacity since manz of them are, so to say, "in a search for the function". Specific self-contained clusters of Cr/Ga/V and Au/Bi/Sn/Tl were identified by this diagonal network display. Acknowledgements. This work was supported by the RCI, Isle of Man, UK, the McDonald's, Zg, CRO, and the MZOS, Zg, CRO, Grants 022-0222412-2403 and 292-0222412-2405.

P118. THE ISOTOPE MASS NUMBER OVERLAP IN THE MULTIELEMENT PROFILE ANALYSIS – THE *CHESHCHUYA* (FISH SKIN) MODEL.
[1]Rastko Momčilović, [2]Juraj Prejac, [3]Glenn Irvin Lykken, [4]Nikola Ivičić, [5] Berislav Momčilović.
[1]Faculty of Electro Engineering and Computing, Zagreb (Zg), Croatia (CRO)
[2]University Hospital Center, Zg, CRO,
[3]Department of Physics, University of North Dakota, ND 58201-7219, USA
[4]Institute for Medical Research and Occupational Health, Zg, CRO
[5]Institute for the Research and Development of the Sustainable Eco Systems, Zg, CRO
berislav.momcilovic@gmail.com

The appearance of the inductively plasma coupled mass spectrometry (IC PMS) was the major analytical advance in the accurate analysis of the minute amount of the large number of elements in the small volume of the same biological matrice sample. Eventually, the very new condition of available multielement profiles was created where, paradoxically, the elements (or some of them), are in a “search” for their biological function. However, the advance of ICP MS increases the importance of up till now “soft” analytical errors due to the great number of the isotope of the different elements sharing the same mass numbers, to the apparently “hard” error status. Today, indeed, the very nature of the existence of the isotopes of different elements sharing the same mass number is at the cutting edge of the scientific research. The aim of this eclectic work is to show the map of isotopes within the element and between the elements [number of isotopes within the element (number of the isotopes sharing the same mass number between the elements)], in order to visualize the spectrum of possible analytical errors. There are 1497 isotopes of the natural 92 elements of the Periodic system. Indium has the highest number of the isotopes [35(136)], whereas antimony (Sb) has the greatest cluster of the isotopes sharing the same mass number [33(223)]. Iodine, the heaviest essential trace element which deficiency appeared to be highly associated with the development of human depression, has 24 isotopes within the cluster of 207 element isotopes sharing the same mass numbers, i.e. [24(207)]. Indeed, Ag, Cd, In, Sn, Sb, Te, Xe, Cs, Ba, La,, Ce, Pr, and Nd, are 13 different elements comprising the iodine cluster by sharing the same mass numbers. It is reasonable to assume that by controlling for the difference in the isotope mass numbers and their valence will allow for a more advanced research in the metabolism of elements. Acknowledgements, this work were supported by the RCI, Isle of Man, United Kingdom; McDonald’s, Zg, CRO; and MZOS, Zg, CRO, Grants 022-0222412 -2403 and 292-0222412-2405.

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